A produção do conhecimento nas Ciências Exatas e da Terra 2

Ingrid Aparecida Gomes
(Organizadora)

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2

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APRESENTAÇÃO

A obra “A produção do conhecimento nas Ciências Exatas e da Terra” aborda uma série de livros de publicação da Atena Editora, em seu II volume, apresenta, em seus 21 capítulos, discussões de diversas abordagens acerca do ensino e educação.

As Ciências Exatas e da Terra englobam, atualmente, alguns dos campos mais promissores em termos de pesquisas atuais. Estas ciências estudam as diversas relações existentes da Astronomia/Física; Biodiversidade; Ciências Biológicas; Ciência da Computação; Engenharias; Geociências; Matemática/ Probabilidade e Estatística e Química.

O conhecimento das mais diversas áreas possibilita o desenvolvimento das habilidades capazes de induzir mudanças de atitudes, resultando na construção de uma nova visão das relações do ser humano com o seu meio, e, portanto, gerando uma crescente demanda por profissionais atuantes nessas áreas.

A ideia moderna das Ciências Exatas e da Terra refere-se a um processo de avanço tecnológico, formulada no sentido positivo e natural, temporalmente progressivo e acumulativo, segue certas regras, etapas específicas e contínuas, de suposto caráter universal. Como se tem visto, a ideia não é só o termo descritivo de um processo e sim um artefato mensurador e normalizador de pesquisas.

Neste sentido, este volume é dedicado aos trabalhos relacionados a ensino e aprendizagem. A importância dos estudos dessa vertente, é notada no cerne da produção do conhecimento, tendo em vista o volume de artigos publicados. Nota-se também uma preocupação dos profissionais de áreas afins em contribuir para o desenvolvimento e disseminação do conhecimento.

Os organizadores da Atena Editora, agradecem especialmente os autores dos diversos capítulos apresentados, parabenizam a dedicação e esforço de cada um, os quais viabilizaram a construção dessa obra no viés da temática apresentada.

Por fim, desejamos que esta obra, fruto do esforço de muitos, seja seminal para todos que vierem a utilizá-la.

Ingrid Aparecida Gomes

SUMÁRIO

CAPÍTULO 15

FT-NIR IN THE CONSTRUCTION OF PLS MODELS FOR DETERMINATION OF TOTAL FLAVONOIDS IN SAMPLES OF PROPOLIS SUBMITTED TO DIFFERENT PROCESSES

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RESUMO: A própolis é um material resinoso natural produzido pelas abelhas para as mais diversas finalidades, dentre elas proteger a colmeia, preencher fendas, manter a temperatura da colônia e embalsamar predadores. A própolis contém resina, cera, óleos essenciais, pólen e outras substâncias tais como flavonoides, ácidos fenólicos, ésteres e cetonas. Atualmente, a própolis veem se destacando na comunidade científica, devido as suas propriedades biológicas e farmacológicas, tais como as antitumorais, antimicrobiana, antisséptica, anti-inflamatória, antioxidante, cicatrizante e anestésica. Dentro deste contexto o objetivo deste trabalho foi determinar o teor de flavonoides totais em amostras de própolis produzidas no sul do Brasil, e verificar qual o tipo de própolis: bruta, macerada, extrato etanólico de própolis (EEP) e extrato etanólico de própolis concentrado (EEPC), associado a diferentes pré-processamentos gera o melhor modelo de calibração multivariada para a determinação de flavonoides totais. Os modelos PLS construídos para as amostras bruta e macerada apresentaram os melhores resultados a partir dos pré-processamentos Savitzky-Golay (SG) + primeira derivada (1D) + Correção do Espalhamento Multiplicativo (MSC) e número de variáveis latentes (VL) igual a 8 . As amostras EEP e EEPC apresentaram os melhores resultados a partir do pré-processamento 2D e VL igual a 9. Por meio de uma análise comparativa conclui-se que o modelo para EEP com pré-processamento 2D foi o melhor para a predição do teor de flavonoides totais nas amostras de própolis, pois apresentou maior valor de correlação (R^2 = 0.88), menor erro RMSECV = 1.12 (RMSECV = 1.12), e maior valor de RPD = 2.9.

PALAVRAS-CHAVE: Antioxidantes, Espectroscopia, NIR, Quimiometria

ABSRACT: Propolis is a natural resin material produced by bees for a variety of purposes, including protecting the hive, filling cracks, maintaining colony temperature, and embalming predators. Propolis contains resin, wax, essential oils, pollen and other substances

such as flavonoids, phenolic acids, esters and ketones. Nowadays, propolis stands out in the scientific community due to its biological and pharmacological properties, such as antitumor, antimicrobial, antiseptic, anti-inflammatory, antioxidant, healing and anesthetic. In this context, the objective of this study was to determine the total flavonoid content in propolis samples produced in southern Brazil and to determine the type of propolis: crude, macerated, Ethanolic Extract of Propolis (EEP) and Ethanolic Extract of Propolis Concentrate (EEPC), associated with different pre-processing results in the best multivariate calibration model for the determination of total flavonoids. The PLS models constructed for the crude and macerated samples presented the best results from Savitzky-Golay (SG) + First Derivative (1D) + Multiplicative Scatter Correction (MSC) and 8 latent variables (VL). The EEP and EEPC samples presented the best results from the 2D preprocessing and VL equals 9. By means of a comparative analysis it was concluded that the 2D pre-processing EEP model was the best for predicting the content of (R^2 = 0.88), lower RMSECV error (RMSECV = 1.12), and higher value of RPD = 2.9.
KEYWORDS: Antioxidants, Spectroscopy, NIR, Chemometrics

1 I INTRODUCTION

Bees have been living with humans since the earliest hominids and their greatest benefits are to provide pollination of natural vegetation (MICHENER, 2000). Several species of insects have been found to be affected by pollination of bees (VAN'T et al., 2005), which guarantees the biodiversity of the ecosystems and the world agricultural production by these small insects (MICHENER, 2000; WINFREE et al., 2011). Several families of farmers have in beekeeping its main income source (JOLLIVET, 1994; PEREIRA et al., 2016; LOPES et al., 2016). Honey, wax, royal jelly and propolis are the main products produced by bees (YILMAZ, 2016; BARGAŃSKA, ŚLEBIODA, NAMIYEKI, 2016).

Propolis is a Greek word that means "pro" that represents "in defense of", and "polis" meaning "city." In other words, it is understood as "defense of the city" or "defense of the hive" when used by bees (SALATINO et al., 2005). In addition, the propolis can be used in a hive for a variety of purposes, such as filling cracks (BARTH, 2004; SALATINO et al., 2005) maintaining the colony temperature at around 35 °C (SALATINO et al., 2005), embalming predators (BARTH, 2004; JÚNIOR et al., 2006) so that this avoids its decomposition as well as antiseptic function, which protects the colony from possible diseases (SAHINLER; KAFTANOGLU, 2005; JÚNIOR et al., 2006). Propolis has been marketed by the pharmaceutical industry as well as by natural products stores for their pharmacological uses (BANSKOTA; TEZUKA; KADOTA, 2001).

Currently, propolis has been highlighting among researchers, due to its biological and pharmacological properties, such as antitumor, antimicrobial, antiseptic (SALATINO et al., 2005), anti-inflammatory, antioxidant (BANKOVA; DE CASTRO; MARCUCCI,

2000; SFORCIN, 2007; OLDONI et al., 2011; OLDONI et al., 2015; CALEGARI et al., 2017, DA SILVA et al., 2018), healing and anesthetic (PARK et al., 2002; JÚNIOR et al., 2006). The antioxidant capacity of propolis represent the combat of cellular oxidative damage, that can stop and eliminate the reactions that occur due to the action of free radicals, which can cause oxidative stress and consequent tissue damage (KUMAR, 2015).

Among the main compounds described in propolis polyphenols are the main responsible by antioxidant activity. The flavonoids belong to the class of polyphenols and have a basic C_6-C_3-C_6 structure. In the flavonoid group are anthocyanidins, flavones, flavanols, aurones, chalcones, isoflavones (SOARES, 2002), flavanones, flavanonols, leucoantocyanidins, deoxyanthyanidins and anthocyanins (VERMERRIS; NICHOLSON, 2008).

The antioxidant and biological activity of flavonoids is related to their chemical structure and depends on this, mainly due to three main characteristics (Figure 1): 1) presence of catechol group in ring B; 2) the 2,3-double bond conjugated with the 4-oxo function of a C-ring carbonyl group and 3) the presence of hydroxyls in positions 3 and 5. The flavonol quercetin shows all these characteristics and therefore has a great antioxidant potential. It is also worth mentioning that the antioxidant potential increases significantly when there is an OH group in the 5 'position of ring B (GÜLÇIN, 2012).

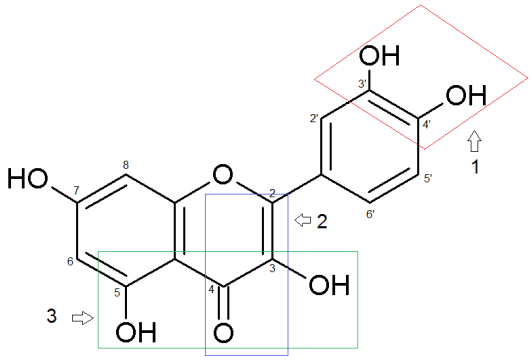

Figure 1 - Structural characteristics of flavonoids conferring maximum antioxidant activity

Source: Own authorship

Many rapid instrumentation techniques have been developed and these have presented great advantages over conventional analysis techniques (WILLARD et al., 1988; SHARMA, 2000). Chromatographic, electrochemical and spectroscopic techniques are examples of evolutions in researches and technologies. Spectroscopy is the study of the interaction of waves with matter, that is, corresponds to the set of techniques that analyze the substances based on the interpretation of emission spectra and absorption of electromagnetic radiation produced, based on the different frequencies of the electromagnetic spectrum (SIESLER et al., 2008; SUN, 2009).

The Infrared (IR) spectroscopy is divided into three regions (near, mid and far infrared) with different wavelengths. The Near Infrared (NIR) Spectroscopy comprises

the wave number range of 13330 - 4000 cm^{-1} (STUART, 2005; SIESLER et al., 2008; SUN, 2009). This region absorbs overtones or combinations of fundamental stretching vibrations of the molecules, and the spectra depend on the chemical composition and physical characteristics of these (HOLLER; SKOOG; CROUCH, 2009; KUMAR; 2015). The overtones in the NIR spectroscopy are formed by combination bands, which are excitations of the electrons at the higher energy levels (SIESLER et al., 2008; SUN, 2009; AGELET; HURBURGH, 2010).

In NIR spectroscopy with Fourier Transform (FT) data are guided through an interferometer, which performs the FT, the interferometer can generate interference patterns in a balanced way, the signal is then measured through the interferogram. FT-NIR spectrophotometers are much cheaper and more sensitive than conventional spectrophotometers because the interferometer is an inexpensive component (SUN, 2009).

The mathematical regression techniques help in the treatment of the data obtained in the researches, since the biggest problem of the data of multivariate origin is the weight of the data, that is to say, the great amount of information contained, since it is known that this makes it difficult to interpret them. In this way the methods to which the multivariate analysis applies are used to reduce the number of variables and even the data themselves (VARMUZA; FILMOSER, 2016).

An important tool that appears in this context is chemometrics. Chemometrics is nothing more than a new area within analytical chemistry, which uses statistical tools, mathematics and other methods in data processing of chemical origin or not, in the vast majority of cases multivariate data, this area aims to evaluate in a way fast and efficient results, by interpreting what is really significant and important (TRYGG; HOLMES; LUNDSTEDT, 2007; REINHOLDS et al., 2015).

As an example PLS is a powerful tool, which aims to use linear combinations of the variables that predict the analysis, instead of using the original variables (VARMUZA; FILMOSER, 2016). In PLS, the variables that present greater correlation with the response variables are attributed greater weight, because according to the model they will be more effective in the prediction (MILLER; MILLER, 2010).

Because the spectra generate a large amount of information, many of them are sometimes not relevant to the construction of the calibration models, and often not related to the information that actually represent the samples, it is necessary in this way a polishing (pre-processing) of the spectroscopic data for the construction of the models (RINNAN; VAN DEN BERG; ENGELSEN, 2009; SOUZA; POPPI, 2012; SOUZA et al., 2013).

Some preprocessing are:

The Multiplicative Scatter Correction (MSC) is the most applied method for the correction of data in NIR (RINNAN; VAN DEN BERG; ENGELSEN, 2009). The MSC helps to remove baseline fluctuations, imperfections, physical aspects of samples (size and shape of particles) from the data matrix, so that only chemical information is used

(SOUZA; POPPI, 2012; SOUZA et al., 2013).

The first and second derivative can remove the additive effects of the models, the first derivative removes the baseline which can cause displacement of this, uses the difference between two points of consecutive spectral measurement. The second derivative eliminates the baseline as well as the linear trend. It is estimated by calculating the difference between two sequential points of the spectra of the first-order derivative (RINNAN; VAN DEN BERG; ENGELSEN, 2009).

The Savitzky-Golay smoothing method was initially proposed in 1964 (SAVITZKY; GOLAY, 1964), and is an ideal method to apply to analytical signals that have narrow peaks between them. To find the derivative of the center point, a given low order polynomial is assembled in a symmetric window, after finding and calculating the parameters of the polynomial the derivative of any order of the function (of the polynomial) can be found analytically, and the value found is used as a derivative estimate for the central point (RINNAN; VAN DEN BERG; ENGELSEN, 2009).

After the development of the multivariate calibration models, it is necessary to verify the validation of these models, since the models may not be true of their predictive capacity and may be over-adjusted. Therefore some parameters can be verified to attest to the quality of the models developed (Table 1).

Parameters		Values	Quality of models	Reference
R^2	Coefficient of determination	>0.93	Good robustness of prediction	Elfadl, Reinbrecht and Claupein, 2012
RPD	Residual prediction deviation	1.5 to 2.0	Model discriminates between minors and the highest values of the responses	Williams and Norris, 2001; Kumar, 2015
		2.5 to 3.0	Good prediction accuracy	
		>3.0	Excellent prediction accuracy	
RER	Range error ratio	>10	Good prevision estimate	Páscoa, Magalhães and Lopes, 2013
RMSEP/RMSECV	Root mean square error of prediction/ Root mean square error of cross validation	≈1.0	Robustness	Li et al., 2011
RMSEP	-	The lower the better	Validation analysis error	Conzen, 2006
RMSEC	Root mean square error of calibration	The lower the better	Calibration analysis error	Oliveira, Braga and Costa, 2015
RMSECV		The lower the better	Previson error	Kumar, 2015

RMSEE	Root mean square error of estimation	The lower the better	Calibration analysis error	Conzen, 2006

Table 1 - Quality Parameters of NIR Spectroscopy Models

Source: Own authorship

In this context, the objective of this work is to define the best type of propolis associated with a given preprocessing produces the best PLS model for the determination of total flavonoids.

2 I MATERIAL AND METHODS

2.1 Sample collection and preparation

The samples of propolis were supplied by Breyer & Cia Ltda, located in the city of União da Vitória, State of Paraná. Thirty-three samples of propolis from the states of Paraná and Santa Catarina (Figure 2) were collected. All of them were received, cleaned, macerated with liquid nitrogen and kept under refrigeration at -6 °C until analysis.

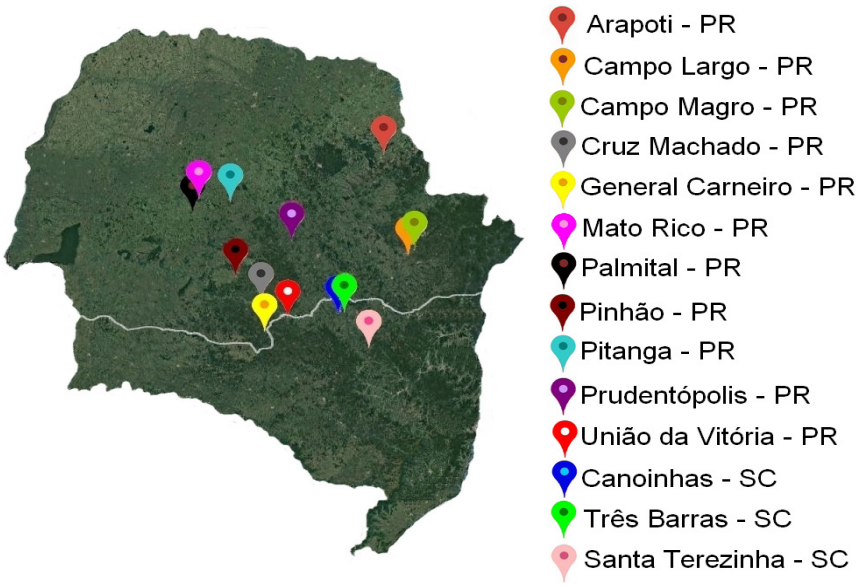

Figure 2 - Geographical location of the cities of origin of the propolis samples

Source: Own authorship

2.2 Preparation of Ethanolic Extract of Propolis (EEP) and Ethanolic Extract of Propolis Concentrate (EEPC)

The production of EEP followed the methodology described by Oldoni et al. (2015). Were weighted 4 g of macerated propolis and 50 mL of ethanol:water (80:20 v v^{-1}) were added and the mixture was maintained to the thermal bath at 45 °C for

45 min. The EEP were concentrated on a rotary evaporator under the conditions of 120 mbar at 40 °C and then the residual water was removed by lyophilization. After concentration, standard extracts (1000 mg L^{-1}) of EEPC were prepared. All extracts were prepared in triplicate. The samples of crude propolis, macerated, ethanolic extracts and concentrated extracts were evaluated in NIR Spectroscopy for the acquisition of spectra. A simplification of the process is exemplified in Figure 3.

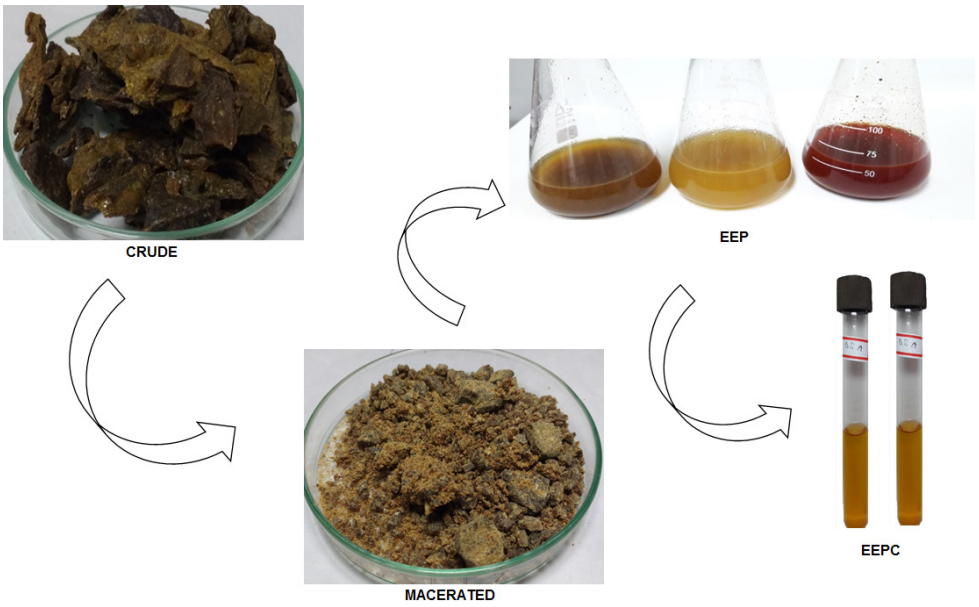

Figure 3 - Sample preparation process

Source: Own authorship

2.3 Total flavonoid content (TFC)

The TFC was determined through the methodology proposed by Jurd and Geissman (1956) and Park et al. (1995). The method consists of mixing 500 µL EEPC (500 µg mL^{-1}) into a series of tubes identified with and without aluminum chloride. In the tubes that received aluminum chloride were added 4.3 mL of 80:20 ethanol:water (v v^{-1}), in the tubes not receiving chloride were added 4.4 ml of 80:20 ethanol:water (v v^{-1}). Then, 100 µl of potassium acetate (CH_3COOK 1 mol L^{-1}) was added to all tubes. The blank sample was prepared with 4.9 mL of 80:20 ethanol:water (v v^{-1}) and 100 µL of potassium acetate. After 40 min the absorbance in spectrophotometer (UV-VIS model Lambda 25, Perkin Elmer) was measured at 415 nm. The TFC determined on the basis of the quercetin calibration curve as standard, the results were expressed in mg EQ g^{-1}.

2.4 FT-NIR analysis

A BRUKER MPA FT-NIR spectrometer was used to obtain the spectra of all samples. In order to obtain the best relation between samples and spectra it was necessary to adjust the equipment for each type of sample. For solid samples of propolis (crude and macerated) the equipment was configured with a 32 cm^{-1} resolution with 64

accumulations using the rotating quartz becker. Each sample of solid propolis (crude and macerated) had its spectrum acquired in triplicate in the equipment. For the liquid propolis extracts (EEP and EEPC) the equipment was configured with 8 cm^{-1} resolution with 32 accumulations, using a quartz cuvette with suction pump as a support. For all samples the region studied will be between 13330 - 4000 cm^{-1}. The liquid extracts (EEP and EEPC) were produced in duplicate and the spectra acquired in triplicate in the equipment.

The construction of multivariate calibration models was performed using Bruker Opus 7.2 quant 2 software (Bremen, Germany). The validation of the models will be performed by leave-one-out cross validation and test group by internal and external validation.

3 I RESULTS AND DISCUSSION

3.1 Total flavonoid content

The EEP were evaluated for total flavonoid content and the range of data used for calibration and validation of the model is showed in Table 2.

Model	Calibration (70% of samples)					Validation (30% of samples)				
	Min	Max	Mean	s.d.	CV (%)	Min	Max	Mean	s.d.	CV (%)
Flavonoids (mg EQ g^{-1})	0.122	16.51	3.65	3.27	89.7	0.117	38.8	5.70	7.03	123

Table 2 - Data obtained from TFC analyses

Source: Search data

It is possible to observe that the values obtained for TFC presented a very broad spectral range in the model and the calculated coefficients of variation (CV), indicating the sample variation.

The sample from the Arapoti - PR city presented the highest value for the flavonoid content, 38.8 ± 6.30 mg EQ g^{-1}. In the work developed by DOS SANTOS et al. (2017), with propolis from the city of Blumenau, state of Santa Catarina, were obtained a content ranging from 0.74 to 0.93 mg EQ g^{-1}. Propolis from the Entre Rios city, state of Bahia and Brumadinho city, state of Minas Gerais were evaluated by CASTRO et al. (2007) that obtained values of 2.47 and 47.31 mg EQ g^{-1} respectively.

3.2 Models for flavonoids

The PLS models of flavonoids constructed presented two particularities. The first one is that for the samples in the solid state, crude and macerated, the best models were molded with the same preprocessing (SG + 1D + MSC) and and 8 LV. The PLS

model for crude propolis presented R^2: 0.65, RMSECV: 3.29 mg EQ g^{-1} and RPD: 1.71, for this model the selected spectral region was between 9411.7 - 7498.5 to 6109.9 - 5446.4 cm^{-1}. The model of macerated propolis presented R^2: 0.69, RMSECV: 2.08 mg EQ g^{-1} and RPD: 1.8 in the spectral region of 6109.9 - 4597.9 cm^{-1} (Table 3).

Propolis	R^2	RMSECV (mg EQ g^{-1})	RPD	Spectral region (cm^{-1})
Crude	0.65	3.29	1.7	9411.7 - 7498.5 a 6109.9 - 5446.4
Macerated	0.69	2.08	1.8	6109.9 - 4597.9
EEP	0.88	1.12	2.9	6102.1 - 5446.4
EEPC	0.70	1.93	1.8	9400 - 6098.3 a 5450.3 - 5022.1

Table 3 - Validation parameters obtained for the different types of propolis

Source: Search data

The second particularity for PLS flavonoid models is that the liquid samples (EEP and EEPC) were best modelled by using 2D preprocessing and the same 9 LV. The EEP model presented R^2: 0.88; RMSECV: 1.12 mg EQ g^{-1} and RPD: 2.91. The EEPC model presented R^2 of 0.70; RMSECV: 1.93 mg EQ g^{-1} and RPD of 1.85. The spectral region for EEP was 6102.1 - 5446.4 cm^{-1} and for EEPC 9400-6098.3 at 5450.3 at 5022.1 cm^{-1}.

Table 3 shows that the best model for TFC was constructed using EEP in this model the R^2 values were very good, especially the R^2 of the calibration of the model with R^2: 0.92 (Table 4). Similarly, the best RPD value was also for the calibration (3.65). The other RPD values also indicate good models with good predictive capacity according to the reference (Table 1).

Flavonoids	Calibration	Validation	
		Cross	*Test Set*
R^2	0.92	0.88	0.86
RMSEC (mg EQ g^{-1})	0.89	-	-
RMSECV (mg EQ g^{-1})	-	1.12	-
RMSEP (mg EQ g^{-1})	-	-	1.1
RMSEE (mg EQ g^{-1})	0.93	-	-
RPD	3.65	2.91	2.77
RER	-	14.9	15.60
RMSEP/RMSECV		0.98	

Table 4 - Calibration and validation results for the flavonoid model

Source: Search data

From the robustness, it is possible to suggest that the flavonoid model for EEP is ideal, indicating a perfect robustness of the model. The RER values found for cross and test set indicate models with a high degree of prediction (Table 1). Figure 4 represents

the internal validation curve (cross).

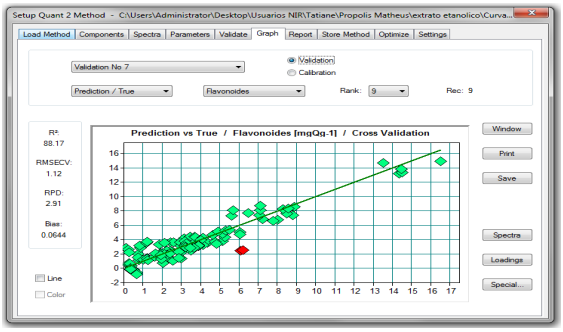

Figure 4 - Prediction vs True (Validation Cross) R²: 0.88

Source: Search data

As the best model obtained for TFC was the model with EEP, Figures 5 and 6 shows the spectrum of EEP across the NIR spectrum range and in the selected region of the model respectively, Figures 7 and 8 represent the spectrum of EEP applied to 2D preprocessing in every region of the spectrum and selected region of the model respectively.

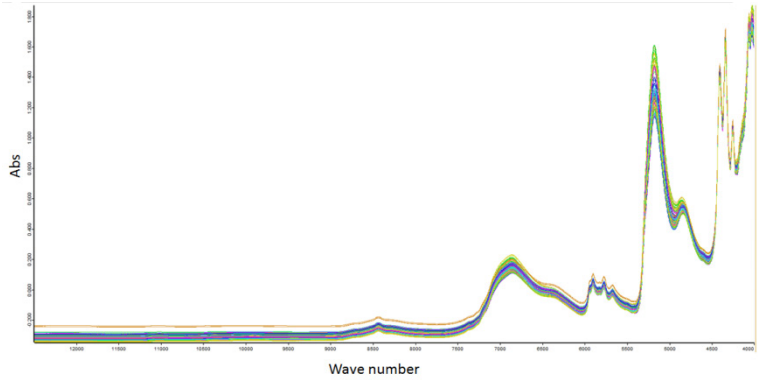

Figure 5 - NIR spectra for EEP without preprocessing (whole region)

Source: Search data

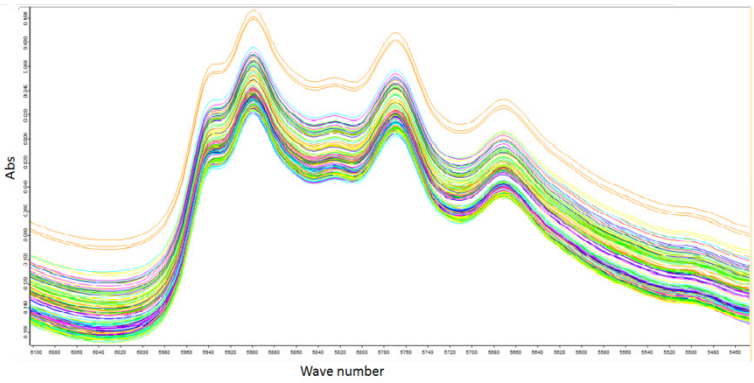

Figure 6 - NIR spectra for EEP without preprocessing (selected region)

Source: Search data

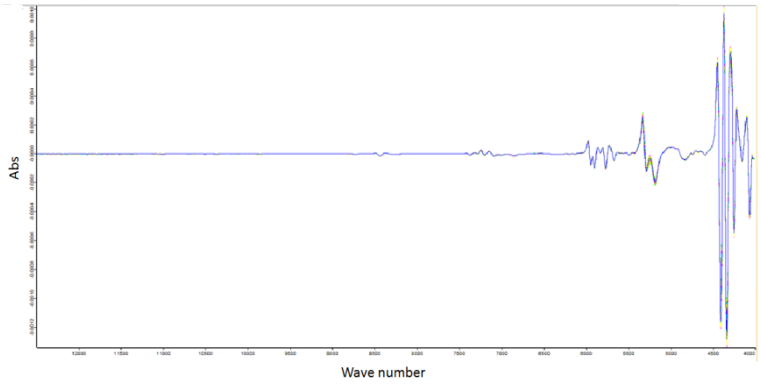

Figure 7 - NIR spectra for EEP with 2D (whole region)
Source: Search data

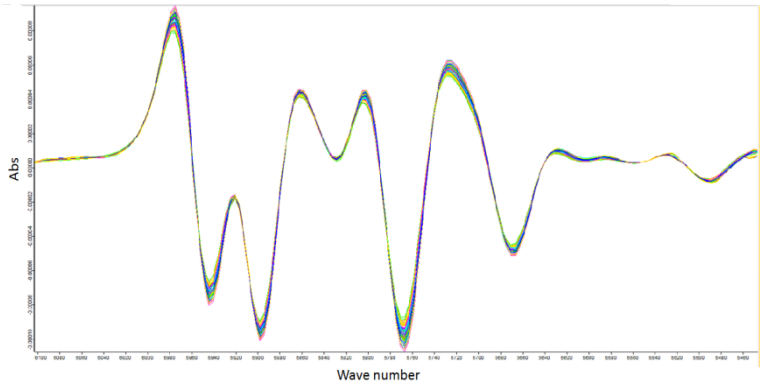

Figure 8 - NIR spectra for EEP with 2D (selected region)
Source: Search data

The importance of the preprocessing is remarkable through the figures above, the spectra of figures 5 and 6 present wide bands, spaced and with diverse amplitudes and when applying the preprocessing is verified in figures 7 and 8 that the 2D removes the baseline and creates a new baseline from the center of the spectrum and the linear trend of this, reducing the amplitude of the spectra, accentuating the bands and highlighting them.

4 I CONCLUSIONS

From the reference analyzes it was possible to generate good calibration models with crude propolis, macerated, EEP and EEPC. However, based on the parameters R^2, RMSEC, RMSECV, RMSEP, RMSEE, RPD, RER and RMSEP / RMSECV ratio, the best model obtained for TFC was with EEP. Optimal ranges of values were found for TFC being the highest value 38.8 mg EQ g^{-1} for the city of Arapoti - PR.

Finally, it can be concluded that the NIR spectroscopy associated with chemometrics can be used in the characterization of the propolis samples, and that the PLS models constructed, using the NIR spectra using preprocessing, stood out for being robust and high ability to predict Flavonoid content.

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