Microchemical Journal 128 (2016) 113–117

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Prediction of Orange juice sensorial attributes from intact fruits by TD-NMR

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ARTICLE INFO

Article history:
Received 7 March 2016
Received in revised form 10 April 2016
Accepted 10 April 2016
Available online 16 April 2016

Keywords:
TD-NMR
Orange
Soluble solids
Titratable acidity
pH
Quality

ABSTRACT

The quality of orange juice is related to its total soluble solids (TSS) content, pH and titratable acidity (TA), which are indexes normally determined by invasive methods. In this study, we demonstrate that time domain nuclear magnetic resonance (TD-NMR) and chemometrics can be used to classify intact oranges in terms of TSS, and to evaluate the sensorial attributes of orange juices. 210, 90 and 170 oranges were used for calibration, prediction and classification sets, respectively. TD-NMR decay signals were obtained using the Carr–Purcell–Meiboom–Gill (CPMG) sequence. The CPMG decay data were normalized between 0 and 1 and smothered with a Savitzky–Golay algorithm. These data were modeled using Partial Least Squares Regression (PLSR) that obtained a Square Error for Prediction (SEP) set for TSS and pH of 0.88 and 0.17, respectively. The noninvasive TD-NMR/PLSR approach allowed grouping oranges in terms of high TSS (Class 2) and low TSS (Class 1) contents. Sensorial evaluation revealed that juices from class 2 were sweeter than juices from class 1 within a confidence interval of 95%. Sensorial analysis results were also successfully associated with pH values, in which orange juices with high pH were sweeter than those exhibiting low pH. A nonparametric qui-quadratic statistics was performed between maturation index (MI) ratio and pH, which revealed a significant correlation ($p < 0.001$); when pH is greater than 3.5, the MI ratio is higher than 11.39. The results confirmed that TD-NMR and chemometrics can be an alternative and powerful noninvasive method for classification of intact oranges.

1. Introduction

Quality attributes of oranges are rated depending on the commercial destination of the fruits. Oranges sold directly to consumers as fresh fruits, for example, must exhibit remarkable visual parameters such as intense color and absence of defects, uniform texture, and excellent sensorial parameters such as aroma and taste. Visual characteristics of oranges are not that important for industrial processing. The most important parameters for industrial applications are related to the chemical composition of the juice and include total soluble solids (TSS) content, pH, titratable acidity (TA) and the maturation index ratio (TSS /TA). The use of automated and noninvasive systems to inspect the external quality of oranges and other fruits have been widely used in industries and packinghouses [1]. On the other hand, the use of such methods to assess internal quality indexes of fruits is much less common. Most of the methods used in internal quality control of fruits are time-consuming, destructive, based on sampling procedures, and they cannot be applied to 100% of the fruits in processing lines.

Most currently noninvasive and automated methods are based on visible and infrared radiation technologies. These methods provide good predictions for fruits with thin peels, such as apples and pears [2,3]. However, fruits with thicker peels, such as citrus, are difficult to be analyzed through light-based methods [4,5]. Hence, the development of noninvasive methods which are independent on reflection effects when measuring internal quality attributes of oranges is of enormous interest to the citrus production.

Time domain nuclear magnetic resonance spectroscopy (TD-NMR) has been used as a noninvasive technique to measure quality indexes of foods and packaged products without sample preparation procedures [6–8]. Although NMR spectroscopy is known to be a very expensive technique [8], this concept is based on high-resolution NMR spectrometers that use high-field superconducting magnets. In this paper, we are using a TD-NMR spectrometer that uses a low cost permanent magnet. This spectrometers have been used as a new way to apply NMR in industrial quality and process control [8–10]. Therefore, TD-NMR is a alternative technology with cost similar to the current, non-invasive methods based on infrared and visible light [4,5].

The TD-NMR analyses are based on relaxation times, which are known as longitudinal or T_1 relaxation time and the transverse relaxation time or T_2, and molecular diffusion [8,11]. TD-NMR has been used to investigate the ripeness of intact apples and pears as well as the effect of mechanical damage and low temperature on the chemical properties of intact fruits such as plums, grapes and bananas [11,12]. TD-NMR has

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http://dx.doi.org/10.1016/j.microc.2016.04.009

also been used to evaluate the quality of navel oranges during storage [13].These studies demonstrated that TD-NMR can be an alternative to the invasive and non-invasive methods, for example near infrared, to measure internal quality indexes of whole fruits, and to assess chemical changes induced by maturation processes and fruit development.

In this paper we demonstrate that transverse relaxation data measured by TD-NMR and modeled with partial least squares regression (PLSR) can be used as a rapid and noninvasive approach to predict internal quality and sensorial attributes of Valencia and Hamlin oranges.

2. Materials and methods

2.1. Samples

Four hundred and seventy oranges of the varieties Hamlin and Valencia were hand harvested in farms located at São Paulo, Brazil. The fruits were further selected by absence of defects and diseases, and then cleaned and stored at 12 °C ± 1.5 at a R.H. of 80% ± 10.

2.2. Time domain nuclear magnetic resonance (TD-NMR)

Oranges were analyzed on a SLK-MRI-1400 spectrometer operating with a static magnetic field (B_0) of 0.21 T (8.5 MHz for ^{1}H) and useful area of 10 cm diameter and 10 cm length. The CPMG sequence developed by Carr-Purcell-Meibom-Gill [17, 18] was used in the measurements, in which the pulse sequence generated an exponential decay with the transverse relaxation time (T_2). The acquisition parameters were: pulse 90 = 32 μ and 180 = 64 μ, echo time τ = 5000 μs, and total number of echoes equal to 1500 with 8 scans. All analyses were performed in duplicate under a laboratory temperature controlled at 21 ± 1 °C.

2.3. Chemical analyses

The total soluble solids (TSS) content of juice extracted from each orange was determined in triplicate using a digital refractometer (*Atago Co*, *Brix-Meter*, *Tokyo*), and the results were expressed in ° Brix. The pH of each fruit was also obtained in triplicate on 20 ml of juice under agitation with the aid of a pHS-3B pH meter equipment. Titratable acidity (TA) was determined by titration of 10 ml of homogenized juice with addition of 10 ml of distilled water, with 0.1 N NaOH solution until pH 8.2, and the results were expressed in g citric acid/100 g sample. The maturity index (MI) was calculated as the ratio between TSS and TA. All these analyses of reference were performed [16].The chi-square test was used to assess the correlation between ranges of pH and MI ratio at a significance level of $p = 0.05$ using the SPSS software, version 20.

2.4. Data processing

TD-NMR data (1500 variables) were normalized between 0 and 1 and smoothed with Savitzky–Golay algorithm (21 points with 2nd degree polynomial function). The first 10 and the last 200 values were excluded because they contained only noise. In addition, none outlier was excluded from the calibration, prediction and classification sets. These procedures were done with the software Origin 8.1 (OriginLab, Northampton, MA, USA) and Pirouette v. 4.5 (Infometrix, Inc. Bothell - WA).

Subsequently, the TD-NMR signals and the results obtained in the analyses of reference were arranged in two sets; the first contained the average data of 300 oranges to build partial least squares regression (PLSR) models. The second set of results contained 170 oranges, which were used for classification and sensory analyses.

The first set of data (300 oranges) was divided into two matrices: the calibration matrix "A" which comprised 70% of the data (210 oranges) and the prediction matrix "B″ which enclosed 30% of the data (90 oranges). The performance of the models was evaluated by leave-one-out cross-validation on the calibration set. For the prediction set, the correlation coefficient (r) and the root mean square error for prediction (RMSEP) were examined. In addition, the residual deviation predictive (RPD) was determined (ratio between standard deviation of the response variables with RMSEP). A RPD of 1.5 and 2 indicates models that discriminate samples of high and low values; a RPD value between 2.5 and 3 or larger indicates excellent models [12].

2.5. Classification of oranges

The classifications were based on the prediction of total soluble solids (TSS) of each individual fruit by TD-NMR, with a total of 170 oranges, using the PLSR model with two repetitions.

The first classification (A) involved 110 oranges, where the average TSS content for this group was obtained. Oranges with TSS content below the average TSS were grouped into the class 1, whereas fruits with TSS higher than the average were grouped into the class 2.

The second classification (B) had the objective of increasing the difference in the classifications by 1 °Brix. 60 oranges were used for this classification, where the average TSS content for this group was also obtained. Again, oranges with TSS 1°Brix lower than the average TSS were grouped into class 1, while the oranges with TSS 1°Brix higher than the average were grouped into class 2.

The accuracy of classification was calculated by Eq. (1), where y1 and y2 represent the wrong number of classified samples and N is the number of samples. Finally, the selectivity (TN/TN + FP) and sensitivity (TP/TP + FN) of the classifications were also calculated, where TN and FN represent the number of true negatives and false negatives, respectively, and TP and FP represent the number of true positives and false positives, respectively.

$$\mathrm{Accuracy}(\%) = 100 - \left\{ \frac{\left(\sum y1 \sum y2\right)}{\mathrm{N}} \times 100 \right\} \quad (1)$$

2.6. Sensory analysis: pairwise comparison test

Orange juice was extracted before sensory tests in order to keep its freshness characteristics. All food safety and hygiene handling practices were adopted as recommended by the ethical committee of University of São Paulo, Brazil.

For the first (A) and second (B) classifications, all oranges grouped into class 1 were used to extract juice, which was filtered through a 4 mm sieve, homogenized and encoded. The same procedure was carried out for oranges belonging to class 2. The pairwise comparison test was applied, in which two encoded samples containing 45 ml of juice for each class accompanied by a glass of water were presented to each panelist to evaluate the sweetness aspect of the samples.

A total of 110 untrained consumers of orange juice participated in the tests. The results were evaluated on the number of right answers to the most sweet juice class (1 or 2), and difference among responses were statistically evaluated at a confidence level of 95% as suggested [19].

3. Results and Discussion

Fig. 1 shows TD-NMR signals obtained with CPMG pulse sequence of two oranges with TSS contents of 9.0 and 13.5 °Brix. The CPMG decay is governed by transverse relaxation time (T_2) that is inversely correlated with sample viscosity. Therefore, the orange with lower TSS content shows longer CPMG decay (longer T_2) than that of the orange with higher TSS content. Therefore, univariate models can be used to predict TSS and pH of intact oranges from T_2 signals. However, the discrete T_2 values obtained by mono or multi-exponential fitting of the CPMG decay may reduce the information contained in the full CPMG data