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Short communication

Tracking thermal degradation on passion fruit juice through Nuclear Magnetic Resonance and chemometrics

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ABSTRACT

Thermal food processing mainly aims to control microorganism in order to extend its shelf life. However, it may induce chemical and nutritional changes in foodstuff. The Nuclear Magnetic Resonance (NMR) coupled to multivariate analysis was used to evaluate the effect of different thermal processing conditions (85 and 140 °C for 4; 15; 30; and 60 s) on the passion fruit juice using an Armfield pasteurizer. Through this approach it was possible to identify the changes in the juice composition. The temperature and the time lead to a hydrolysis of the sucrose to glucose and fructose. Additionally, juice submitted to 140 °C for 60 s results in the degradation of the sucrose and the formation of 5-(hydroxymethyl)-2-furfural (HMF). Despite no novel chemical marker has been identified, the ^{1}H NMR chemometrics approach may contribute in the choice of the temperature and time to be employed in the juice processing.

1. Introduction

Brazil is the world's largest passion fruit producer (776,000 tons) and consumer (Bellon et al., 2007). Although there is a great diversity of passion fruit species, *Passiflora edulis* f. flavicarpa is the only one with an established market (Dhawan, Dhawan, & Sharma, 2004; Zeraik, Pereira, Zuin, & Yariwake, 2010). Passion fruit has essential nutrients and functional compounds with antioxidant properties, such as polyphenolic compounds, carotenoids, vitamins and amino acids (Dhawan et al., 2004). Nontraditional food processing has been proposed for passion fruit juice, such as deacidification, microfiltration and membrane concentration (Domingues, Ramos, Cardoso, & Reis, 2014; Vera et al., 2009). However, thermal processing remains as the main industrial process for passion fruit producing countries (Sun, 2012). Heating is applied in juice processing to inactivate enzymes and microorganisms that might affect the quality and safety of the product (Awuah, Ramaswamy, & Economides, 2007). Nevertheless, heating may induce irreversible chemical and nutritional changes in the food product, such as browning, color changes, and formation of undesirable constituents (Butz & Tauscher, 2002). Therefore, a general understanding of the effects that the thermal processing promotes in the quality attributes of juices is important to produce high-standard products.

Nuclear magnetic resonance (NMR) spectroscopy is rapidly achieving significance in food analysis driven by quality control (Grandizoli, Campos, Simonelli, & Barison, 2014; Spraul et al., 2009). NMR is an adequate tool for the food screening as it allows the study of complex mixtures in small concentrations and the changes of several metabolites simultaneously without extensive sample pretreatments. However, due to highly complex NMR datasets from food matrices and the inherent similarity between the samples, applications of chemometric methods to complement the analytical methodologies are indispensable (Aguiar et al., 2013; de Oliveira, Carneiro, & Ferreira, 2014; Le Gall, Puaud, & Colquhoun, 2001; Silva, Alves Filho, Choze, Lião, & Alcantara, 2012). In the present study, the effects of different thermal conditions were studied to identify chemical markers (revealed by the

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multivariate statistical analysis techniques) related to the thermal process of the passion fruit juice.

2. Material and methods

2.1. Chemicals

Tetra-deuterated methanol (CD_3OD-MeOD) with 99.8% of deuterium and the sodium-3-trimethylsilyl propionate (TSP-d_4) were bought from Cambridge Isotope Laboratories, Inc. (Apeldoorn, The Netherlands). The ethylenediaminetetraacetic acid (EDTA) (99.9% purity) was purchased from Tedia (Rio de Janeiro, Brazil).

2.2. Sample preparation

The passion fruits (*Passiflora edulis* f. flavicarpa) were randomly purchased from the local market (Fortaleza, Ceará, Brazil) during February of 2014. The fresh fruits, previously sanitized with deionized water, were peeled and their pulps were manually squeezed, producing a yellowish juice. The juice was subjected to processing on a FT74 UHT/HTST Armfield pasteurizer, employing the following conditions: 85 °C for 15; 30; or 60 s and 140 °C for 4; 15; 30; or 60 s. Each thermal treatment was performed two times. Also, an aliquot of the thermally untreated juice was used as the control sample (STR) and analyzed. All the analytical samples were prepared in duplicates.

For ^{1}H NMR analysis, 3 g of juice were centrifuged at 1232*g* for 15 min. The supernatant (130 μL) was mixed with 470 μL of MeOD containing 14 mM of EDTA, 350 μL of MeOD and 1 % TSP-d_4. It was transferred to 5 mm NMR tubes for data acquisition (Biais et al., 2009).

2.3. NMR spectroscopy

The NMR experiments were performed in quintuplicate on an Agilent 600-MHz spectrometer equipped with a 5 mm (H-F/^{15}N-^{31}P) inverse detection One Probe™, at 298 K, using a pulse sequence for the saturation of the residual water signal (PRESAT, Agilent code). The TSP-d_4 was used as internal standard (0.0 ppm). The 90° pulse width was calibrated to each sample and the longitudinal relaxation time (T_1) was estimated through an inversion-recovery experiment prior the ^{1}H NMR analysis resulting in 15.0 s of relaxation delay and 5.0 s of acquisition time. The spectra were recorded with 64 free induction decays (FID) into 66 k data points in a 13,157.9 Hz spectral window. The spectra were processed by applying exponential multiplication of the FIDs by a factor of 0.3 Hz, Fourier transformation of 128 k points and a zero fill of 64 K. Phase corrections was manually performed and the baseline correction was applied over the entire spectral range. The integration of the signals was performed automatically choosing the same width for each quantified compound, e.g. sucrose from 5.36 to 5.43; glucose from 4.52 to 4.56; citric acid from 2.85 to 8.91; and 5-(hydroxymethyl)-2-furfural (HMF) from 9.47 to 9.50.

2.4. Molecular identification and quantification analysis

The constituents from the juice samples were identified by using the data obtained through ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and ^{1}H-^{13}C HMBC experiments. The results were compared to the existing NMR data in open access databases and literature reports.

The amount of sucrose, glucose, citric acid, and HMF were estimated by an external reference method. In this method, the absolute integration of a 24 mmol.L^{-1} standard solution of sucrose (⩾99.5%, Sigma, USA) was used to calibrate the equipment. Sucrose was chosen since due it high purity, stability (low reactivity), non-toxicity and cheap availability. Afterward, the probe file was updated with all the parameters required for concentration determination of an unknown sample.

2.5. Chemometric analysis

To test the statistical significance of the treatments on the sucrose, glucose, citric acid and HMF contents a Multivariate Analysis of Variance (MANOVA) was employed, followed by Tukey HSD (honest significant difference) test. The normality and homoscedasticity of the residuals were evaluated by Jarque-Bera goodness-of-fit test of composite normality and Hartley's and Cochran's tests of heteroskedasticity. To overcome the observed heteroskedasticity and lack of normality of the residues for sucrose and HMF, the samples replication (batch preparation) were considered as a covariate.

For the Principal Component Analysis (PCA) the matrix data was reduced (averaged) along variables by a factor of 20. Afterward the spectral area were normalized and aligned by Correlation Optimized Warping (COW) algorithm using a segment of 20 data points and a slack of 10 data points. The COW alignment was performed in the spectral ranges of 4.83–4.41; 3.11–2.64; and 1.35–1.10 ppm. The chemometrics analysis was performed by excluding the HOD (4.68 to 5.32 ppm) and MeOD signals (3.28 to 3.34 ppm) for whole spectra analysis. For quantification purpose all the representative signals for glucose and sucrose were considered.

Besides this unsupervised analysis, to improve the identification of chemical changes due to the thermal treatment a supervised Partial Least Square – Discriminant Analysis (PLS-DA) was employed using the time and the temperature of the thermal treatments as categorical variables. All the multivariate (PCA and PLS-DA) models were evaluated by segment cross-validation where all the replicates of each treatment were left out from the calibration data set and the sub-models were calibrated on the remaining data points. All the chemometrics were performed at The Unscramble® X (CAMO) program.

3. Results and discussion

The ^{1}H NMR spectrum (Fig. 1) shows characteristic hydrogens of amino acids and organic acids at 0.90 to 3.00 ppm; of carbohydrates residues between 3.00 to 6.00 ppm; and above 6.00 ppm from aromatic compounds. Based on known database (Wishart et al., 2007) and literature (de Oliveira et al., 2014; Le Gall et al., 2001; Silva et al., 2012; Spraul et al., 2009) the main constituents were identified as indicated in Fig. 1.

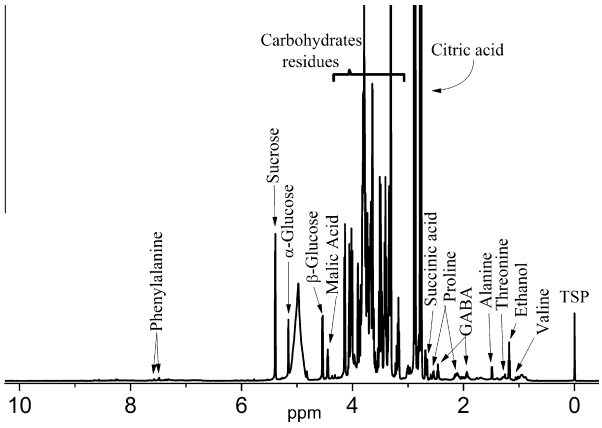

Fig. 1. ^{1}H NMR spectrum of the main components of the passion fruit juice. Legend: GABA: γ-aminobutyric acid; TSP: Trimethylsilyl propanoic acid.