Physico-Chemical Changes in the Fruits of Two Coconut (*Cocos nucifera L.*) Hybrids during Ripening. A NIRS-boosted Study

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Abstract

Near infrared spectroscopy was used to assess total soluble sugar (TSS) and lipid contents in the freeze-dried kernel of coconuts from two cultivars: PB121 or MYD x WAT from Ivory Coast and VRD x VTT from Vanuatu. The spectra of 385 samples were acquired on a NIRSystems 6500 monochromator. Using this spectral library, a classification algorithm was applied to extract 128 samples representative of the library. The latter were used to construct calibration equations in reference to HPLC laboratory analyses for sugar contents and automatic extraction by organic solvent for lipid contents. The TSS and lipid contents predicted by the model provided information on coconuts quality from the two cultivars studied at different stages of ripeness. This study demonstrated the suitability of a near infrared spectroscopy tool for assessing the quality of coconut palm fruits. The methodology chosen was shown to be relevant for the research and production constraints encountered in the coconut commodity chain. The cost of the study was lowered by 70%, whilst the volume of organic solvents was reduced by more than 90%.

INTRODUCTION

Copra oil (from dried coconut kernel), which ranks seventh in the world oils and fats trade, is a major outlet for coconut growing (FAOSTAT, 2004), but it is not the only one. Over the last few years, new coconut-based products like canned coconut milk have been appearing on the markets in producing countries and are gradually establishing a foothold on the European market. For these outlets, a coconut variety is not required to provide tonnes of copra per hectare, but tonnes of fresh kernel (Ranasinghe, 1996). For instance, new criteria for assessing and selecting coconut varieties are appearing (Baudoin and Rouzière, 1996) and need to be measured. As a corollary, analytical methods adapted to products and production contexts have to be developed.

The coconut palm is a perennial plant whose continuous production throughout the year undergoes climatic variations. The most common varieties are diploid and cross−fertilizing, which leads to substantial variability in the phenotypic traits of the fruit. Monitoring a single variety over a period of three years generates around 350 kernel's samples. Given the number of varieties to be assessed, the structure of the farms (Kullaya and Sangare, 1996) and the uncertainty of the coconut world market (Voituriez, 2000), it is clear that proposing a methodology making it possible to reduce the number of

laboratory analyses whilst using inexpensive, versatile and robust tools (Quinsac and Ribailler, 1998) would be advantageous.
Near infrared spectroscopy (NIRS) is one of the methods that satisfied our operating conditions. It does not require much equipment in the field and can be used to monitor several fruit quality criteria. This study set out to test the ability of NIRS to assess the different coconut cultivars according to predefined quality criteria. It also set out to develop a sustainable research methodology, in adequation with the coconut production context.

MATERIAL AND METHODS

Harvesting and Sample Preparation

The study involved two coconut hybrids: PB121 from Ivory Coast (Nucé de Lamothe and Bénard, 1985), cultivated worldwide, which is a cross between the Malayan Yellow Dwarf and the West African Tall (MYD x WAT) and the Vanuatu hybrid which is a cross between the Vanuatu Red Dwarf and the Vanuatu Tall (VRD x VTT) (Labouisse et al., 2005). Six palms were selected per study plot for each of the cultivars. Bunches of increasing ripeness were harvested from the six palms. In the coconut palm, the degree of ripeness is determined by the rank number of the frond subtending the fruit bunch. Rank 15, the most immature fruit stage in our study, corresponded to 4-month-old nuts and rank 24 to mature coconuts (12 to 13 months), harvested for copra preparation. There was an interval of around one month between two successive ranks.

Three nuts were taken from each of the harvested bunches. A 50 g sample of kernel was taken from each nut and freeze-dried. Three hundred and eighty five kernel samples, which were representative of the different stages of ripeness, were harvested in Vanuatu (246) and Ivory Coast (139) during the 2001 to 2004 period. The freeze-dried samples were transported to the laboratory and ground in liquid nitrogen using an IKA–Werke (Germany) A10 M20 type grinder equipped with blades, until a particle size of 200 µm was obtained.

Biochemical Analyses

1. Determination of Lipid Contents. Lipids were extracted from the freeze-dried kernels using an automatic extractor: ASE® 200 (Accelerated Solvent Extraction, DIONEX Inc., USA). After homogenization of the thawed samples, a 2 g sample aliquot was taken and placed in the extraction unit along with 2 g of Fontainebleau sand. The extraction solvent was petroleum ether at 60°C. The flush was set at 100% and the number of cycles at 5, with a static time of 7 min. The lipid content of the sample was the ratio between the weight of fatty matter extracted and the weight of the test-piece. It was expressed in g of lipids per 100 g of dry matter (% db).
2. Determination of Total Soluble Sugar Content. Sugars were extracted on the same apparatus: ASE® 200. Extraction was carried out immediately after lipid extraction on the meal remaining in the extraction unit. The solvent used was an 80% ethanol solution at 60°C. There were 5 cycles, a static time of 7 min, and a flush of 100%. The sugar extracts recovered were diluted and filtered. They were injected into a DIONEX-DX 600 high performance liquid chromatograph fitted with a Carbopac MA-1 column. The eluant was 612.10^{-3} mol sodium hydroxide at a flow rate of 0.4 $ml.min^{-1}$. After separation, sugars were detected by a Dionex ED50 pulsed amperometric system. The total sugar content corresponded to the sum of the contents of the different carbohydrate compounds detected

in the kernel and was expressed in g of sugars per 100 g of dry matter (% db).

Spectral Analyses

NIRS acquisitions were carried out in mini-cups on a Foss-Perstorp 6500 analyser (FOSS NIRSystems Inc., USA) with a spinning module equipped with an automatic sampler taking up to 50 cups. The spectral data were collected and processed by WinISI version 1.5 (Infrasoft International, USA).

Three grams of ground kernel were analysed by diffuse reflection from 400 to 2500 nm at 2 nm intervals. For each sample, a sequence of 32/32 measurements (32 measurements of the reference ceramic, then 32 measurements of the sample) was performed. The sample measurements were averaged to obtain the absorbance spectrum in log(1/R) where 1 was the reflection of the ceramic and R the reflection of the sample for each wavelength.

Statistical Analyses

The descriptive statistics of the reference and NIRS predicted data were computed using STATISTICA 6.1 Software (StatSoft Inc., USA).

Relevant information was extracted from the matrix of spectral data by Principal Components Analysis (PCA). Based on the PC matrix, the Mahalanobis H distance from the average spectrum was calculated for each spectrum. An H distance over 3 for a given sample corresponded to a probability of less than 0.01 that the sample belonged to the population. Sample selection was based on this distance (Shenk and Westerhaus, 1991).

Partial least squares or PLS regression was applied to establish mathematical models between the spectral and chemical data (Prévot, 2004). The number of terms (factors) to be introduced was determined by cross−validation.

The efficiency of the models developed was estimated by statistical criteria, which reflected quality of fit and precision (Workman, 1992), such as the coefficient of determination (RSQ), the standard error of calibration (SEC), which represented the precision of the model depending on the number of terms introduced. The standard error of cross-validation (calculated through 4 sub-groups) was used to select the optimum number of PLS factors and to estimate the predictive error of the model (Wold, 1978).

The relationship between the standard deviation (SD) of the reference data and the SECV, expressed as the ratio of performance to deviation (RPD) is a useful criterion for the effectiveness of a calibration (Williams, 1993). Indeed, a calibration for which the RPD is over 3 can be used to predict a criterion with a precision approaching the reference analysis; a RPD between 2 and 3 corresponds to a model applicable to "rough" sample sorting.

RESULTS AND DISCUSSION

Spectral Analysis

Even though the spectral profiles of the two varieties/countries were identical, the absorbance levels were clearly different. It suggested that the average biochemical compositions of the two cultivars were similar in terms of constituent types; the same compounds were found throughout the ripening process and only the relative contents of those compounds varied from one cultivar/country pair to the next.

The graph of the standard deviations (Fig.1), calculated for each wavelength for all of the 385 samples, clearly confirmed that the major variations in the spectra were

located at the fats and/or cellulose bands. This result clearly reflected a spectral population that was representative of all stages of nut ripening. Indeed, as nuts ripen, the two major metabolic phenomena that occur are the synthesis of lipids, which make up the reserves of the seed, and construction of the kernel cell walls, which switches from a gelatinous to a solid state.

Principal Components Analysis

Three successive PCA were calculated, based on the second derivatives of the spectra for the wavelength segment between 908 nm and 2500 nm. This iterative procedure allowed to identify and eliminate 23 outliers samples (H>3). Thirteen principal components were chosen and explained 99.7% of total variance. The first 4 PCs explained 52.9%, 37.0%, 5.1% and 2.5% respectively.

The cluster of points was uniformly distributed according to the first main plane, which explained 89.9% of the initial variance. There was no apparent group structure, be it according to cultivar types and/or countries. Superimposing the samples from Ivory Coast on the first two main planes of the PCA calculated using the samples from Vanuatu (Fig.2) clearly confirmed the choice of a single population for calibration development. In other words, the spectral variability of the samples from Ivory Coast was described by that of the Vanuatu samples.

Sample Selection

In order to save time and money, it was useful to be able to base selection on the spectra of the samples that were most representative of the entire population, so as only to carry out reference analyses on those samples. As a spectrum is the result of the elementary absorption of different constituents depending on their concentration, it is correct to imagine that once artefacts due to physical properties (particle size) and atypical spectra (oxidized, etc.) have been overcome, a spectrum is representative of its composition (types of constituents and concentration). Based on this, it was assumed that one sample was sufficient to represent its neighbourhood.

This approach was applied to the 362 samples kept, by fixing a neighbourhood global H distance of 0.6. The relatively small number (128) of selected samples, i.e. 35% of the whole, reflected relatively low variability in the library. Of the 128 samples, 96 were from Vanuatu and 32 from Ivory Coast.

Biochemical Analyses

The total soluble sugar (TSS) contents of the 128 samples were between 3.5 and 25.5% db, the lipid contents between 34.0 and 76.8% db (Tab.1).

Changes in the TSS and lipid contents of the kernel in the two hybrids was antagonistic (Fig.3), which argued in favour of an assumption whereby sugars contribute towards the synthesis of lipid reserves in the coconut (Rajagopal and Ramadasan, 1999). The highest sugar contents and lowest lipid contents corresponded to the most immature ranks: ranks 15 to 20 depending on the hybrids. The correlation between the lipid content and the TSS content showed a coefficient of determination of 0.61.

According to these initial analyses, the ripening processes of the two hybrids were different. The appearance of kernel in the nut, which was later in Ivory Coast, did not seem to affect lipid contents once ripe. Whilst the kernel of the Vanuatu hybrid seemed to stabilize at maximum lipid content as of rank 20, the kernel of the Ivorian hybrid did not reveal any plateau at the most evolved stages of the fruit.

NIRS Calibration

The value of the RPD coefficient for total sugars was equal to 5.1 (Tab.2). This value, like the SECV value (0.817%), corresponded to an efficient model for the prediction of total soluble sugar contents in the kernels. The coefficient of determination (RSQ) of the reference values depending on the predicted values (Fig.4) was equal to 0.983, the slope of the regression was 1.00 and bias was nil. In comparison, Tarkosova and Copikova, 2000 found a SECV of 1.04 and a RPD of 4.17 for total sugar content of Cavendish bananas.

The fit of the model for lipid contents was not efficient (RPD = 3.1 and SECV = 2.75), but the distribution of this component was highly dissymmetrical; 78% of samples had a lipid content over 55%. The RSQ of the regression (Fig.4) of the reference values depending on the predicted values fell from 0.92 to 0.65 if only those values over 55% were kept. The model developed to predict lipid contents could be applied to classify samples, e.g. by class of contents centred on 30%, 40%, 50%.

Analysis of the Predicted Values and Conclusions of the Study

As the calibration model was judged to fit well for total sugars, the contents of the remaining 234 samples were predicted. The average of the TSS contents for all the samples was 8.4±0.3%db, that of the Vanuatu hybrid was 8.2 and 8.6 for PB121. For lipid content, only a sample screening was possible. The spectral library needs to be developed further, but the prediction indicators are heading in the right direction.

Using NIRS as a sample selection tool led to the laboratory analysis of 128 samples+23 samples removed from the spectral library after the first PCA, i.e. 151 samples in the whole. Laboratory analysis of 385 samples for the constituents described here would have taken 40 weeks in a so-called conventional study using standardized methods. With the combined use of near infrared spectroscopy tools and automatic extraction, it only took 10 weeks (Tab.3), which broke down into 9 weeks of chemical analyses and 4 days of spectroscopy to construct the spectral library. Moreover, the methodology used made it possible to use only 9 litres of solvents rather than 109 (Tab.3). Pollution level was thus reduced by 92%.

The duration, as long as the cost of the study (cost of solvents +cost of equipment, based on a 5 years linear amortization + labour, based on the French Ministry of Foreign Affairs 2003 technician tariff), was 70% less than a so-called conventional study. Time and cost were closely correlated since 97% of the overall cost consisted of labour costs.

Thus, this study demonstrated the feasibility of using near infrared spectroscopy to assess the quality of coconut fruits at different ripening stages. It also highlighted that implementing an analytical methodology combining automatic extraction methods and NIRS enables substantial savings to be made, whilst protecting the environment. The coconut commodity chain has a new tool at its disposal to assess the numerous and various cultivars, which would make it possible to improve the production management in terms of both quantity and quality. However, the full potential of this methodology and, in particular, the ability of NIRS instrument to work in coconut production context has still to be demonstrated.

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Tables

Table 1: Descriptive statistics for the constituents of the 128 selected samples (% db)

	TSS content	Lipid content
Mean	9.0	59.3
Standard deviation	4.2	9.2
Range	3.5-25.5	34.0-76.8
Standard Error of Laboratory	0.2	0.4

Table 2: Calibration and validation results (% db)

Constituent	N[1]	Mean	SD[2]	SEC[3]	RSQ[4]	SECV[5]	Nb PLS[6]	RPD[7]=SD/SECV
TSS	119	8.824	4.138	0.572	0.981	0.817	10	5.1
Lipid	113	60.170	8.451	2.485	0.914	2.750	1	3.1

[1]Number of samples kept by the model (test *t*) out of 128, [2]standard deviation of the calibrating population, [3]standard error of calibration, [4]coefficient of determination, [5]standard error of cross validation, [6]Number of PLS terms, [7]Ratio performance deviation

Table 3: Comparison of solvent volume, time and cost of the study according to the type of methodology

Type of methodology	with NIRS with ASE®	with NIRS without ASE®	without NIRS with ASE®	without NIRS without ASE®
	n=151	n=151	n=385	n=385
Organic solvents (L)	9	71	21	109
% reduction	**92%**	**35%**	**81%**	
Time (week)	10	16	23	40
% reduction	**75%**	**60%**	**43%**	
Cost (euros)	16613	24742	34234	54858
% reduction	**70%**	**55%**	**38%**	

Figures

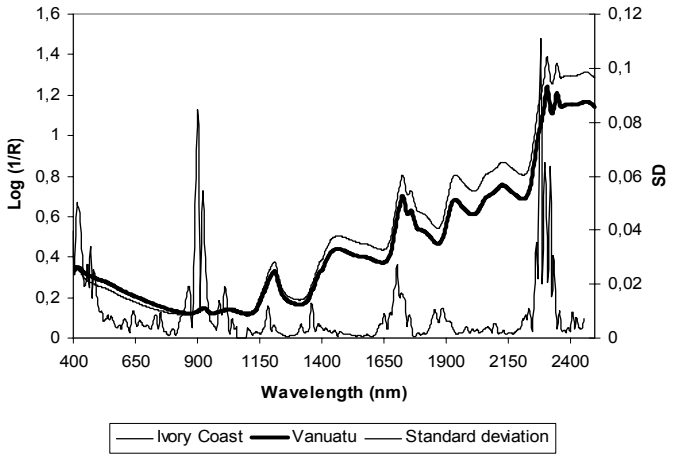

Fig. 1: Average spectra of freeze-dried kernels from the two coconut hybrids and standard deviation at each wavelength

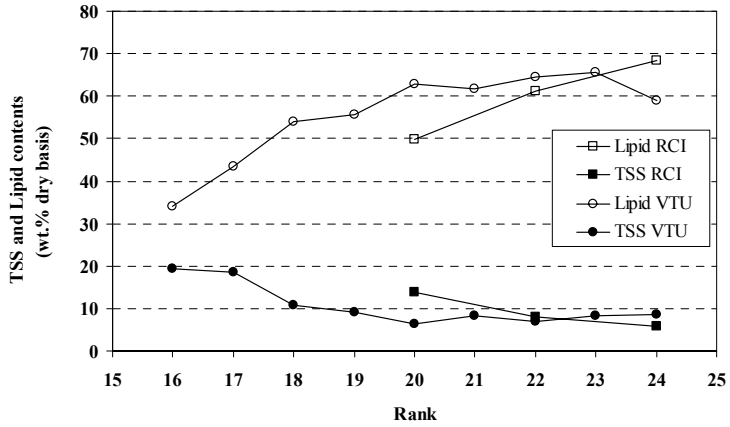

Fig. 2: Two-dimensional scatter plot of the 246 coconut kernel samples from Vanuatu (VTU) and 139 from Ivory Coast (RCI) for the first two PCs

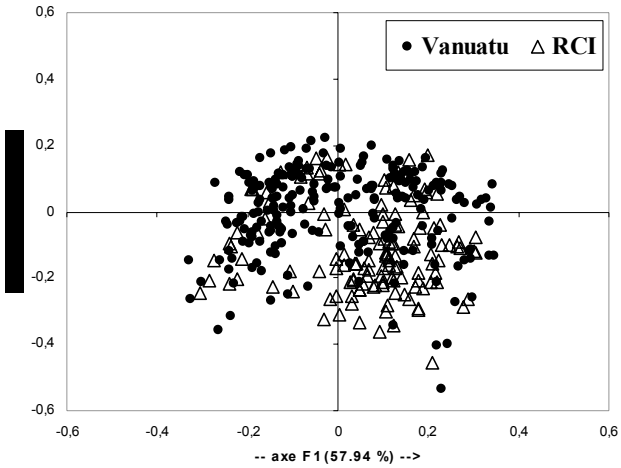

Fig. 3: Total soluble sugar and lipid contents (% db) of the 128 selected freeze-dried coconut kernels according to the ripening stage

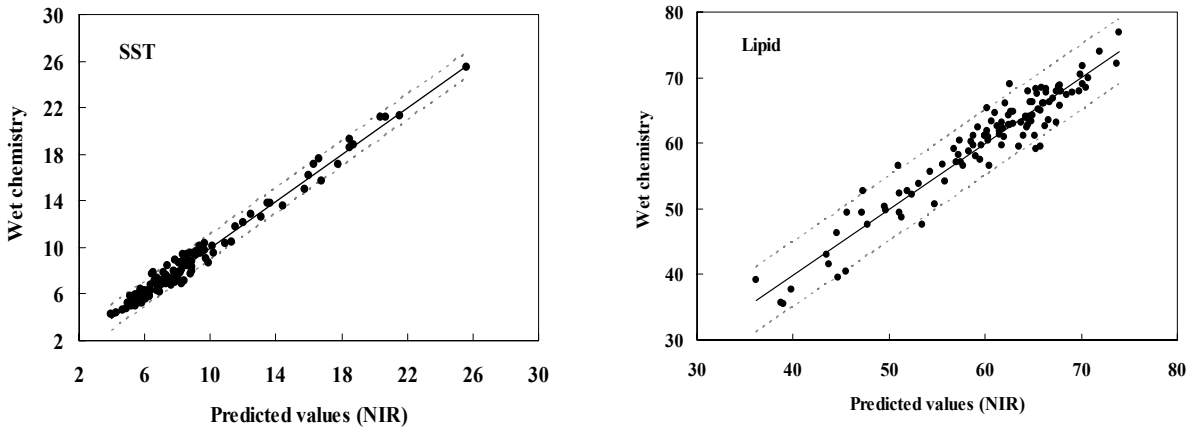

Fig. 4: Correlation between wet chemistry and NIR predicted values for total soluble sugar content and lipid content (Confidence interval 95%)