RAPID ANALISYS OF VITAMIN-C CONTENT IN ACEROLA EXTRACT BY FT–NIR SPECTROSCOPY

ANÁLISE RÁPIDA DO CONTEÚDO DE VITAMINA-C EM EXTRATO DE ACEROLA POR ESPECTROSCOPIA FT–NIR.

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Abstract. The acerola fruit, also known as cherry-of-Antilles or cherry-of-Barbados was originated in the West Indies and belongs to the *Malpighiaceae* family. It has pro-vitamin A carotenoids, vitamins B_1 (thiamin), B_2 (riboflavin), B_3 (niacin), calcium, phosphorus, iron and especially vitamin-C, which is its main nutritional appeal. In consequence of this, develop methodologies for a rapid and accurate determination of vitamin-C content in the extract of this fruit is of academic and commercial interest, since the levels of this vitamin decay in function of time. The application of FT-NIR technology for predicting the quantification of vitamin-C content in acerola extract is a new methodology that has being adapted and exploited to provide reliable results. To develop this work, the vitamin-C content was originally determined by the iodide ions reduction and the results were used to calibrate the FT-NIR apparatus in order to develop a calibration curve by using PLS (Partial Least Squares) treatment. The calibration range obtained lies between 0.38 to 2.15% of vitamin-C which has an associated prediction error of 0.067%. The curve obtained proved to be enough accuracy to be routinely used in rapid analysis of the vitamin-C content in acerola extract.

Keywords: Acerola; vitamin-C; FT-NIR.

Resumo. A acerola, também conhecida como cereja-das-Antilhas ou cereja-de-Barbados, tem origem nas Antilhas e pertence a família das *Malpighiaceae*. Possui carotenóides pró-vitamina A, vitaminas B_1 (tiamina), B_2 (riboflavina), B_3 (niacina), cálcio, fósforo, fer-

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ro e principalmente vitamina-C, sendo este o principal atrativo em termos nutricionais. Em função disso, desenvolver metodologias rápidas e precisas para determinação do teor de vitamina-C no extrato desta fruta, é de interesse acadêmico e comercial, uma vez que os teores desta vitamina decaem em função do tempo. A aplicação da tecnologia FT-NIR na quantificação por predição do teor de vitamina-C em extrato de acerola, é uma metodologia nova que foi adaptada e explorada para fornecer resultados confiáveis. Para isso, o teor de vitamina-C foi originalmente determinado por redução de íons iodeto e os resultados utilizados para a calibração do aparelho FT-NIR de forma a desenvolver uma curva de calibração pelo método PLS (Mínimos Quadrados Parciais). A faixa de calibração obtida foi de 0,38 a 2,15% de vitamina-C, com um erro associado à predição de 0,067%. A curva obtida se mostrou precisa o suficiente para ser utilizada rotineiramente em análises rápidas do teor de vitamina-C em extrato de acerola.

Palavras-chave. Acerola; vitamina-C; FT-NIR.

INTRODUCTION

Acerola (*Malpighia glabra*) is a fruit widely known and consumed throughout Brazil. Its main nutritional value is related to high content of vitamin C, or ascorbic acid, so that the pure acerola juice is consumed "*in natura*" and also used to enrich other foods (MATSUURA *et al.*, 2001; MATSUURA *et al.*, 2002; ROLIM, 2002).

There are multiple possibilities for the consumption of this fruit, which can be both "*in natura*" by the commercialization of its frozen pulp or in the processed form, passing by the fabrication of liqueurs, jams, candy syrup and ice creams.

Because of its potential as a natural source of vitamin-C and its capability of industrial utilization, acerola has attracted the interest of fruit growers and went on to have economic importance in several regions of Brazil (PETINARI; TARSITANO, 2002). Besides the vitamin-C, acerola also has a high content of antioxidant pigments like anthocyanins and carotenoids that when combined, are responsible for the red color of these fruits (LIMA *et al.*, 2003).

Regarding its nutritional role, vitamin-C has multiple functions in the body. As examples, it is necessary for the production and maintenance of collagen, participates in the process of healing wounds, fractures, bruises and bleeding gums, also reduces the susceptibility to infection, plays a role in the formation of teeth and bones, increases iron absorption and prevent scurvy (MAHAN; SCOTT-STUMP, 2005). In this way, the vitamin-C is important in the development and maintenance of the whole human body.

However, ascorbic acid is easily degraded in juices and fruit pulps, both in aerobic or anaerobic conditions, which leads to browning of the product with the consequent economic and nutritional losses. The oxidizing agents have an important role in the degradation of this vitamin however, the action of light is in part also responsible for its destruction (PIMENTEL *et al.*, 2001). All these factors together directly affect the content of vitamin-C in pulp and other preparations of commercially sold acerola.

Despite to be an important source of it nutritional constituent, industry does not invest in the commercialization potential of the fresh acerola, but just in the processing and conservation of its pulp and production of its juice (MAIA *et al.*, 1999; MAIA *et al.*, 2007).

The cultivated area in Brazil is estimated to be 10,000 ha, especially at Bahia, Ceará, Paraíba and Pernambuco, which together hold 60% of national production, be-

ing most of the orchards formed by seedlings from acerola seeds (PETINARI; TARSITANO, 2002). In the state of Parana, stands out the region around the city of Pérola, this holds a cooperative formed by sixty small farmers that focus the production, processing and commercialization of this fruit.

Considering the economic and nutritional importance of the acerola for Brazil, to develop analytical physical-chemical methodologies for determine quite rapidly the vitamin-C content in this fruit is of interest for both producers and industry, aiming the quality improvement of the product.

A technique that has been increasing and gaining recognition in physical-chemical analysis is the near infrared absorption spectroscopy. This technique uses the near infrared spectrophotometer which is a device that performs high-precision analysis of food and a lot of other organic samples (and some inorganic) by through the principle of emission of electromagnetic radiation, where the infrared radiant energy is used to characterize organic matter in a qualitative and quantitative way. New generations of FT-NIR spectrometers (Fourier Transform - Near Infrared) are equipped with interferometer devices, a recent advance that increases their sensibility.

The FT-NIR technology is based upon the application of spectrophotometry and mathematics coupled with the analytical chemistry (chemometrics) as a technique to integrate spectroscopy, statistical and data computing (FERREIRA, *et al*., 1999) for detection and quantification of organic compounds in samples solid, liquid, pasty or powder states, without the need of destruction or pretreatment of it. Furthermore, the analysis of an organic sample only by FT-NIR is free of generation of chemical waste, which could be harmful to the environment. This methodology is already used successfully in the areas of quality control in food industry to detect proteins, carbohydrates, lipids, moisture, fibers, salts, pH, among others in samples of meat, milk, chocolate, mayonnaise, beans, etc, as also other fields of chemical and pharmaceutical industries (BURNS; CIURCZAK, 2007).

However, the technique of FT-NIR is a secondary analytical methodology, in other words, to make a prediction of the content of some substance by this technique, requires that the database of the equipment has been previously supplied with reference spectra from samples of known concentrations. These determinations should be pre-established by primary and reliable analytical techniques like Kjeldahl, HPLC, titration, etc.

Moreover, the detection limit of this technique is about 0.1%, which limits the possibilities of analysis to the most abundant components of the sample.

Given the nutritional importance of active vitamin-C to the body's physiology, their commercial importance as food antioxidant and its rapid degradation in function of time, the aim of this work was to establish a calibration curve for vitamin-C in acerola extract starting from its absorption spectra in the near infrared region, so that from these data, to make possible to determine the vitamin-C content only by spectroscopic analysis, without the use of chemical reactants, which reduce the cost and time of the analysis and do not generate chemical waste being this methodology less aggressive for the environment.

The application of FT-NIR technology in the prediction of the vitamin-C content in acerola extract is a recent methodology that can be adapted and exploited in a way to benefit both the producers and food industry with a quality control over cheap.

MATERIAL AND METHODS

The samples were collected among October 2008 to March 2009 from acerola orchards in the region of Pérola-PR, and then were directly taken to analysis at

the laboratory of Food Chemistry in the Universidade Estadual de Maringá, campus Umuarama.

The extract was obtained by pressing method, which was then filtered and added to the centrifuge tubes with screw cap to be centrifuged at a speed of 2,220 rpm for 5 minutes for clarification purposes.

After this time, 70 mL of the extract were placed in a beaker and quantities of pure vitamin-C (Merk) were added to them in increasing concentrations from 0.0 to 2.0 g. After the addition of vitamin-C, 20 mL of solution were transferred to an erlenmeyer in order to determine the content of vitamin-C by titration. The method for determining consisted of adding 1 mL of potassium iodide (10% m/v), 1mL of starch (1% m/v) and 4 mL of sulfuric acid (20% v/v) in the sample, where the titration was performed with standardized 2 mM potassium iodate (IAL, 2005). At the turning point titration the color changes from red to black. By this way the anthocyanin pigments do not interfere on turning point achievement. This procedure was made in triplicate. Pure vitamin-C solutions were used as reference.

Paralelly, 5 mL of the same sample to be titrated were added into a Petri dish and this coupled in the sample port of a spectrophotometer FT-NIR model N-200 (Büchi, Switzerland). The NIR absorbance spectra were acquired in the range from 1000-2500 nm, and then exported to the software NIR-Cal 4.01 (Büchi, Switzerland) where the relative values of the concentration of vitamin-C inherent in each sample were assigned to their spectra which were used in the procedures of multivariate calibration (chemometrics).

Fifty-six samples were used in these procedures, which were divided into two sets of data by the NIRCal software. The first set contained 36 spectra were used to create the calibration protocol by extracting them the relevant information. The second set contained 20 spectra were used in the validation of calibration (cross validation).

The spectra were initially pretreated by smoothing (Savitzky-Golay Smooth 9 points), as described by the following equation:

$$gm_1 = \frac{A_{i-4} + A_{i-3} + A_{i-2} + A_{i-1} + A_i + A_{i+1} + A_{i+2} + A_{i+3} + A_{i+4}}{9} \quad (1)$$

where ***A*** represents the absorbance at wavelength ***i***.

To avoid the interference from other chemical compounds that were also present in the sample, there was made a selection of regions of the spectra in a way that the counting of their areas by Savitzky-Golay method could be attributed to vitamin-C. Those selected regions for wavelengths calibration set are summarized in Table 1.

The calibration curve obtained is a mathematical algorithm that contains the necessary information to carry out the pretreatment of the obtained spectrum, more a linear function of Abs *versus* concentration that can be used to predict the concentration of the chemical component of interest in samples of unknown composition using the instrumental response of them.

After the determination of the calibration curve it was inserted into the prediction software of the FT-NIR device and then its predictive capability was tested against 16 new samples of "*in natura*" acerola fruit extract. No vitamin-C enrichment was made in this set of samples. The device output were compared with those obtained by traditional methods (IAL, 2005) by means of linear correlation, using the program Origin 6.0 (Northampton, MA, USA).

RESULTS

The spectra utilized in the construction of the calibration curve as well the respective wavelength range analyzed are shown in Figure 1.

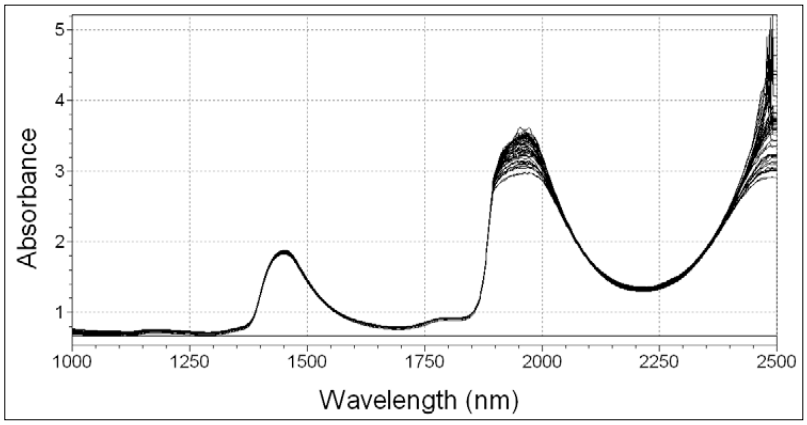

Figure 1. Pretreated NIR spectra from acerola extract.

Spectra pretreatment was necessary to avoid baseline fluctuations. Several methods of pretreatment have been tested, however, only the smoothing gave the best results.

The multivariate calibration method chosen for this work was the PLS, english abbreviature for Partial Least Squares. This is a regression method that uses principal component modeling. It is a method of multivariate statistics to assess relationships between two or more sets of variables measured in the same entity. PLS analyses the covariance between sets of variables using the information of concentration on obtaining the factors, which can only be justified if such concentrations be reliable values (FERREIRA *et al*., 1999).

As a result of multivariate calibration by PLS method performed by the software NIRCal we obtained a calibration curve with good predictive ability. Curve parameters of calibration and validation are summarized in Table 1.

Table 1. Summary of the parameters obtained with the multivariate calibration.

CALIBRATION PARAMETERS	RESULTS
Photometric range (nm)	1000 – 2500 (total 1557/1557)
Wavelengths Calibration Set (nm)	1000 – 1282, 1514 – 1854, 2082 – 2276 (total 994/1557)
N° of calibration spectra	36
N° of validation spectra	20
Chemometric method	PLS
Pretreatment applied to spectra	Smooth Savitzky-Golay 9 points
Primary factors	8
Secondary factors	1-8
Calibration range	0.38 – 2.15%
Consistency	86.9
Standard error of prediction (RMSEP)	0.067%

Figure 2 shows the linear regression obtained applying PLS method to the experimental data. The linear regression made between the points that represent the predicted content of vitamin-C against the measured points was 0.994. In cross-validation test, this regression was 0.990.

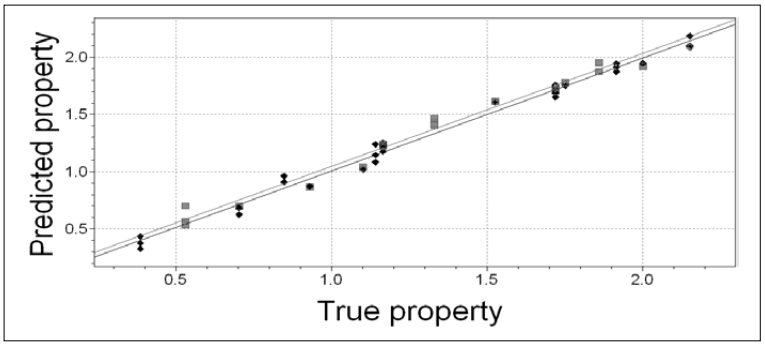

Figure 2. Correlation between the methods of titration and FT-NIR for dosing vitamin-C content in acerola extract by the PLS method. (•) Dataset used for calibration. (■) Dataset used for model cross-validation.

The secondary factors described in this method are used by the program NIRCal for the separation of different substances and are responsible for tolerance in their grouping.

The estimated error of prediction (RMSEP) is actually a standard deviation, which is defined by the expression:

$$RMSEP = \sqrt{\frac{\sum_{n}(x_n - y_n)^2}{n}} \qquad (2)$$

where ***x*** represents the predictive value of concentrations previously known, ***y*** the data of removed spectra (cross validation) and ***n***, the number of samples of the calibration set. The $(x_n - y_n)$ term represents the prediction error.

Consistency is defined as the percentage of the standard error of estimation (SEE) divided by the standard error of prediction (SEP) and must be closest possible to 100, with acceptable values between 80 and 110. The consistency value of 86.9 found in our results supports the acceptable quality of the calibration curve.

The calibration range constructed from 0.38 to 2.15% represents the minimum and the maximum concentrations of vitamin-C which can be predicted from the calibration curve established by the FT-NIR methodology. Predicted values above or below this range are unreliable. This limit is due to the limited amount of vitamin C found in fruits of this crop, as well as the methodology used and not from the analytical method itself.

Test for prediction efficiency.

After determining the mathematical algorithm of prediction (linear function of the calibration curve more the data pre-treatment condition), it was inserted into the prediction software of the FT-NIR equipment. Then, its predictive capability was tested in an independent data set containing 16 new samples of acerola extract in which the vitamin-C content was determined as previous described methodology without any kind of enrichment.

The output data were then inserted into the Origin software. In this independent dataset, the linear regression between the points that represents the traditional methodology of iodine ions reduction (IAL, 2005) against the predicted by FT-NIR methodology was 0.98 ± 0.05. The correlation (r) was 0.985 with a standard deviation of 0.066 and $p<0.0001$ (Figure 3).

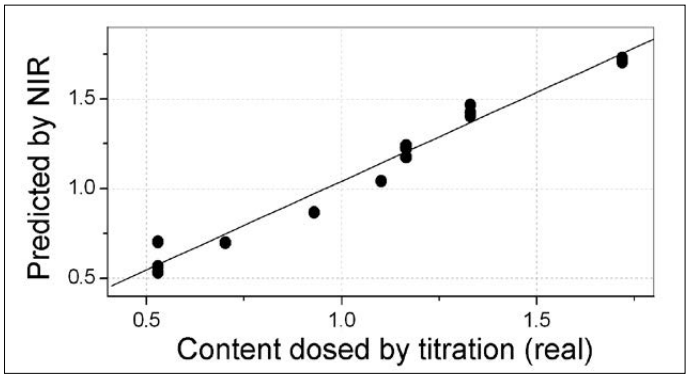

Figure 3. Comparison of vitamin-C content dosed by the IAL methodology and predicted by the FT-NIR calibration curve constructed in this work.

Recovery assay.

Three amounts of vitamin-C (0.5 g, 1.0 g and 1.7 g) were added into a sample of acerola extract that naturally contained 0.695 grams of vitamin-C, in order to carry out recovery assays using NIR prediction. The recovery percentage was 92.2%, 90.0% and 90.0% respectively.

DISCUSSION

The titration methodology adopted in these assays can appear outdated regarding modern HPLC techniques. However it still is a reference methodology recommended by IAL for determination of vitamin-C content in fruit juices (IAL, 2005).

The need to enrich the samples of acerola extracts with solutions of pure vitamin-C was due to the low variation range of the content of this vitamin found in the samples at the beginning. Perhaps this low range was due to the fact that they were samples from the just one crop and from trees of the same species (BRUNINI *et al.*, 2004).

Within a low range of vitamin-C content, it was very difficult to establish a significative correlation between methodologies because under these conditions, the experimental error was huge in relation to the calibration range, being not possible to perform the multivariate calibration.

Similar work was done by LIU *et al.*, (2008) for prediction of vitamin-C in tangerine juice. The methodology used by these authors to construct the calibration curve was very similar to ours, but they used only samples of juice "*in natura*", ie without enrichment with pure vitamin-C. Perhaps this is why their results were inconclusive while promising, and they did not find a significant correlation between the two methodologies.

YANG & IRUDAYARAJ (2002) demonstrated that the application of the PLS chemometric method, the same used in our experiments was quite effective to construct a calibration curve for prediction of vitamin-C content in drugs by FT-NIR methodology. The correlation found by these authors was 0.992 with an estimated error of prediction of 0.2 to 3.0%, demonstrating that the application of this chemometric method was also very effective for analyzing and predicting the content of such constituent. Others work involving the PLS method applied to FT-NIR for prediction of the vitamin-C content in drugs also showed a correlation above 95% and high predictive capability (BLANCO *et al.*, 1993; BRUNINI *et al.*, 2004; BURNS; CIURCZAK, 2007; DU *et al.*, 2000).

According to our results obtained with the prediction of vitamin-C content in acerola extracts from the independent set of samples, it was observed that both methodologies are well correlated (R = 0.985) and now, this methodology that use the absorption spec-

tra in the near infrared region can be used routinely to predict the vitamin-C content in acerola extract with high reliability.

However, the calibration range that emerged from this work was not wide enough to cover all the range of vitamin-C content that can be found in acerola fruits and, by now, can be applied only in a range from 0.38% to 2.15%. The limits of this range were constructed only for this work and its limitation are due to the low vitamin-C content that was found in the fruits that were used. The limits of the range of the calibration curve by this methodology are the maximum and minimum content of a compound in the matrix (sample).

It is necessary to conduct new tests to obtain a greater range of calibration, since fruits from trees of a single harvest and from the same species were used here. Although this calibration range is reliable, there are other studies in the literature that describe certain varieties of acerola which may be up to 4.83% of vitamin-C content (NOGUEIRA *et al.*, 2002) indicating that the range of the calibration curve obtained in this work needs to be amplified to cover all the variation scale.

However the widening of this range will occur naturally when new acerola samples with higher vitamin-C content appear and be incorporated to the model. Nevertheless the widening of this range will not change the final algorithm used in determining vitamin-C content and this small range will not be the limitating factor of this method.

CONCLUSION

The calibration curve for determination of vitamin-C content in acerola extract constructed by FT-NIR methodology in this work, presented quite reliable parameters, showing that this curve can be applied in routine testing for prediction of vitamin-C content in acerola extracts. The limits of the range for vitamin-C determination constructed here are the same track used in experimental trials and tuning functions, ranging from 0.38 to 2.15% due to limited vitamin-C content that were found in the matrix utilized. This standard curve may be expanded in the mode that new extracts of acerola with higher or lower concentrations of vitamin-C be incorporated into the model.

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