NANCY TAERA IBRAIMO SAMAMAD

CASHEW APPLE QUALITY BY NEAR INFRARED SPECTROSCOPY TECHNIQUE

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fisiologia Vegetal, para obtenção do título de Doctor Scientiae.

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SUMÁRIO

ABSTRACT

SAMAMAD, Nancy Taera Ibraimo, DSc., Universidade Federal de Viçosa, March, 2016, **Cashew apple quality by near infrared spectroscopy technique.** Advisor: Rolf Puschmann. Co-Advisor: Ebenézer de Oliveira Silva

The cashew tree (Anacardium occidentale L.) is a plant with great economic importance for the Brazilian Northeast, due to diversity of products and the amount of jobs generated. Growing demand for healthy products associated an increase in table cashew consumption encouraged the development of technologies to monitor quality criteria. These criteria are determined by destructive analyses, which are usually time-consuming, with high costs and do not take into account the individual cashew variability. Aiming to replace these analyses, the near-infrared spectroscopy (NIRS) allows simultaneous and nondestructive determination of multiple quality attributes. NIRS is a rapid technique that correlates the energy absorption properties in regions of the electromagnetic spectrum with the composition and concentration of molecules through regression models developed by chemometrics. The aim of this study was to develop predictive models in NIRS for bench top and portable device to estimate physical-chemical properties such as firmness, pH, total soluble solids (TSS), soluble sugars (SSC), titratable acidity (TA), flavor, ascorbic acid (vitamin C), carotenoids, total flavonoids, total extractable polyphenols (TEP) and antioxidant activity for monitoring cashew apple quality. For the bench device, the models were constructed with 34 genotypes of 17 samples collected from the reflectance spectra mode. The predictive models obtained for firmness and pH showed determination coefficient values for cross-validation (R^2_{CV}) of 0.92 and 0.84, respectively, while for external validation or prediction, coefficients of determination (R^2_P) were 0.87 for firmness and 0.78 for pH. The residual prediction deviation of cross-validation (RPD_{CV}) have presented values of 3.0 e 2.4 and for external validation values of 2.4 and 2.2 were obtained for firmness and pH, respectively, indicating a good predictive ability. For variables of primary metabolism, the obtained values for R^2_{CV} were 0.86 for SSC, 0.83 for TSS, 0.90 for TA and 0.80 for flavor and the R^2_P values were respectively of 0.78, 0.75, 0.85 and 0.73. The presented RPD_{CV} values were 2.6, 2.4, 3.1 and 2.1 for SSC, TSS, TA and flavor, while RPD_P obtained values were 2.0 for SSC and TSS, 3.0 for TA and 1.8 for flavor. In secondary metabolism, models with 0.87 values for R^2_{CV} were obtained with R^2_P of 0.85 for vitamin C. These models presented good ability to predict both cross-validation and external validation with RPD_{CV} and RPD_P values of 2.6 and 2.8, respectively. Carotenoids models presented R^2_{CV} and R^2_P values of 0.89 and 0.79, with RPD_{CV} and RPD_P of 2.9 and 2.0, respectively, while for total flavonóides, models were obtained with values of 0.86 for both R^2_{CV} and R^2_P as well as RPD_{CV} values of 2.6 and 2.0 to RPD_P. Models obtained for TEP has presented values of 0.90 for R^2_{CV} and 0.89 to R^2_P and RPD_{CV} values of 3.2 as well as 2.5 for RPD_P. Antioxidant activity models were obtained with R^2_{CV} and R^2_P values of 0.87 and 0.81, respectively, and RPD_{CV} values of 2.7 and 2.2 for RPD_P. For portable device predictive models, 75 samples from 21 different genotypes were collected and evaluated of which firmness, pH, TSS, TA, flavor and vitamin C presented R^2_{CV} values of 0.77, 0.75, 0.90, 0.85, 0.80 and 0.89, respectively, with the average relative error of -1.1%, 0.2%, 0.5%, -1.3%, 2.6% and 4.9%. For these variables were obtained coefficients of determination values for prediction (R^2_P) of 0.76, 0.72, 0.88, 0.85, 0.82 and 0.83 with standard error of prediction (SEP) coefficient of variability of 18.2%, 3.0%, 5.6%, 19.6%, 15.4% and 12.1%. Besides these, a quality monitoring experiment in cold storage was evaluated by NIRS over nine days. Four genotypes were used with tree repetitions for TSS, vitamin C and pH assessment evaluated in split plot in time.

RESUMO

SAMAMAD, Nancy Taera Ibraimo, D.Sc., Universidade Federal de Viçosa, Março, 2016, **Qualidade do pedúnculo de caju por técnica de espectroscopia de infravermelho próximo.** Orientador: Rolf Puschmann. Coorientador: Ebenézer de Oliveira Silva

O cajueiro (Anacardium occidentale L.) é uma planta de grande importância econômica para o Nordeste brasileiro, pela diversidade de produtos e quantidade de empregos gerados. A crescente demanda por produtos saudáveis associados ao aumento no consumo do caju de mesa incentivou o desenvolvimento de tecnologias para monitorar os critérios de qualidade. Esses critérios são determinados por meio de análises destrutivas, que são normalmente demoradas, com custos altos e não leva em consideração a variabilidade individual do caju. Visando substituir essas análises, a espectroscopia de infravermelho próximo (NIRS) permite a determinação simultânea e não destrutiva de vários atributos de qualidade. NIRS é uma técnica rápida que correlaciona as propriedades de absorção de energia em regiões do espectro electromagnético com a composição e concentração de moléculas através de modelos de regressão desenvolvidos por meio da quimiometria. O objetivo deste estudo foi desenvolver modelos preditivos em NIRS para o dispositivo de bancada e portátil visando estimar propriedades físico-químicas tais como firmeza, pH, sólidos solúveis totais (TSS), açúcares solúveis (SSC), acidez titulável (TA), sabor, ácido ascórbico (vitamina C), carotenóides, flavonóides totais, polifenóis extraíveis totais (TEP) e atividade antioxidante para o monitoramento da qualidade do pedúnculo de caju. Para o dispositivo de bancada, os modelos foram construídos com 34 amostras de 17 genótipos a partir de espectros coletados no modo de refletância. Os modelos preditivos obtidos para firmeza e pH apresentaram valores de coeficiente de determinação para validação cruzada (R^2_{CV}) de 0.92 e 0.84, respectivamente, enquanto que para os coeficientes de determinação para a validação externa (R^2_P) foram de 0.87 para firmeza e 0.78 para pH. A relação do desempenho do desvio para validação cruzada (RPD_{CV}) apresentaram valores de 3.0 e 2.4 e, para a validação externa (RPD_P) se obtiveram valores de 2.4 e 2.2 para firmeza e pH, respectivamente, indicando boa capacidade de predição. Para as variáveis do metabolismo primário, os valores obtidos para o R^2_{CV} foram de 0.86 para SSC, 0.83 para TSS, 0.90 para TA e de 0.80 para sabor, sendo que os valores de R^2_P foram de 0.78, 0.75, 0.85 e 0.73, respectivamente. Os valores apresentados para RPD_{CV} foram, respectivamente, de 2.6, 2.4, 3.1 e 2.1 para SSC, TSS, TA e sabor, enquanto que os valores de RPD_P foram de 2.0 para SSC e TSS, 3.0 para TA e 1.8 para sabor. No metabolismo secundário, foram obtidos modelos com valores de 0.87 para R^2_{CV} e de 0.85 R^2_P para vitamina C, apresentando boa capacidade de predição tanto para validação cruzada como para validação externa, com valores de RPD_{CV} e de RPD_P de 2.6 e 2.8. Para carotenoides, modelos com R^2_{CV} e R^2_P de 0.89 e 0.79 foram obtidos, com RPD_{CV} e de RPD_P 2.9 e 2.0, respectivamente, enquanto que para flavonóides totais, foram obtidos valores de 0.86 para ambos R^2_{CV} e R^2_P e, valores de RPD_{CV} de 2.6 e de 2.0 para RPD_P. Modelos obtidos para TEP tiveram valores 0.90 para R^2_{CV} e de 0.89 para R^2_P, bem como valores de RPD_{CV} de 3.2 e 2.5 de RPD_P, sendo que para atividade antioxidante foram obtidos modelos com valores de R^2_{CV} e R^2_P de 0.87 e de 0.81, respectivamente, com valores de RPD_{CV} de 2.7 e 2.2 para RPD_P. Para o dispositivo portátil, foram obtidos modelos preditivos de 75 amostras provenientes de 21 diferentes genótipos sendo que para firmeza, pH, TSS, TA, sabor e vitamina C os valores de R^2_{CV} foram de 0.77, 0.75, 0.90, 0.85, 0.80 e 0.89, respectivamente com a média do erro relativo de -1.1%, 0.2%, 0.5%, -1.3%, 2.6% e 4.9%. Para essas variáveis, foram obtidos valores de coeficientes de determinação para previsão (R^2_P) de 0.76, 0.72, 0.88, 0.85, 0.82 e 0.83 com os coeficientes de variabilidade do erro padrão de previsão (SEP) de 18.2%, 3.0%, 5.6%, 19.6%, 15.4% e 12.1%. Além destes, realizou-se um experimento de monitoramento de qualidade do caju de mesa refrigerado por NIRS ao longo de nove dias. Foram utilizados quatro genótipos com três repetições onde se avaliou TSS, vitamina C e pH analisados em parcelas subdividida no tempo.

GENERAL INTRODUCTION

The cashew tree (Anacardium occidentale L.) is a tropical plant, native from Brazil, dispersed in almost all the national territory and widely cultivated in other tropical countries such as Vietnam, India, Nigeria, Tanzania, Ivory Coast, Mozambique and Benin (RUFINO et al., 2010). Cashew agribusiness plays an important role in producing regions by a significant number of opportunities of direct jobs in the field and industry, as well as the significant participation in the generation of foreign exchange. The market cashew peduncle/false fruit for fresh consumption has grown significantly in recent years and its processing is responsible for the production of natural and concentrated juices, jams, honey, nectar, fruit pulp, soft drinks, among other derivatives, which, largely, are intended the internal market (MOURA et al., 2013). The importance of studying the chemical, physical and physic-chemical properties of cashew apple is a result of a growing demand for quality products, an increasing concern for healthy food and for its conservation methods. Physical and chemical properties are of fundamental importance to quality characterization, for post harvest conservation and for good acceptance of the product by consumers. Among the peduncle main qualitative attributes the most important for conservation are: firmness, pH and total soluble solids (TSS) as physical properties, soluble sugar content (SSC), titratable acidity (TA) and flavor can be considered primary metabolic products and vitamin C, carotenoids, total flavonóides, total extractable polifenols (TEP) and antioxidant activities are secondary products from plant metabolism (CHITARRA; CHITARRA, 2005). For firmness, Lopes (2011), working with peduncles of four commercial clones (CCP 09, CCP 76, BRS 189 and BRS 265) of dwarf cashew reported values between 7 N (CCP 09) and 14 N (BRS 265) in mature false fruits. This characteristic is associated to cell wall structures as well as its integrity. With the peduncle ripening occurs improvement of their sensory characteristics, being developed specific flavors and odors, together with increased sweetness, reduction of acidity and astringency. In the same work, practically constant values were reported for the variable pH during maturation. In early development, the average value found for the evaluated clones (CCP 09, CCP 76, BRS 189 and BRS 265) was 4.52 in stage 1, reaching 4.43 at the stage 7 (mature stage). With regard to acidity, for CCP 76 there was a decrease of 0.32 % to 0.25% and 0.28% to 0.16% in BRS 189 for stages 1 and 7, respectively. At early stages of development (stage 1), the peduncle had a mean value of 7.3 ° Brix and obtained at the ideal stage of consumption, 12.6 ° Brix values for CCP 09, 12.4 ° Brix for CCP 76 and 12 ° Brix for BRS 189 in the mature stage.

For mature peduncles of ten clones evaluated by Abreu (2007), the soluble solids content ranged from 10.47 to 12.90 ° Brix and for SSC, the minimum value obtained was 6.76% and the maximum was 10.83%. Lopes (2011) for the clone CCP 09, found flavor, which are values of SS/TA ratio near Abreu (2007) at mature stage (67.40), but this characteristic is widely influenced by environmental factors such as rainfall, fertilizing, insolation among others.

With the wide genetic variability in the cashew tree, it is necessary to select peduncles (genetic material) with desirable characteristics for production, conservation and with the requirements for commercialization asked by consumers.

Organic acids have great importance for the fruit, and ascorbic acid (vitamin C) is one of the most important for vegetables and for human nutrition. Some fruits are considered exceptional sources and cashew apple have about five times more vitamin C than orange juice and 10 times more than the pineapple juice (MOURA et al., 2013).

Peduncles of the cashew tree have great variability for vitamin C and Lopes (2011) found a range of 73 mg/100g a 279 mg/100 of vitamin C among clones in different stages of development. Also according to this author, in addition to vitamin C, carotenoids and phenolic compounds are mainly responsible for antioxidant activity in cashew. These compounds are secondary products of plant metabolism, exercising essential function in reproduction and growth, acting as a defense mechanism against pathogens and parasites, as well as contributing to plants diverse coloration. Besides these functions, these compounds can bring health benefits associated with reduced risk of chronic and degenerative diseases for humans and they are mainly composed by flavonoids and others phenolic acids with high antioxidant activity (BALSANO & ALISI, 2009; UTTARA et al., 2009). Values of phenolic content from 65 mg to 370 mg of gallic acid per 100 g of pulp have been reported in the work of Lopes (2011), who also reported values of 34 uM Trolox g^{-1} to 41 uM Trolox g^{-1} for the antioxidant activity. However, traditional analytical methods applied to the measurement of these parameters for cashew apple are slow, laborious, expensive and destructive (BITTNER et al., 2013).

Near Infrared Spectroscopy (NIRS) is a rapid and non-destructive technique, that requires minimal sample processing before analysis and, coupled with chemometrics, appears to be a convenient analytical tool to study fruit quality and ripeness. Karl Norris was indicated as a pioneer researcher in NIRS to determination of moisture in agricultural products (PASQUINI, 2003).

NIRS allows simultaneous determination of more than one quality characteristic, reducing the number of sample measurements by monitoring physiological changes on the same fruits during the storage.

In the last decades, NIRS has received increasing importance for nondestructive measurements of several characters in a wide range of fruit and vegetable products including food industry (SANTOS et al., 2013; COZZOLINO, 2014).

In fruits, previous works have demonstrated the feasibility of NIR for a rapid analysis for both quantitative and qualitative analysis of main components of fruit, such as TSS, firmness and functional properties (total anthocyanins, total flavonoids, total polyphenols and ascorbic acid of fresh berries and homogenized samples of blueberries (GUIDETTI et al, 2009), fruit quality in plum (COSTA & LIMA, 2013), Mango (MARQUES, et al. 2015).

Portable NIR spectrometers are powerful instruments offering several advantages for nondestructive, online, or in situ analysis: small size, low cost, robustness, simplicity of analysis, sample user interface, portability, and ergonomic design.

The applications of these devices have been divided into fruits and vegetables, meat and fish, beverages and dairy, cereals and feedstock, soils, manures, viticulture and olive growing (SANTOS, 2013). Spectral information captured with portable instruments is processed using standard chemometric methods.

Chemometrics, usually described as the application of mathematics, multivariate statistics, and computer science, plays a fundamental role in the extraction of important and relevant information hidden in NIR spectra.

Some preprocessing technique is sometimes necessary to remove noise and the effects of accumulating data sets so that only the absolute changes are shown. Reducing instrumental noise or background information is usually performed using smoothing techniques and derivatives are a common method used to eliminate unimportant baseline signals from samples (NICOLAI et al., 2007). Path length effects, scattering effects, source or detector variations, and other general instrument sensitivity effects are usually corrected using normalization preprocessing methods, such as standard normal variate (SNV) and multiplicative signal correction (MSC).

There is an arsenal of chemometric tools dedicated to make use of NIR spectroscopic information. The most common are Multiple Linear Regression (MLR), Principal Component Regression (PCR) and Partial Least Square Regression (PLS).

PLS regression is a mathematical approach that is typically employed in a large number of applications for the qualitative and quantitative analyses of fruit and vegetables.

The most widespread approach is often called PLS1, because the models approximate the spectra variables (x) with the true concentration, and it is possible to obtain a scores matrix that is common to both the concentrations (c) and measurements (x) (BRERETON, 2000).

NIRS regions lies between the mid-infrared (MIR) and visible parts of the electromagnetic spectrum and covers a wavelength range from 750 – 2500 nm which corresponds to a wavenumber range from 13.300 – 4000 cm^{-1} (PASQUINI, 2003). Spectral data are usually related to quality attributes by chemometric methods that apply mathematical and statistical methods to create prediction models. Prediction models are developed by software packages to perform necessary mathematical calculations for data preprocessing. This preprocessing, such as derivatives (first and second), multiplicative scatter correction (MSC), standard normal variate (SNV), normalization, baseline corrections and smoothing eliminate systematic disturbances so that spectral features can be enhanced. After preprocessing, regression models such as partial least square (PLS) can be applied for development of calibration models. There are several quality parameters such as root mean square errors (RMSE) of calibration(C), cross validation (CV), prediction (P), coefficient of determination (R^2), residual prediction deviation (RPD) and others available to assess the quality of a calibration model.

This study aims to estimate physical and chemical properties from primary and secondary metabolism in cashew apple for quality monitoring by non destructive method through NIRS application associated with chemometric methods.

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I. ARTICLE 1: DEVELOPMENT OF MULTIVARIATE MODELS FOR NONDESTRUCTIVE ANALYSIS OF CASHEW APPLE QUALITY

ABSTRACT: NIR Spectroscopy ability was investigated to assess cashew apple internal characterization effect on prediction performance of physico-chemical properties, primary and secondary metabolism compounds. The spectra of 34 samples of cashew apple were obtained on reflectance mode from a bench spectrometer using spectral range from 9000 cm^{-1} to 4800 cm^{-1}. Relationships between spectral wavenumber and evaluated parameters through the application of chemometric techniques based on full spectrum were established. The obtained models for firmness and pH presented root mean square error of cross-validation (RMSECV) and external validation or prediction (RMSEP) values of 0.70N and 0.71N for firmness and for pH, the RMSECV and RMSEP values were 0.17. The coefficient of determination (R^2) for firmness was 0.92 for cross validation and 0.87 for prediction and for pH, the R^2 for cross-validation and prediction was of 0.84 and 0.78, respectively. For soluble sugar content (SSC) the models presented RMSECV of 0.96% and RMSEP values of 0.99% with R^2_{CV} values of 0.86 and R^2_P values of 0.78, while total soluble solids (TSS) models obtained 0.7 °brix for both RMSECV and RMSEP with R^2_{CV} values of 0.83 and R^2_P values of 0.75. The obtained models for titratable acidity (TA) presented RMSECV and RMSEP values of 0.049% of malic acid and 0.055% of malic acid with R^2_{CV} values of 0.90 and R^2_P values of 0.85. Flavor (TSS/TA) obtained models presented, respectively, RMSECV and RMSEP values of 6.5 and 7.5 with R^2_{CV} values of 0.80 and 0.73 for R^2_P. For vitamin C, carotenoids, flavonóides, total extractable polyphenols (TEP) and antioxidant activity the RMSECV values were 37mg $100mg^{-1}$, 0.079mg g^{-1}, 0.85µg g^{-1}, 25 mg $100mg^{-1}$ and 32µg mL^{-1}, and the RMSEP obtained values were 38mg $100mg^{-1}$, 0.089mg g^{-1}, 0.86µg g^{-1}, 27mg $100mg^{-1}$ and 34µg mL^{-1} respectively. Good prediction performance was obtained for cashew apple with residual prediction deviation (RPD_{CV}) over 2.5 for firmness, SSC, TA, vitamin C, carotenoids, total flavonoids, TEP and antioxidant activity. The remaining parameters, such as pH, TSS and flavor regression models presented RPD_{CV} values above 2.0, showing that NIR technology can be used to evaluate cashew apple internal quality.

1. INTRODUCTION

The cashew tree is a native South American plant that grows in different countries of the world for production of cashew nut and more recently and increasingly, the cashew apple and its products (BRITO et al., 2007).

The cashew agribusiness in northeastern Brazil occupies a prominent place in the economic and social context, with considerable job creation in rural and agribusiness activities (LIMA, 2013).

Growing demand for healthy products associated with an increase in cashew consumption and the purpose of ensuring its acceptance encourages the technologies development to monitor quality. These parameters, such as soluble sugar content (SSC), total soluble solids (TSS), ascorbic acid (vitamin C) and others are products of primary and secondary metabolism that can be used for fruit development, harvesting and postharvest characterization. SSC consists mainly on soluble sugars dissolved in the cell and TSS corresponds to all substances which are dissolved, consisting of sugars, organic acids and others, varying with species, cultivar, ripening stage and climate.

In addition, there is a growing recognition for fruits and vegetables nutritional importance due to their bioactive compounds, which are beneficial phytochemicals to health when consumed in adequate amounts, such as vitamin C, carotenoids, flavonóides and polyphenols (BRITO et al., 2007; MCGOVERIN et al., 2010; BITTNER et al, 2013). Most of these compounds are usually product of secondary metabolism and are related to quality and fruit conservation in post harvest (BEGHI et al., 2013).

These phytochemicals consumption are associated with fight against chronic and degenerative diseases, such as cancer and cardiovascular disease, due to reactive oxygen species reduction (ROS) and they are also known as bioactive compounds (LIU, 2013).

The use of near infrared spectroscopy (NIRS) as a rapid and non destructive technique to measure vegetable internal quality attributes that has been extensively investigated during the past decade (NICOLAI et al., 2007). This technique relies on absorption of energy from molecules in regions of the electromagnetic spectrum (WANG et al., 2014).

Near infrared molecular vibrations result in anaharmonic transitions and are responsible for energy absorption in this region. The wavelengths at which these vibrations occur vary depending on molecules structure and composition.

In the near infrared region the variations observed are mainly due to overtones, combination bands and deformation transitions of bonds such as C-H, N-H, O-H, S-H, aromatic groups and others. Other bands vibration such as C = C and C = O can influence the intensity and position of NIR spectral data combination, especially those at NIRS equivalent wavenumber from 5000 to 4000 cm^{-1}(SANTOS, 2013).

Spectral data are usually related to quality attributes by chemometric methods that apply mathematical and statistical methods to create prediction models through regression that translate spectral data in useful chemical information.

In chemometric methods, partial least square regression (PLS) and its variations are widely used. It refers to optimal least-squares fit computation of a correlation or covariance matrix. PLS is quite similar to principal components regression (PCR), but defines latent variables (principal components) based on covariance between the independent and dependent variables, rather than variance of independent variables alone (TEÓFILO, 2013). PLS regression advantage is the ability to analyze data with noisy, collinear and takes into account errors both in the concentration estimates and spectra (NICOLAI et al., 2007).

Chemometric methods assists plant metabolism analyses through developed prediction models to estimate parameters of interest (TEÓFILO, 2013). Bobelyn et al. (2010) have studied postharvest quality prediction in apple with NIRS using predictive models for total soluble solids and firmness quantification and they have concluded that the rating source (geographic and seasonal), for cultivating and shelf life can be determined non-destructively through the developed models.

Over the past years NIRS associated with chemometrics have become a useful tool to quickly quantify non-destructively various compounds in agricultural products and other complex mixtures (COZZOLINO, 2015). Thus, this study aims to estimate physico-chemical properties from primary and secondary metabolism in cashew apple by non destructive method through NIRS application associated with chemometric methods.

2. MATERIAL AND METHODS

2.1. Plant material

A total of 34 mature peduncles from 17 different genotypes were collected at the experimental field of EMBRAPA Tropical Agroindustry, Pacajus, (latitude 4º 11 '26.62' 'S, longitude 38º 29' 50.78 " W and altitude of 60 meters above sea level), Ceará, Brazil. Being non climacteric (not ripen after harvest), the peduncle must be harvested fully ripe, when it has the best flavor and aroma characteristics (maximum sugar content, lower acidity and astringency) (MOURA, et al., 2011). For this to be harvested properly, it must be made a slight twist to release the panicle branch of the peduncle. If the peduncle offers resistance to loose, is a sign that he is not yet ready to harvest (MOURA et al., 2013). After harvest, the cashews were transported to laboratory analyses where samples spectra were taken from top, middle and basal region of the peduncle (figure 1). After sample spectra were recorded, cashew was individually identified and held reference analysis.

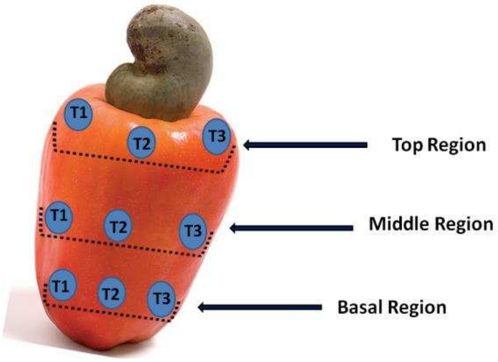

Figure 1. Peduncle regions sampled on the top, middle and basal regions of cashew apple.

2.2. Reference analysis

For quality attributes evaluation of physical and chemical parameters of metabolites from primary and secondary metabolism were carried out. The firmness analyses were performed using a bench texturometer (Stable Micro Systems TA-XT2) equipped with 6 mm diameter probe and pH was measured with a pHmeter, using a potentiometer with glass membrane, as recommended by the Association of Official Analytical Chemistry (AOAC, 1992).

The metabolites from primary metabolism such soluble sugar content (SSC) were measured by the anthrone method described by Yemm and Willis (1954) and total soluble solids (TSS) that were obtained through a portable refractometer (AOAC, 1992). Titratable acidity (TA) was determined by titration with NaOH solution (0.1N) until light pink color and the results were expressed as a percentage of malic acid as recommended by methodology of the Institute Adolfo Lutz-IAL (1985) and flavor obtained through TSS/TA ratio, which is also responsible for palatability.

Compounds with antioxidant properties such as ascorbic acid (vitamin C) were analyzed by titration with DFI solution (2.6 dichlorophenolindophenol 0.02%) until a permanent pink color light (Strohecker & Henning, 1967), carotenoids were determined by Higby (1962) method, total flavonoids (WOISKY & SALATINO, 1998), total extractable polyphenols (TEP) determined by the Folin Ciocalteu method, using a standard curve of gallic acid as a reference, according to the methodology described by Larrauri et al. (1997) and Obanda & Owuor (1997). Total antioxidant activities were analyzed by reaction of radical ABTS with potassium persulphate (LARRAURI et al.; 1997; RE et al.; 1999).

2.3. FT-NIR technique

In this technique the product is irradiated with NIR spectroscopy and the radiation is measured generating a spectra. FT-NIR technique relates changes in spectral data with the changes in the sample and those spectra depend on chemical composition of the product, as well as on its light scattering properties, which are related with microstructure.

Cashew apple samples were analyzed by reflectance mode using a probe with a analyzer FTIR spectrometer (Espectro Varian 660, Agilant FTIR) equipped with a detector SAM at room temperature 24 ± 3 °C, wavelength of 12000 - 4000cm^{-1} (830 - 2,500nm) and relative humidity of 28 ± 3%, with 4cm^{-1} of resolution.

The background measurement was done initially by using reference spectra before collect the sample's spectra. The spectra were measured in log (1/R) mode directly from the region. For each sample, nine spectra were recorded at three different regions of cashew apple. The spectra average of these regions was taken for analysis. All measurements were carried out under controlled room condition in triplicate.

2.4. Spectral data pre-processing

Data analysis was performed using Unscrumbler package (software version 9.7, CAMO). Calibration models for evaluated quality parameters were obtained by application of spectral treatment first derivative Savitzky Golay polynomial algorithm of second order with window size of 31 points.

The optimum pre-processing method and the best calibration model was selected on the basis of minimum PLS factors/rank, lowest RMSECV values, maximum coefficient of determination (R^2) and lowest Bias for cross and external validation. In this work, all data was mean centered.

2.5. Calibration and validation of developed model

Cashew apple samples were divided into calibration set with 2/3 of samples (n=23) and prediction with 1/3 of samples (n=11) by applying the classic Kennard-Stone (KS) selection using MATLAB software version 7.10 (the Math-Works, Natick, Massachusetts, USA), specifically the PLS-toolbox (Eigenvector Research, Inc., Wenatchee, WA, USA, version 6.01). After group formation, calibration and prediction models were developed on Unscrumbler software package (version 9.7, CAMO).

Cashew apple samples were used for developing the models. 23 samples were used for the calibration and cross-validation through partial least squares (PLS) regression since the number of samples was limited. In leave one sample out cross-validation, the spectrum of one sample of the calibration set was deleted from this set, and a PLS model was built with the remaining calibration set spectra. The left out sample was predicted with this model and the procedure was repeated with all calibration set samples.

The models were optimized by elimination of samples considered outliers because they presented high leverage for reference values or spectral data.

Spectra of samples with known value of evaluated characteristics were collected as described previously and the average was used to multivariate analysis.

Calibration (C), cross validation (CV) and prediction model trueness were judged by root mean square errors (RMSE) calculated as follows:

$$\text{RMSE}= \sqrt{\frac{\sum_{i=1}^{n}(yi-y)^2}{n}}$$

Where n is the number of samples in calibration set, y is the reference measurement for sample i and y_i is the estimated result for sample i when the model is constructed with the sample i removed.

This calculation is repeated for each of pre-processed spectra and the coefficient of determination (R^2) between NIR predictive and reference measurement value is calculated:

$$R^2 = 1 - \frac{SSE}{\sum(yi-ym)^2}$$

Where SSE is the squared errors sum and y_m is the mean of reference results for all samples in calibration set. The residual prediction deviation (RPD) was also used to evaluate the models and is defined as the ratio of standard deviation (SD) of response variable to RMSECV and indicates the model predictive capacity (NICOLAI et al, 2007).

$$RPD = \frac{SD}{RMSECV}$$

Prediction accuracy of models was regarded as excellent or good when RPD was above 2.5. The models could be applied for a rough prediction when RPD ranged from 2.0 to 2.5 (WANG et al., 2014).

The significant test was performed on bias values admitting that a non-significant bias at 5% probability level (t calculated is less that the critical t value for $\lim_{n\to\infty}$ t =1.960). The bias of each group (cross validation and prediction) of results was evaluated for their significance using the equation:

$$t = \left|bias\right|\sqrt{N/SEP}$$

3. RESULTS AND DISCUSSION

3.1. Calibration model

Spectra extracted information can be condensed in latent variables (LV), which are used in calibration and prediction steps (TEÓFILO, 2013).

Prior to PLS modeling, spectral pre processing techniques were used to remove any irrelevant information which cannot be handled properly by the regression techniques (figure 2).

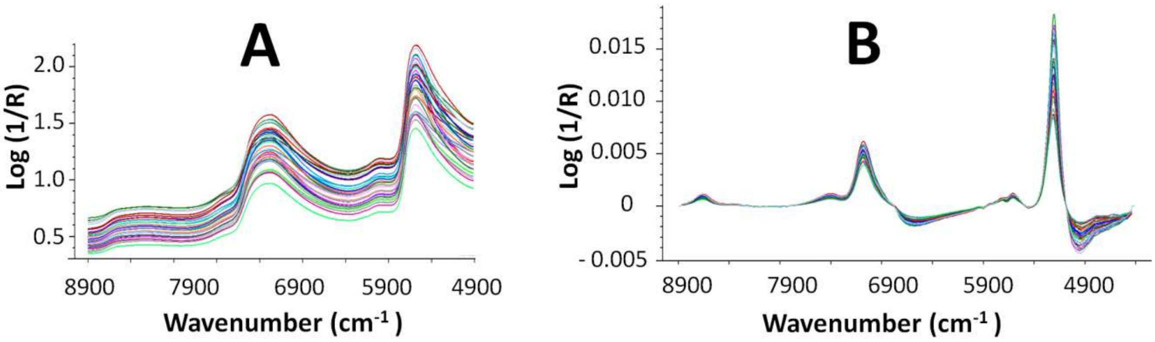

Figure 2. Spectrum of cashew samples without spectral treatment (A) and with first Derivative Savitzky Golay polynomial algorithm of second order with window size of 31points (B).

Best calibration models were achieved after using first derivative Savitzky Golay polynomial algorithm of second order with window size of 31 points to eliminate variables that do not directly correlate with the property of interest or irrelevant information.

3.2. Physico-chemical analysis

For quality evaluation on vegetables, some physico-chemical parameters are required, such as firmness and pH. The firmness or hardness is related to the required force for a product to reach a given deformation, and pH quantifies the ions in the pulp representing -log of hydrogen ions (H^+) concentration. Both models (firmness and pH) were developed with 8 latent variables (LV), firmness models were obtained with RMSECV and RMSEP of 0.70N and 0.71N, R^2_{CV} and R^2_P of 0.92 and 0.87, respectively with RPD values of 3.0 for cross-validation and 2.4 for external validation (prediction). pH models presented values of 0.17 for both RMSECV and RMSEP, R^2_{CV} values of 0.84 with RPD_{CV} values of 2.4 and R^2_P values of 0.78 with RPD_P of 2.2 (table 1). The plots that generated the table can be found in attachment 1.

Table 1. Summary calibration, cross validation and external validation results of cashew apple regression models for firmness (N), and pH obtained by the optimized PLS1 models.

Quality parameters		**Calibration**			**Cross Validation**			**External Validation**				**Range**
	N	LV*	R^{2**}	RMSEC***	R^{2}	RMSECV	RPD****	N	R^{2}	RMSEP	RPD	
Firmness	21	8	0.99	0.23	0.92	0.70	3.0	8	0.87	0.71	2.4	4 - 14
pH	19	8	0.99	0.007	0.84	0.17	2.4	12	0.78	0.17	2.2	3.5 - 5.2

* Coefficient of determination between reference and predicted constituent values (R^2). ** Mean square error (RMSE) of calibration (C) and cross validation (CV). **** Residual predictive deviation (RPD). **** Number of latent variables (LV). Number of samples (N).

Firmness and pH models presented low bias values, respectively, of -0.061 and -0.004 for cross validation, 0.29 and -0.076 for prediction, and they were not significant at 5% of probability by t$_{bias}$ test. These models can predict firmness and pH with efficacy for a large range of values and with low predictive errors.

Eisenstecken et al. (2015) found firmness regression models with 9 LV, R^2 of 0.11 for calibration model with standard error for both calibration (SEC) and external prediction (SEP) of 9.4 N for cultivar Cripps Pink, with R^2_P values of 0.14. For cultivar Braeburn these authors found calibration R^2 of 0.56 and external prediction R^2 of 0.55 with 12 LV, SEC values of 7.8 N and 7.9 N for SEP values. For pH models were found for cultivar Cripps Pink with R^2 of 0.81 and SE of 0.06 for both calibration and prediction sets, with 12 LV. For cultivar Braeburn was found models with also 12 LV, R^2 of 0.62 and SEC of 0.05 for both calibration and validation models.

3.3. Metabolites from primary metabolism

For each set of transformed spectra and each chemical characteristic (quality parameter), PLS models were established (table 2).

Table 2. Summary calibration, cross validation and external validation results of cashew apple regression models for SSC (%), TSS (expressed as °brix), TA (expressed as % malic acid) and flavor obtained by the optimized PLS1 models.

Quality parameters		**Calibration**			**Cross Validation**			**External Validation**				**Range**
	N	LV*	R^{2**}	RMSEC***	R^{2}	RMSECV	RPD****	N	R^{2}	RMSEP	RPD	
SSC	20	5	0.94	0.62	0.86	0.96	2.6	12	0.78	0.99	2.0	5 – 15
TSS	21	5	0.90	0.5	0.83	0.7	2.4	10	0.75	0.7	2.0	10 – 17
TA	18	4	0.96	0.029	0.90	0.049	3.1	11	0.85	0.055	3.0	0.2 – 0.8
Flavor	18	3	0.87	4.8	0.80	6.5	2.1	10	0.73	7.5	1.8	25 – 80

* Coefficient of determination between reference and predicted constituent values (R^2). ** Mean square error (RMSE) of calibration (C) and cross validation (CV). **** Residual predictive deviation (RPD). **** Number of latent variables (LV). Number of samples (N).

For properties of primary metabolism, SSC and TSS presented good modeling, both with 5 LV, R^2_{CV} and R^2_{P} values of 0.86 and 0.78, with RPD_{CV} of 2.6 and RPD_{P} of 2.0, respectively, for SSC. For this property, the RMSECV and RMSEP obtained values for SSC was 0.96% and 0.99%, respectively. For TSS models, the obtained RMSECV and RMSEP values were 0.7 °brix with R^2 of 0.83 for calibration and 0.75 for prediction set and bias values was not significant at 5% of probability by t_{bias} test. RPD_{CV} and RPD_{P} values for TSS models were, respectively, of 2.4 and 2.0. According with Nicolai et al (2007) and Wang et al. (2014), RPD above 2 indicates that quantitative predictions are possible and values above 2.5 corresponds to good accuracy prediction, therefore the calibration models for SSC and TSS have good predictive ability. Total acid (TA) and hydrogenic potential (pH) are the primary methods used to measure the acidity of the fruits and vegetables. The acids are found in vacuoles of cells in free or in combined form with salts, esters and glucosides and they are important energy sources for fruit during the ripening process. In these work, TA best models were obtained with 4 LV, R^2 of 0.90 and 0.85 with RPD values of 3.1 and 3.0 for calibration and prediction sets and RMSECV and RMPSEP of 0.049 and 0.055 % of malic acid, respectively. The models for flavor were developed with 3 LV, R^2 of 0.80 and 0.73 with RPD values of 2.1 and 1.8 for calibration and prediction sets and RMSECV and RMPSEP of 6.5 and 7.5.

Although flavor is not a metabolism product, it is an important parameter for determining quality in postharvest obtained from the ratio of TSS and TA values and can be estimated directly with NIRS. As more samples and biological variability is taken into account the prediction and calibration models become more robust against future changes, but may simultaneously reduce the accuracy of the models (NICOLAI et al., 2007).

Eisenstecken et al. (2015) worked with application of near infrared spectroscopy (NIRS) in the wavelength range of 1000–2500 nm for predicting quality parameters in two cultivars of apples (“Braeburn” and “Cripps Pink”) during the pre- and post-storage periods. These authors found TSS prediction models with 3 LV, R^2 of 0.03 for calibration and 0.02 for validation model with SEC and SEP of 0.57 °brix and 0.56 °brix, respectively, for cultivar Cripps Pink. Still for the same cultivar, TA models were found with 8 LV, R^2_{CV} and of R^2_{P} of 0.85 and 0.69, respectively. For cultivar Braeburn R^2_{CV} values of 0.49 and 0.38 for R^2_{P} were found with 6 LV and both SEC and SEP values of 0.52 °brix. Models for TA was found with R^2_{CV} of 0.52 and R^2_{P} of 0.50 with SEC of 0.43 g/L of malic acid and SEP 0.45 g/L of malic acid.

NIRS can also be used for other physiological studies of several plants or plant parts such plant leaves, measuring water contents, chlorophyll content, minerals or products of metabolic pathways (SANTOS et al., 2013) .

3.4. Metabolites from secondary metabolism

The evaluated compounds of secondary metabolism are very important in postharvest because they are related with quality and shelf life, because of their antioxidant capacity and their ability to neutralize ROS. From secondary metabolism, smaller and bigger groups of compounds with antioxidant capacity were evaluated such as vitamin C and total extractible polyphenols, respectively (table 3).

Table 3. Summary calibration, cross validation and external validation results of cashew apple regression models for vitamin C (expressed as mg 100g^{-1} ascorbic acid), carotenoids (mg g^{-1}), total flavonoids (µg g^{-1} of quercetin), TEP (mg 100g^{-1}) and antioxidant activities (µg mL^{-1} of trolox) obtained by the optimized PLS1 models.

Quality parameters		Calibration			Cross Validation			External Validation				
	N	LV*	R^{2**}	RMSEC***	R^2	RMSECV	RPD****	N	R^2	RMSEP	RPD	Range
Vitamin C	21	5	0.92	26	0.87	37	2.6	10	0.86	38	2.8	200-500
Carotenoids	18	5	0.97	0.038	0.89	0.079	2.9	9	0.79	0.089	2.0	0.1 – 0.8
Total flavonoids	22	7	0.95	0.47	0.86	0.85	2.6	9	0.79	0.86	2.0	2 – 10
TEP	20	4	0.94	19	0.90	25	3.2	10	0.89	27	2.5	50 – 350
Antioxidant activity	19	4	0.93	22	0.87	32	2.7	12	0.81	34	2.2	75 – 400

* Coefficient of determination between reference and predicted constituent values (R^2). ** Mean square error (RMSE) of calibration (C) and cross validation (CV). **** Residual predictive deviation (RPD). **** Number of latent variables (LV). Number of samples (N).

Ascorbic acid also called vitamin C, has an important role in cell and tissue defense acting against ROS and its damages. For this property good model was obtained with 5 LV, R^2_{CV} and R^2_P values of 0.87 and 0.86, with RPD_{CV} of 2.6 and RPD_P of 2.8, respectively, and RMSECV of 37mg 100g^{-1} and 38mg 100g^{-1} for RMSEP with not significant bias values at 5% of probability by t_{bias} test.

The accuracy of the prediction models is usually dependent on the accuracy of the reference method. Although vitamin C scavenge ROS separately, they have long been considered to function together with the Acetylsalicylic (AsA) - GSH and the water-water cycles to metabolize H_2O_2, and to dissipate excess excitation energy in chloroplasts (FOYER & SHIGEOKA, 2011). In the literature, several authors consider the regeneration of AA fundamental for cell homeostasis, allowing for re-use of AA in the removal of ROS (MA et al., 2012; XU et al., 2012). Malegori et al. (2015) employed a bench top and portable NIR instrument for measuring fruit quality in acerola fruit. Partial least squares (PLS) regression and support vector machine (SVM) was used to generate the calibration equations for titratable acidity and ascorbic acid. For calibration, the authors found R^2 of 0.78 for titratable acidity with RMSEC of 0.11g $100g^{-1}$ of malic acid and R^2 of 0.71 with RMSEC of 221mg $100g^{-1}$ of ascorbic acid for vitamin C and in external calibration they reported R^2 of 0.65 and 0.72 for titrable acidity and vitamin C, respectively.

Carotenoids are a group of pigments responsible for the colours of fruits and vegetables. The models for carotenoids and total flavonoids were built with 5 and 7 LV, R^2 values of 0.89 and 0.86 for cross-validation models and RPD_{CV} of 2.9 and 2.6, respectively. For external prediction, R^2 values obtained were 0.79 with RPD values of 2.0 for both carotenoids and total flavonoids 2.4. The RMSECV and RMSEP for carotenoids were 0.079 mg g^{-1} and 0.089 mg g^{-1}, while for total flavonoids the RMSECV and RMSEP values were 0.85 µg g^{-1} and 0.86µg g^{-1}, respectively.

Flavonoids are a group of polyphenols with antioxidant activities involved in growing, developing and reproducing processes. Phenolic compounds or polyphenols are compounds that have at least one aromatic ring in their constitution. These compounds have considerable physiological and morphological importance in plants participating in the growth and reproduction of plants, providing protection against pathogens and predators besides contributing to the sensory quality of fruits and vegetables and its oxidative balance.

For total extractible polyphenols, models were obtained models with 4 LV, R^2 values of 0.90, RPD values of 3.2 and RMSE of 25 mg $100g^{-1}$ for cross-validation and for external prediction, model with R^2 values of 0.89, RPD of 2.5 and RMSE of 27 mg $100g^{-1}$ were presented. For total antioxidant activity model were built with 4 LV, R^2 values of 0.87 and 0.81 with RPD of 2.7 and 2.2 and RMSE of 32 µg ml^{-1} and 34 µg ml^{-1} for cross-validation and external prediction, respectively.

The balance between ROS production and ROS scavenging in chloroplasts (photosynthesis) and mitochondria (respiration) is delicate and must be strictly controlled by compounds with antioxidant activities, which can be quantified. Szuvandzsiev et al. (2013) working with estimation of antioxidant components of tomato using vis-nir reflectance data by handheld portable spectrometer with range of 500-1000nm, obtained models with 6 LV for TSS with R^2 of 0.77 and for RMSECV 0.51 and for polyphenols the model with 4 LV presented R^2 of 0.72, with RMSECV of 7.63. Wang et al. (2014) have analyzed the nutritional composition from 244 samples of faba bean (Vicia faba L.) seed and estimation models were developed for total polyphenol by PLS regression using NIRS. It was used a range of spectra from 4000 – 12,500cm^{-1} and the model was developed with cross validation and external prediction set where values for R^2_{CV} were 0.79, RMSECV of 0.40 mg.g^{-1}, RPD of 2.20 and for external prediction set, the obtained R^2 was 0.78, RMSEP of 0.37 mg.g^{-1} and RPD 2.20 for milling powder. For intact seed, models were obtained with R^2 of 0.70, RMSECV with 0.42 mg g^{-1} and RPD of 1.83 and for validation set the models had R^2 with 0.69, RMSEP of 0.38 mg g^{-1} and RPD of 1.80.

The development of predictive models using infrared spectroscopy can be a great advantage for development of strategies in the area of production, distribution and conservation of plant products (JANNOK et al., 2014).

The models developed for cashew apple in general has high values of R^2, good values for RPD, low prediction errors, low bias and a big range of measurement probably because of the genotypic variability of the samples used for development of the models.

4. CONCLUSIONS

The developed NIR method has a potential application to estimate compounds from primary and secondary metabolism as well as physical characteristics of cashew apple and can be used for quality characterization of this peduncle.

The evaluated metabolites from primary and secondary metabolism are usually used for determine the quality at the harvest and post harvest of cashew apple. To make the models more robust more samples should be included.

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6. ATTACHMENT I

1. Physico-chemical analysis

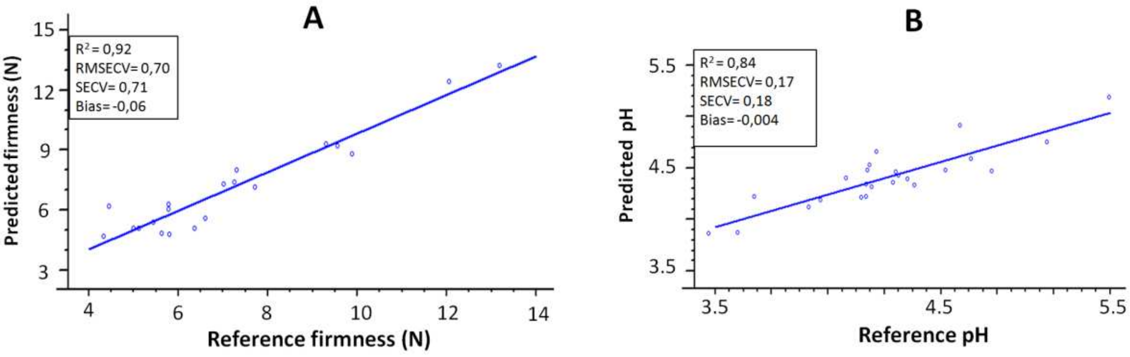

Figure 1. Predicted versus reference values of cashew apple regression models for firmness (A) and pH (B).

2. Metabolites from primary metabolism

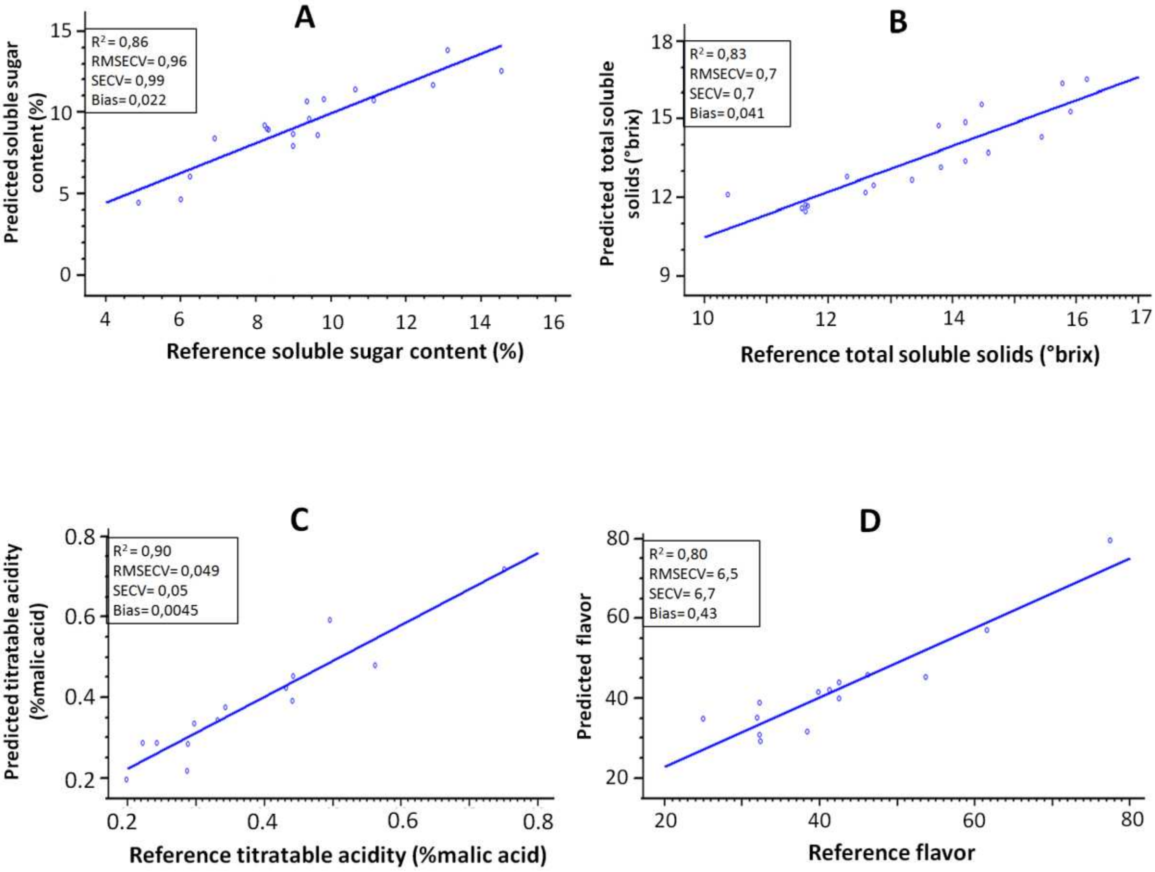

Figure 2. Predicted versus reference values of cashew apple regression models for SSC (A), TSS (B), TA (C) and flavor (D).

3. Metabolites from secondary metabolism

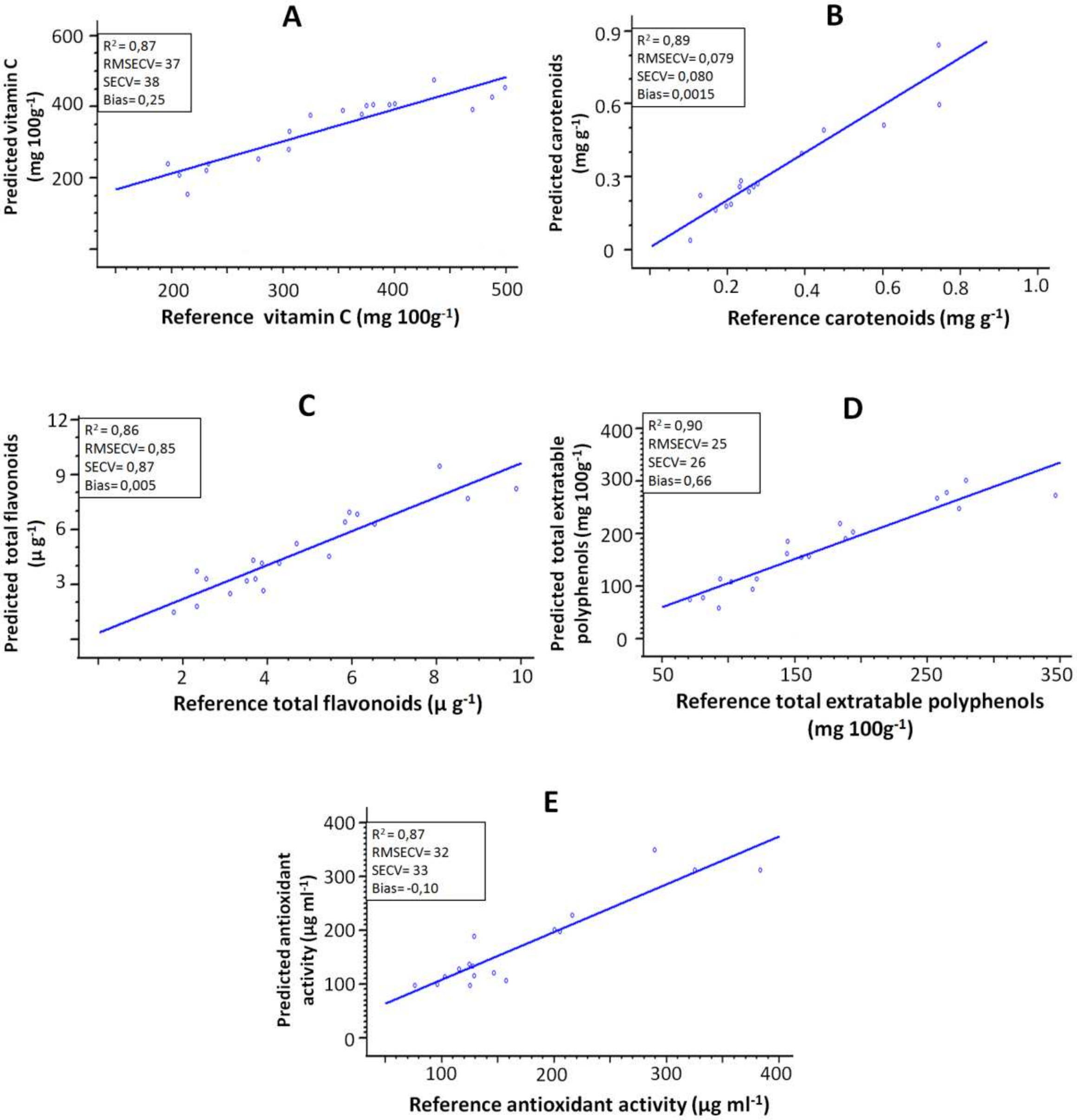

Figure 3. Predicted versus reference values of cashew apple regression models for vitamin C (A), carotenoids (B), total flavonoids (C), TEP (D) and antioxidant activity (E).

II. ARTICLE 2: USING NIRS TO PREDICT POSTHARVEST QUALITY OF CASHEW APPLE

ABSTRACT: The usefulness of portable equipment of near infrared (NIR) spectroscopy as a simple and efficient method to determine some of quality traits of cashew apple peduncles was evaluated in this work. Partial least squares (PLS) calibration models were developed using spectra of 75 samples of cashew apple obtained on reflectance mode with spectral range from 1100nm to 2500nm. The evaluated quality parameters of interest were: Total soluble solids (TSS), titratable acidity (TA), flavor (TSS/TA ratio), firmness, pH and ascorbic acid (vitamin C), which resulted in models with wide ranges and good prediction accuracy. The model was validated using an independent set of samples. Validation results gave root mean square error of prediction (RMSEP) values of 0.06% malic acid, 0.7°brix, 6.7, 0.8N, 0.14 and 34mg 100mg^{-1} for TA, TSS, flavor, firmness pH and vitamin C, respectively. The results showed that accurate models with coefficient of determination (R^2) between referenced and predicted cashew apple samples were higher than 0.80 for titratable acidity (TA), total soluble solids (TSS), flavor and ascorbic acid (vitamin C). R^2 values were higher than 0.70 for firmness and pH in both cross-validation and prediction results. A portable NIRS equipment was used for cashew apple quality monitoring in cold storage for nine days by evaluation of TSS, vitamin C and pH content. The developed models were efficient for monitoring post harvest quality of cashew apple. During storage, there was significant effect for time, genotypes and for interaction time x genotypes, at 1% of probability by F test. After nine days of cold storage, there were significant differences between the genotypes for all evaluated parameters at 1% of probability of Tukey test. Among the evaluated genotypes, B1537 showed significant increases in TSS and vitamin C and lowest values of pH during storage at 4 ºC. According to the results, portable spectrometer can be used to monitor and control cashew apple postharvest quality.

1. **INTRODUCTION**

Cashew apple breeding programs involves a set of procedures aimed at altering the characteristics of cultivars, so that the obtained new materials allow increased productivity and better quality with lower cost.

Cashew apple (Anacardium occidentale) is native to Tropical America. Originating in Brazil, it has become common and financially important crop in many tropical countries such as Vietnam, India, Nigeria, Tanzania, Ivory Coast, Mozambique and Benin (RUFINO et al., 2010).

In order to achieve maximum quality and ensure that this quality is maintained during postharvest storage, it is essential to harvest cashew apple at optimum stage of ripening. Since cashew apple is a non-climacteric fruit, it should be harvested at stage approaching commercial ripeness, in order to guarantee maximum sensory quality in terms of taste, color and texture. Fruit selection is based both on external attributes, such as color, fruit size and shape, absence of physiological defects, and on internal quality parameters including firmness, sweetness, acidity, flavor and vitamin C (SANCHÉZ et. al, 2012).

Total soluble solids, vitamin C and pH are some of important characteristics that determine storage postharvest quality. However, these characteristics determination is still performed in a destructive manner, which requires the destruction of samples, it limits the number of processed samples and involves a huge amount of manual work. Besides, these chemical analysis do not provide all necessary information for quality control, especially when considering the fruit individual variability (MACHADO et al., 2012).

Industry has created the need for a cost effective and nondestructive quality-control analysis system. This requirement has increased interest in near-infrared (NIR) spectroscopy, leading to development and marketing of handheld devices that enable new applications that can be implemented ´´in situ´´. Portable NIR spectrometers are powerful instruments offering several advantages for nondestructive, online or in situ analysis: small size, low cost, robustness, simplicity of analysis, sample user interface, portability and ergonomic design (SANTOS et al., 2013).

Near infrared spectroscopy (NIRS) is a non-destructive technique highly-suited to quality attributes measurement in fruit products: it is rapid, requires little sample preparation, allows reduction of experiment size and enables simultaneous determination of several parameters using a single measurement (BEGHI et. al., 2013; SANCHÉZ et. al, 2012).

NIRS is reported to be a rapid, low cost, versatile and non destructive analytical tool for chemical and physical analysis based on physical matter interaction with light in near infrared spectral region (700-2500nm). The reflectance spectra of samples is mathematically compared with reference sample results that previously have been assayed by standardized and industry approved chemical analysis to develop prediction models (SANTOS et. al., 2013). NIR absorption bands are produced when NIR radiation at specific frequencies (wavelengths) resonates on the same frequency as a molecular bond in test sample. This allows association of a specific wavelength with a specific chemical bond vibration such as C-H, C-C, N-H, O-H, S-H, C-O-C, C=O, C=C, generating a specific spectra that is related to a specific feed component or molecule concentration. More complex molecular structures lead to additional absorption bands and more complex spectra. Prediction models are developed by using software packages to perform necessary mathematical calculations to correlate reference samples NIR spectra with chemical composition of those samples. This mathematical process is called “chemometrics”. The mathematical equations developed are termed “prediction models,” although they are also called “calibrations”.

In chemometric methods, partial least square (PLS) regression and its variations are widely used (FU et al., 2008; EISENSTECKEN et al., 2015). It refers to computation of optimal least-squares fit to part of a correlation or covariance matrix. PLS regression is quite similar to principal components regression (PCR), but defines latent variables (principal components) based on covariance between the independent and dependent variables, rather than the variance of independent variables alone (TEÓFILO, 2013). The aim of this study was to develop non-destructive techniques in portable NIR spectroscopy for monitoring post harvest quality in cashew apple.

2. MATERIAL AND METHODS

2.1. Plant Material

A total of 75 mature cashew apple of 21 different genotypes were collected from experimental field of EMBRAPA Tropical Agroindustry, Pacajus (latitude 4º 11 '26.62' 'S, longitude 38º 29' 50.78 " W and altitude of 60 meters above sea level), CE, Brazil. Being non climacteric (not ripen after harvest), the peduncle must be harvested fully ripe, when it has the best flavor and aroma characteristics (maximum sugar content, lower acidity and astringency) (MOURA, et al., 2011). After collected the peduncles were kept under ambient room conditions (18-25 °C, RH 60-80%) for at least 3 hours before performing NIR spectral measurements, for temperature normalization. After harvest, the cashews were transported to laboratory analyses where samples spectra were taken from top, middle and basal region of the peduncle (figure 1). After sample spectra were recorded, cashew was individually identified and held reference analysis.

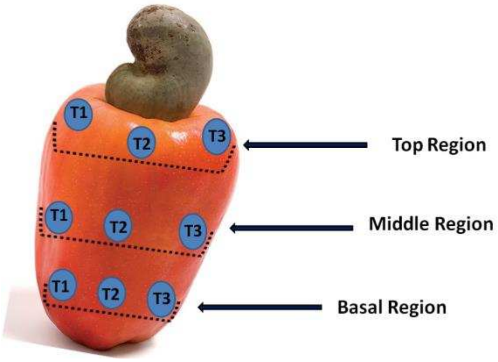

Figure 1. Fruit regions sampled at top, middle and basal regions of cashew apple.

2.2. Reference analysis

For quality attributes physical chemical analysis was performed. Firmness analysis were carried out using a bench texturometer (Stable Micro Systems TA-XT2) equipped with 6 mm diameter plunger and pH was measured with a pHmeter, using a potentiometer with glass membrane, as recommended by the Association of Official Analytical Chemistry (AOAC, 1992). Fresh juice from the samples was extracted by using an electronic fruit mixer and total soluble solids (TSS) determination was performed with a portable refratometer of Quimis brand that has a range of 0-45°brix (AOAC, 1992). Titratable acidity (TA) was determined by titration with NaOH solution (0.1N) until light pink color and the results were expressed as a percentage of malic acid as recommended by methodology of the Institute Adolfo Lutz-IAL (1985) and flavor obtained through TSS/TA ratio, which is also responsible for palatability.

Compounds with antioxidant properties such as ascorbic acid (vitamin C) were analyzed by titration with DFI solution (2.6 dichlorophenolindophenol 0.02%) until a permanent pink color light (Strohecker & Henning, 1967).

2.3. NIR technique

Samples of cashew apple were analyzed by a portable micro spectrometer (JDSU, microNIR), in the wavelength range between 1100 – 2500nm, equipped with a tungsten light source and reflectance mode measures. Measurements were performed placing the probe's head perpendicularly to the peduncle surface to avoid external light noise. These spectral measurements were performed in laboratory considering a white and dark calibration (variable in function of internal and external light), the instrumental for spectra integration time (light acquisition time) and subtracting background noise (variable in function of instrument temperature). Spectra were obtained by one hundred scans per spectra and average value from nine different locations of each sample was properly calculated and stored.

2.4. Spectral pre-processing

Data analysis was performed using Unscrambler software version 9.7. In the present study, different spectral pre-processing methods were applied to original data matrix and they were all mean centered. For data pre processing were used Standard Normal Variate (SNV) and smoothing Savitzky-Golay with 13 points that is an averaging algorithm that fits a polynomial equation to data points. Performances of these pre-processing methods were compared based on calibration (C), cross validation (CV) and prediction (P) models for TA, TSS, firmness, flavor, pH and vitamin C parameters. Samples were divided into calibration set with 2/3 of samples (n=50) and prediction with 1/3 of samples (n=25) by applying the classic Kennard-Stone (KS) selection using MATLAB software version 7.10 (the Math-Works, Natick, Massachusetts, USA), specifically the PLS-toolbox (Eigenvector Research, Inc., Wenatchee, WA, USA, version 6.01).

The lowest root mean square error (RMSE) was obtained using the optimum number of partial least square (PLS) factors, which is found using the variance of matrix instrumental responses. The external validation set was used to test the predictive ability of calibration models.

2.5. Calibration and validation of developed model for cashew apple peduncles

All 75 samples of cashew apple were individually numbered followed by non-destructive analysis (spectra). It were juiced and reference analysis were performed on pulp of each sample in order to verify if nondestructive correlations existed between parameters and reference values for TA, TSS, firmness, flavor, pH and vitamin C.

Estimation of chemical content on samples was performed by PLS regression model on spectral values basis. Two subsets data were separated using a KSxy algorithm from Matlab software, the calibration set with 2/3 of samples (n=50) that was validated internally using full cross-validation and the external validation set with 1/3 of samples (n=25) used for independent validation test.

The accuracy of calibration and validation models were judged by coefficient of determination (R^2) and root mean square error of cross validation (RMSECV) between the NIR predictive and the reference values. Root mean square error of prediction (RMSEP) and residual prediction deviation (RPD) indicates the model predictive accuracy (NICOLAI et al., 2007). Relative error of models and standard error of prediction (SEP) variability coefficient were also used for model accuracy evaluation.

Relative errors are used to evaluate the predictive models accuracy by differences between reference and predicted values and are calculated as it follows:

$$\text{Relative error} = \frac{(Predicted\ values - Reference\ values)}{Reference\ values} \mathrm{x}100$$

Predictive model average of relative error values for all evaluated characteristics are in line with the limits recommended by validation guide MAPA between -20 and + 10% (BOTELHO et al., 2013).

The models were optimized by elimination of samples considered outliers because they presented high leverage for reference values or spectral data.

2.6. Monitoring cashew apple quality

Mature cashew apple of four different genotypes were harvested, two accesses of active germplasm bank (BAG), respectively B1537 and Comum BM1, and two commercial germplasm, BRS189 and CCP76, with three repetitions for each genotype. These fruits were packed in plastic boxes with only one fruit layer, the bottom of which contained an inner lining of foam, about 1 cm thick and transported into postharvest laboratory at EMBRAPA. In the laboratory, cashew apple were selected and packed in polystyrene trays (PET) with no film. After this process the spectra was collected and cashew was stored in a cold room at 6 ± 2 °C, 90-95% RU. Every 3 days for a total of 9 days, the peduncles were evaluated by collected spectra from the same samples. For this experiment, 12 fruits were used reducing the size of the experiment. The experiment was conducted in split plot in time design with 4 genotypes, 3 repetitions and 4 evaluation times (day 0, 3, 6 and 9).

The analysis consisted on taking several spectra of each fruit individually, resulting in a rapid and simple analysis with no need of samples preparation. Once calibration model was developed, several quality characteristics could be estimated by reading the obtained spectra (figure 3, 4 and 5).

3. RESULTS AND DISCUSSION

3.1. Calibration model

Multivariate analysis methods coupled with spectral preprocessing techniques are the fundamentals of chemometrics for appropriate analytical information treatment contained in NIR spectra.

Spectral information captured with portable instruments usually has instrumental noise or background information that need to be removed to enhance calibration model robustness and predictive ability. Therefore some preprocessing technique is sometimes used to remove the accumulating data sets effects from physical variation so that only the absolute changes for chemical properties are shown (figure 2). The figure shows the raw average reflectance spectra (A) and the pretreated spectra (B) collected from cashew apple samples.

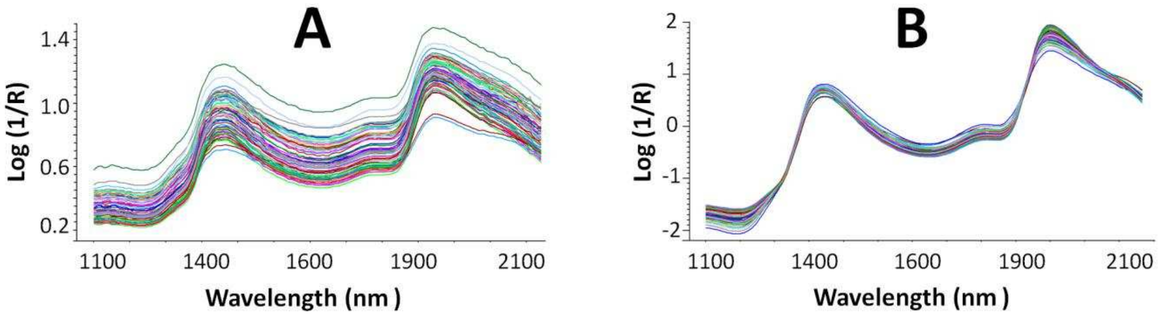

Figure 2. Spectrum of cashew samples without spectra pre-treatment (A) and with Standard Normal Variate and Smothing Savitzky Golay (B).

After preprocessing, a PLS1 regression method was performed on all spectra to develop calibration models and thereby nondestructively predict TA, TSS, flavor, firmness, pH and vitamin C from cashew apple samples. All models were developed with low bias showing no significance at 5% probability for t_{bias} test.

Calibration models for TA and TSS were both developed with 10 latent variables (LV), firmness, pH and flavor presented the highest LV values respectively, 15, 14 and 11 LV and lowest LV values were observed for vitamin C with 7 LV. Calibration and validation results for the models are shown in table 1 and plots that generated the table can be found in attachment 2.

Table 1. Calibration, cross validation and external validation of cashew apple predictive models for physical chemical parameters

Quality parameters	**Calibration**			**Cross Validation**			**External Validation**		
	LV*	R^{2**}	RMSEC***	R^2	RMSECV	RPD****	R^2	RMSEP	RPD
TA (% malic acid)	10	0.94	0.035	0.85	0.057	2.7	0.85	0.059	2.7
TSS (° brix)	10	0.96	0.47	0.90	0.74	3.1	0.88	0.70	3.0
Flavor	11	0.92	4.89	0.80	7.68	2.3	0.82	6.7	2.4
Firmness (N)	15	0.95	0.77	0.77	1.70	2.1	0.76	0.80	1.8
pH	14	0.95	0.064	0.75	0.13	2.1	0.72	0.14	2.6
Vitamin C (mg/100g)	7	0.93	17.19	0.89	22.48	3.0	0.83	33.6	2.5

* Number of latent variables (LV). ** Correlation between reference and predicted constituent values (R^2). *** Mean square error (RMSE) of calibration (C), cross validation (CV) and prediction (P). **** Residual predictive deviation (RPD).

All parameters presented good calibration indexes with 45 elements resulting in good prediction models with good RPD values on validation and cross validation. RPD values between 2 and 2.5 indicates that quantitative predictions are possible, and values above 2.5 and 3 corresponds to good or excellent predictive accuracy, respectively (NICOLAI et al., 2007; WANG et al., 2014). Therefore, based on the RPD values, calibration models for TA, TSS and vitamin C presented good and excellent prediction accuracy, respectively.

Calibration models for flavor, firmness and pH have coarse prediction accuracy, although they all presented good calibration and cross validation R^2 values. The calibration model with the worst indexes were obtained for firmness and pH with R^2 values of 0.95, for cross validation and external validation R^2 values of 0.77 and 0.76 for firmness and for models the pH R^2 values were 0.75 and 0.72, respectively, with low RPD values of 2.1 for both sets. Although presented low RPD parameters, the models also shown low RMSEC and RMSECV for calibration and cross validation sets which allow good predictive model with variation of 1,70N for firmness and 0.13 for pH.

Larger RMSEC and RMSECV values were observed for TA and vitamin C models, probably because of reference method low accuracy. More precision at chemical analysis results in more accurate models with lower RMSEC and RMSECV.

Models for vitamin C also showed one of the highest RPD values (3) and R^2 of 0.93 for calibration, 0.89 and 0.83 for cross validation and external validation, respectively. Best calibration model was obtained for TSS that presented the highest R^2 values of 0.96 for calibration, 0.90 and 0.84 for cross validation and external validation, models with RPD values of 3.1, indicating the regression model excellent predictive capacity with acceptable RMSECV and RMSEP values of 0.78 °brix and 0.77 °brix, respectively. Reference values range, average relative error as well as standard error of prediction (SEP) coefficient of variability are presented in table 2.

Table 2. Reference values and calculated indexes for predictive model evaluation of physical chemical parameters

	TA (% malic acid)	TSS (%)	Flavor	Firmness (N)	pH	Vitamin C (mg/100g)
Reference values range	0.15-0.80	8 - 20	18 - 100	5 - 25	4.0-5.2	50 - 400
Average relative error (%)	-1.3	0.5	2.6	-1.1	0.2	4.9
SEP coefficient of variability (%)	19.6	5.6	15.4	18.2	3.0	12.1

Increasing the number of samples and its heterogeneity can improve predictive models accuracy (MACHADO et al., 2012). Therefore, several samples of 21 different genotypes were used and they presented high variability for evaluated characteristics resulting in models with relatively wide range of reference values (table 2) in order to improve calibration models consistency. This work presented models with higher range than obtained by Moura et al. (2011) for cashew apple different development stages using different genotypes. For these authors, the reference values for TA, TSS, flavor and pH were, respectively, 0.16 to 0.33% of malic acid, 6.48 to 12.80 °brix, 20.61 to 76.66 and 3.97 to 4.57. The predictive models developed in the present work can be applied on pre and postharvest determining cashew apple developing stage. Machado et al. (2012) working with pear reported lower ranges of predictive models with values between 9.60 to 16.20°brix for TSS. Moura et al. (2013) reported vitamin C for cashew in ranges between 73 to 279 mg/100g.

Firmness range with values between 5 to 25 N found in this work was superior than reported by Moura et al. (2010) and Moura et al. (2013) for cashew apple (7-14 N).

The highest predictive model relative error was found for vitamin C (4.9%) and the lowest for TA (-1.3%).

The SEP coefficient of variability, calculated as SEP, is divided by the average of total observed values and it was higher for TA of 19.5 %, followed by vitamin C values of 14.3%, probably because of high genetic variability and range values of cashew apple evaluated parameters. The lower SEP coefficient of variability was found for pH of 2.9% followed by TSS with 5.7% variability. The present work showed higher values for SEP coefficient of variability, comparing with Antonucci et al. (2010) that reported SEP coefficient of variability for TA and TSS of 3.8% and 4%, respectively. Although these authors found lower values of SEP coefficient of variability, the values found in the present work are acceptable because the limit variation is reported on literature for pre and post harvest quality of peduncles (MOURA et al., 2011). The cashew clones BRS 265, CCP 1001, CCP 09, CCP 06 and Embrapa 51 showed the highest SS ratio values / AT: 74.32; 63.78; 61.23; 55.89 and 55.63, respectively (MOURA, et al., 2013). The higher this ratio, the more representative is the amount of solids in the form of sugars relative to the amount of organic acids present in peduncle.

The possibility of acquiring more detailed information, varying either in space and time, when compared with the standard chemical analysis, should prove to be a useful tool to select cashew apple on the base of their quality fastening an important post-harvest operation.

Root mean square error of cross validation (RMSECV) are parameters that measure how well predictive models estimate the reference values in calibration set when one sample is selectively removed from calibration process. Root mean square error of calibration

(RMSEC) defines how well predictive model estimate the reference values on calibration set and RMSEP when predictive model is build with an independent external set samples.

3.2. Monitoring cashew apple quality

During cashew apple storage there is a series of chemical and physical changes which reduce the quality, leading to senescence and death of the peduncle. These changes are due to the active functions of plant metabolism such as respiration that lead to oxidative degradation of cell content, such as total soluble solids (TSS) and vitamin C.

The TSS content corresponds mainly to soluble sugars, vitamins, amino acids, organic acids and others, considered an important quality criterion, as it is related to taste and control of the fruit maturity (CHITARRA & CHITARRA, 2005).

During storage, there was an increase of TSS content in cashew apple, probably associated with water loss, which is reported by Russo et al. (2012), who claimed that an increase of simple sugars content could be a result of biosynthesis pathways, polysaccharides degradation or by fruit water loss resulting in higher TSS solids. Among the genotypes, B1537 showed almost a linear increase of TSS, while others showed variations throughout the storage period ending with almost no change in TSS content (figure 3).

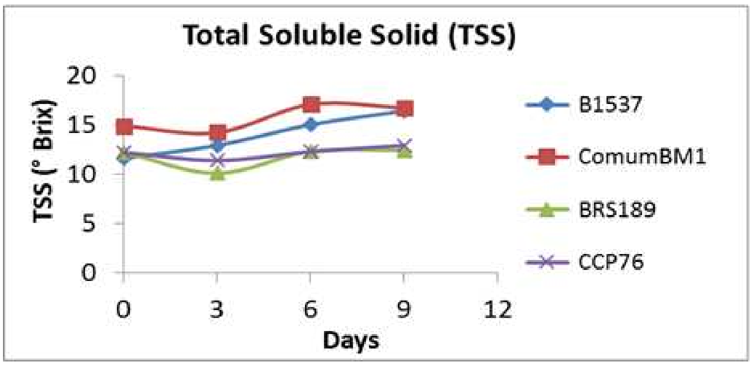

Figure 3. NIRS determination of total soluble solids for cashew apple genotypes throughout cold storage.

Different of TSS, all genotypes showed a significant increase in vitamin C content throughout storage, which may have been a response to higher oxidative stress caused by water loss and senescence processes (figure 4).

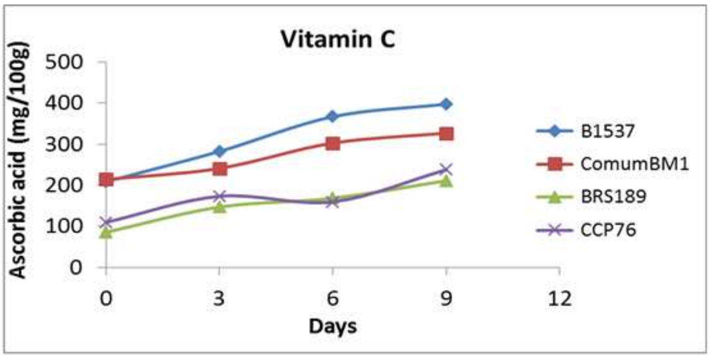

Figure 4. NIRS determination of vitamin C for cashew apple genotypes throughout cold storage.

The maintenance of ascorbic acid (vitamin C) levels involves a recycling mechanism rigidly controlled within the plant cell. Because its antioxidant function, this recycling route in fruit is especially important during oxidative stress response, because in this condition the reduced ascorbic acid is oxidized to the unstable form dehydroascorbate, which is easily degraded and it needs to be converted by reductase enzymes that have its expression enhanced in oxidative stress conditions leading to ascorbic acid increase.

Environmental as well as genetic factors are known to be greatly responsible for variability in vitamin C content and fruit quality. The recycling route and synthesis processes of ascorbic acid can explain the maintenance and increase of vitamin C content, especially in response to stress situation such as water loss (COLTRO et al., 2014). This same trend was reported for TSS and vitamin C by Silva et al. (2013), studying effect of postharvest temperature on gabiroba fruit (Campomanesia pubescens) shelflife and they associated that effect with physiologic processes such as postharvest respiration. These same authors found an increasing pH, similar to Comum BM1 genotype studied in this work that showed a small increase on pH values, different from the other genotypes that showed small decreases of pH values during the storage (figure 5).

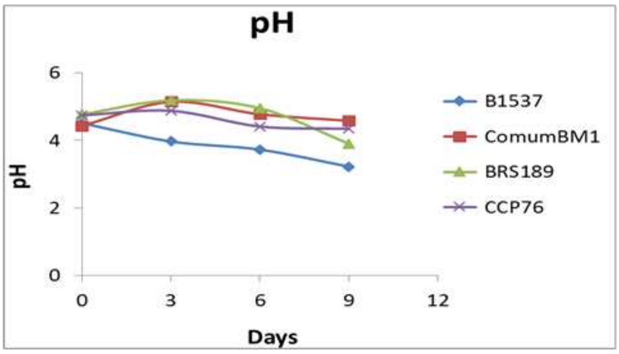

Figure 5. NIRS determination of hydrogenionic potential (pH) from cashew apple genotypes throughout cold storage.

Acidity and pH determine the levels of organic acids that influence the taste, odor, color, stability and quality maintenance.

The first physiological studies on cashew (Białe; Barcus, 1967) showed that the fruit has a high metabolic rate as evidenced by the high breathing rate (74 - 76ml O^2 kg^{-1} h^{-1} and 62 - 72 ml CO^2 kg^{-1} h^{-1}) at 20 ° C. However, according Biale and Young (1981), respiratory behavior is not climacteric and ethylene production is very low (200 - 400 ml kg^{-1} h^{-1}) at 20 ° C. In this sense, cashew apple is a non climacteric fruit but it has high rates of respiration. This condition may cause some controversial behavior in storage conditions such as the observed variation in TSS, vitamin C and pH. After data processing, analysis of variance (ANOVA) were performed (table 3).

Table 3. Analysis of variance of physical chemical parameters for cashew apple genotypes

VARIATION SOURCE	DF	**TSS**		**Vitamin C**		**pH**	
		MS[1]	P[2]	MS	p	MS	P
Genotypes (Gen)	3	38.2	<0.01	72733	<0.01	1.94	<0.01
Plot error*	8	4.4		2655		0.27	
Days	3	17.7	<0.01	41634	<0.01	1.4	<0.01
Genot x days	9	2.6	<0.01	1666	<0.01	0.3	<0.01
Subplot error*	24	0.7		394		0.1	
Mean		13.45		226.77		4.46	
Cvplot**		15.53		22.72		11.67	
Cvsubplot		6.00		8.75		6.34	

*Plot error was the variation between the samples in the respective genotypes during storage; and subplot error was the variation during evaluation time through cold storage;
**Coefficient of variation for plot and subplot;
Degree of freedom (DF);
[1] Mean square;
[2] Probability of F test

The ANOVA showed significant effect for genotypes, meaning that at least one of them is significantly different from the others at F test at 1% of probability. The test also showed significant effect for days and for the interaction genotypes and days for all evaluated parameters.

The figures 3, 4 and 5 showed variations on evaluated content during storage. That variation was proved statistically significant at 1% of probability for genotypes, time of storage and for interaction genotypes x days by test F (table 3). For TSS, this interaction was proved significant at 1% of probability for linear regression for genotypes B1537 and Comum BM1, and significant at 5% probability for quadratic regression for genotypes

BRS189 and CCP76. The B1537 and CCP76 genotypes showed no significant deviation for TSS, while BRS189 and Comum BM1 showed significant regression deviation at 1% probability by test F (table 4). The plots that generated the table can be found in attachment 2.

Table 4. Regression analysis of physical chemical parameters for cashew apple genotypes

			Mean Square		
Genotypes	**Regression**	**DF**	**TSS**	**Vitamin C**	**pH**
B1537	LR[1]	1	40.6**	63540**	2.5**
	QR[2]	1	0.0^{ns}	1389.6^{ns}	0.0^{ns}
	Deviation	1	0.3^{ns}	626.1^{ns}	0.0^{ns}
BRS189	LR	1	1.2^{ns}	23740.2**	1.4**
	QR	1	3.7*	263.4^{ns}	1.1**
	Deviation	1	6.2**	560.7^{ns}	0.1^{ns}
CCP76	LR	1	3.3*	20621.4**	0.4*
	QR	1	3.0*	161.1^{ns}	0.0^{ns}
	Deviation	1	0.3^{ns}	4243.2**	0.1^{ns}
ComumBM1	LR	1	10.5**	23975.7**	0.0^{ns}
	QR	1	0.1^{ns}	5.1^{ns}	0.6*
	Deviation	1	6.7**	769.5^{ns}	0.2^{ns}
	Error	24	0.7	394	0.1

* e **Singificant at 5 and 1% of probability of test F, respectively;
^{ns} Not significant;
[1, 2] Linear and quadratic regression respectively

For vitamin C all genotypes presented significant interaction by test F at 1% of probability for linear regression. Except CCP76, that presented significant interaction by test F at 1% of probability, all other evaluated genotypes showed no significant deviation.

For pH, linear regression with significant interaction was found for B1537 and CCP76, while BRS189 and Comum BM1 presented quadratic regression with significant interaction by test F at 1 and 5% of probability, respectively.

Up to nine days storage, there were genotypes significant differences for TSS and vitamin C content. The genotypes Comum BM1 and B1537 are statistically not different for Tukey test at 1% of probability and they were higher than BRS 189 and CCP76. For vitamin C all genotypes were statistically different, were B1537 showed highest content and BRS 189 showed the lowest. B1537 genotype was statistically different from the others that had no statically differences between them for tukey test at 1% probability for pH during cold storage (table 3).

Table 5. Average of physical chemical parameters for cashew apple genotypes during cold storage at 6°C

Genotypes	Quality parameters		
	TSS	Vitamin C	pH
B1537	14.0 ^{ab}	313.5 ^{a}	3.9 ^{b}
BRS 189	11.8 ^{b}	152.7 ^{d}	4.6 ^{a}
CCP 76	12.3 ^{b}	169.9 ^{c}	4.6 ^{a}
Comum BM1	15.7 ^{a}	270.9 ^{b}	4.7 ^{a}

* Values followed by the same letters in the same column do not differ statistically by Tukey test at 1% probability.

The results of this study showed that a system based on a portable spectrophotometer can provide quickly and accurately information in a shorter period of time for a wide range of cashew apple cultivars and it can be used to define maturation and fruit quality, as reported for mango, carambola, mandarin, peaches, plum and others done (ANTONUCCI et al., 2010; BOBELYN et al., 2010; SANTOS et al., 2013; OMAR & MATJAFRI, 2013; SZUVANDZSIEV et al., 2014; and COZZOLINO, 2015).

4. CONCLUSIONS

In general, the developed calibration models presented good quality parameter indexes with good prediction accuracy and acceptable error variation.

These are robust prediction models and allow a rapid and repeatable assay procedure for nutritional values that help detect and manage variability in composition among and within cashew apple genotypes.

Developed regression models for portable device was tested and applied for postharvest cashew apple quality determination. These models can also assist in cashew apple preharvest quality and breeding programs access characterization of evaluated physical chemical quality parameters.

For NIRS quality determination of cashew apple genotypes throughout cold storage, TSS and vitamin C showed significant increases while pH showed small decreases during storage. ANOVA showed statistically significant variation at 1% of probability for genotypes, time of storage and for interaction genotypes and time storage (days) by test F for all evaluated parameters.

During storage, B1537 presented higher contents of TSS and vitamin C and lowest content of pH.

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6. ATTACHMENT II

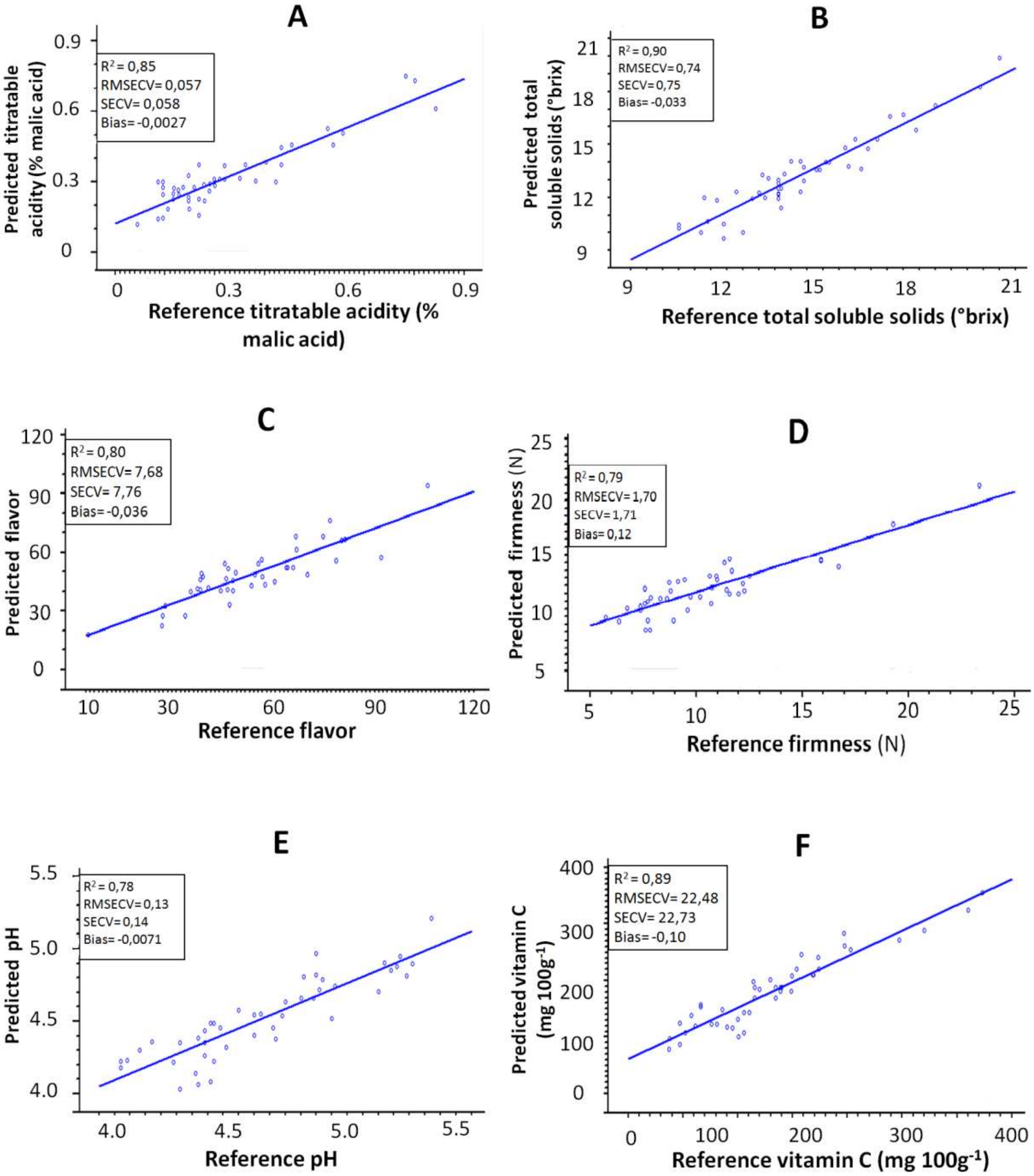

Figure 1. Predicted versus reference values of cashew apple regression models for TA (A), TSS (B), flavor (C), firmness (D), pH (E) and vitamin C (F).

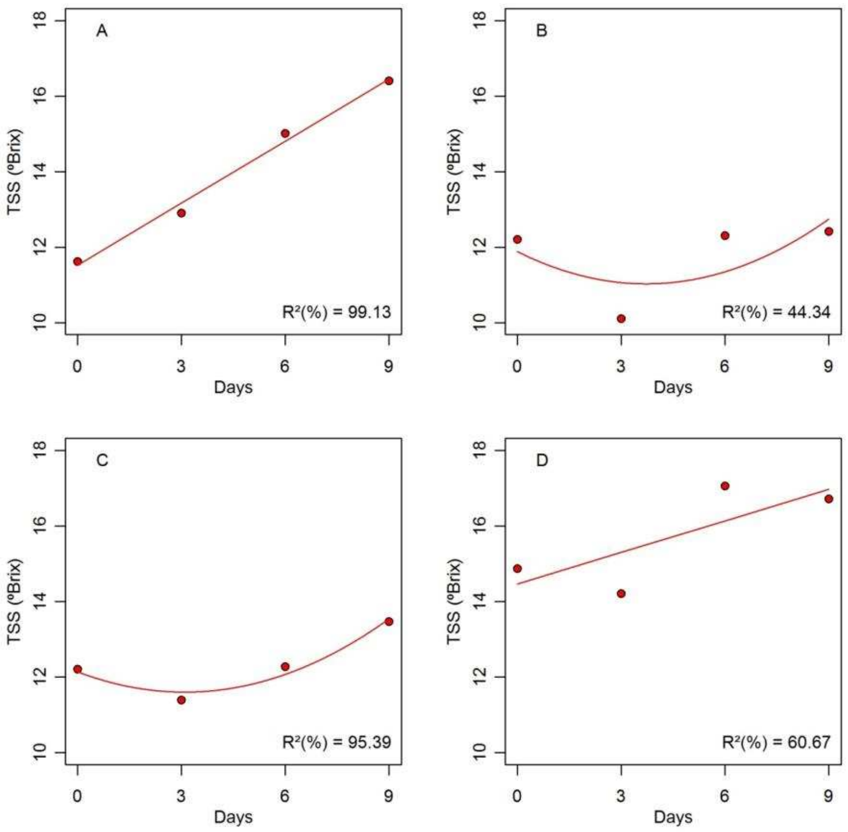

Figure 2. Regression analysis for monitoring total soluble solids (TSS) of cashew apple genotypes (**A** – B1537; **B** – BRS189; **C** – CCP76 and **D** – Comum BM1) through cold storage.

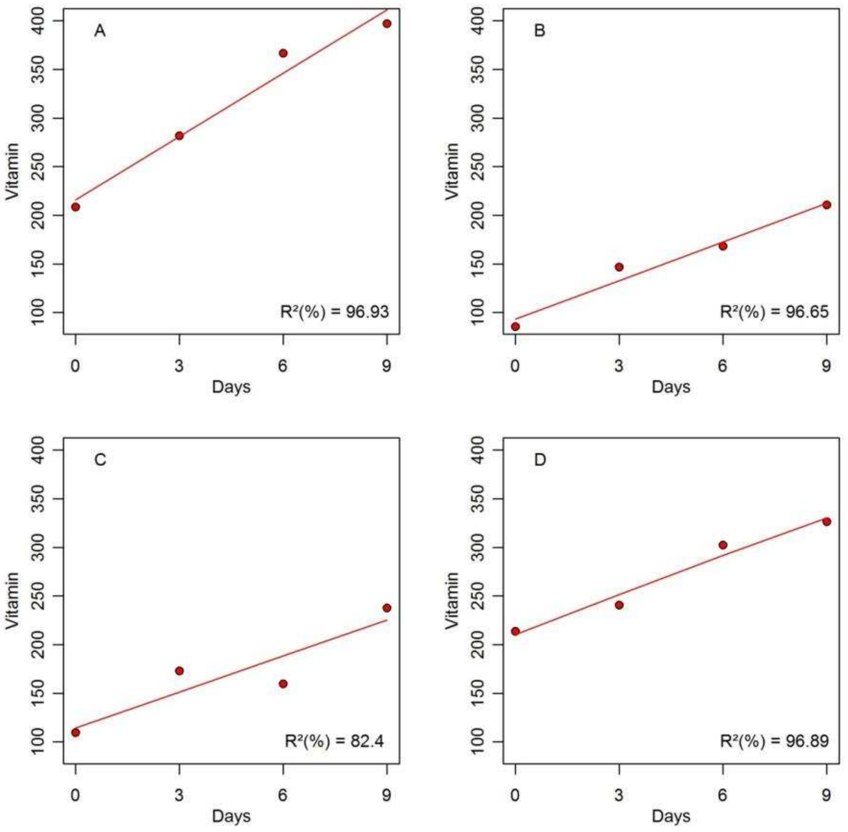

Figure 3. Regression analysis for monitoring vitamin C of cashew apple genotypes (**A** – B1537; **B** – BRS189; **C** – CCP76 and **D** – Comum BM1) through cold storage.

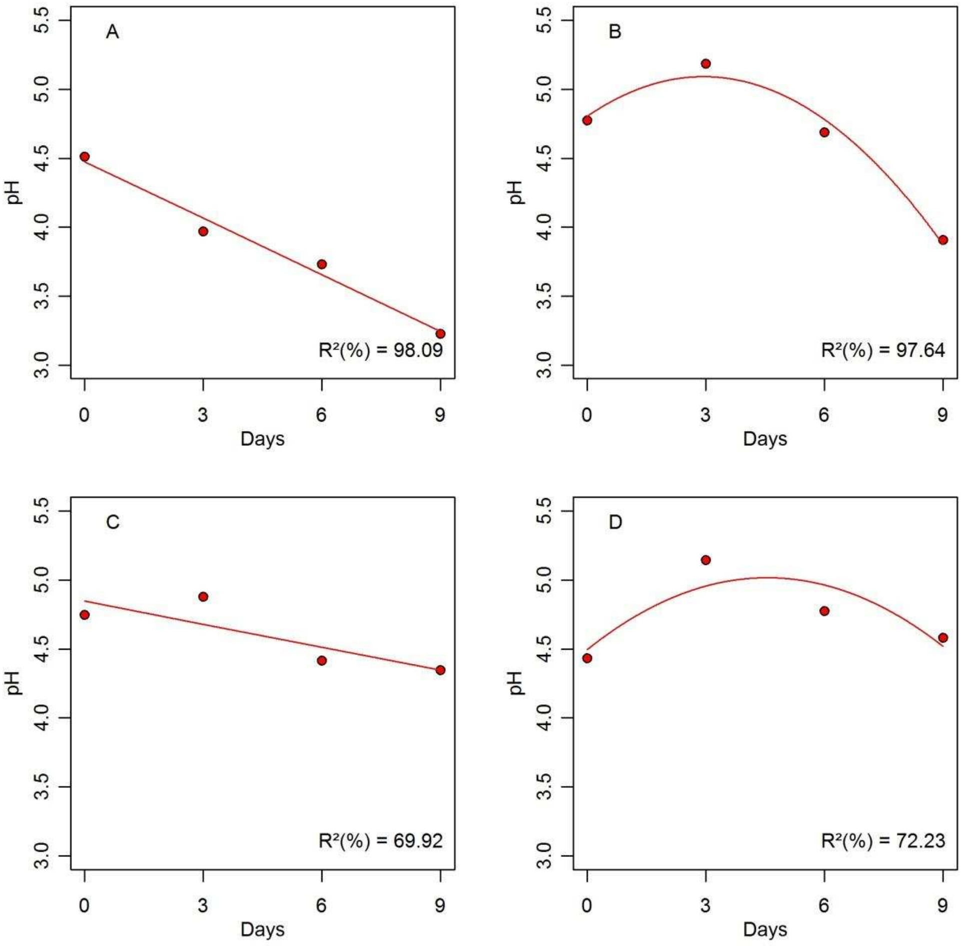

Figure 4. Regression analysis for monitoring pH of cashew apple genotypes (**A** – B1537; **B** – BRS189; **C** – CCP76 and **D** – Comum BM1) through cold storage.

III. GENERAL CONSIDERATIONS

In general, the developed calibration models for bench and portable spectrometers presented good parameters evaluation indexes for physical and metabolic characterization of cashew apple quality. These models presented good prediction with high RPD values, high accuracy and acceptable error of variation. These prediction models allow a rapid and repeatable assay procedure for nutritional values that help detect and manage variability in composition among and within cashew apple.

Calibration models for portable device were developed with wide range of prediction values, acceptable prediction coefficient of variability and they were tested and applied for postharvest cashew apple quality determination. These models can also assist characterization of pre and post harvest quality of established crops and breeding programs access.

NIRS technology using portable instrument and chemometric regression strategies proved highly suited to prediction of cashew apple internal quality parameters although there is scope for improvement.