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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.

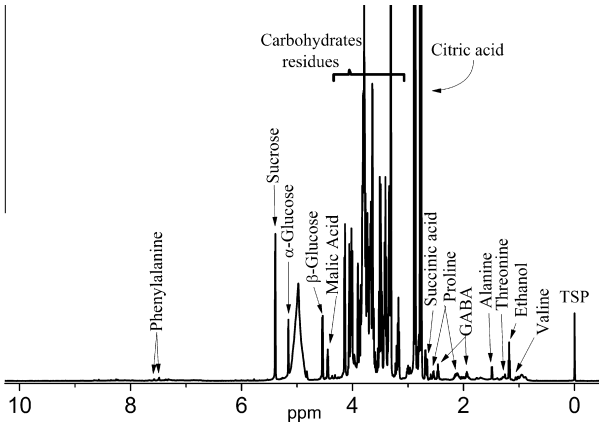

Fig. 1. ^{1}H NMR spectrum of the main components of the passion fruit juice. Legend: GABA: γ-aminobutyric acid; TSP: Trimethylsilyl propanoic acid.

The PCA is a valuable tool to: detect the data structure due to variable correlations, i.e. peak intensity correlations by different chemical groups from the same compounds or correlations, positive or negative, between different compounds; to separate sources of variations, due to the orthogonality of the principal components; and disclose existent samples distribution patterns (Alves Filho et al., 2016; Granato, de Araújo Calado, & Jarvis, 2014; Monakhova, Kuballa, & Lachenmeier, 2013; Rusilowicz, O'Keefe, Wilson, & Charlton, 2006). Since in this controlled experiment, the only sources of variations, barring the uncontrolled random experimental "error", are the treatment temperature and time, it is expected that the extracted principal components reveal this variability drives. Therefore, the PCA was used to highlight the information regarding the thermal processing.

The two first Principal Components (PC) from the full NMR spectra (0.12 to 11.01 ppm) explained 71% of the total variance. The PCA scores (Fig. 2a) show clearly the existence of three groups in PC1 (46 % of total variance). The PC2 is responsible for 25% of the total variance, however there was no clear grouping of samples. Regarding PC1 axis, the first group was composed of control and 85 °C samples at positive scores. The second group is composed of samples submitted to 140 °C for 4, 15, and 30 s at negative scores on the same axis, followed finally, by the third group composed of samples pasteurized to 140 °C for 60 s with the lowest PC1 scores. Therefore, the main driver of this PC was the intensity of the thermal treatment and the time as the juice pasteurized to 140 °C for 60 s presented higher compositional differences (higher scores on PC1).

A careful inspection of the PC1 loadings plots (Fig. 2b) provides relevant information regarding the variables responsible for the formation of the groups observed in the score plots. In general, the samples located in positive scores of PC1 (control and 85 °C) showed higher amount of sucrose (5.46 and 4.20 ppm), whereas the samples processed at 140 °C at any time, with higher amount of α-glucose (5.15 ppm); β-glucose (4.54 ppm); and free fructose (4.00 ppm). This finding revealed that the thermal process affected the sugar composition by converting sucrose to glucose and free fructose. Furthermore, the complete degradation of sucrose occurred when the passion fruit juice was submitted to 140 °C for 60 s (Fig. 3).

In order to get a better understanding of the effect of the thermal processing on the particular components of the juice, the loadings plot was carefully evaluated for some specific regions of the NMR spectrum: aliphatic (0.8 to 2.7 ppm) and aromatic (6.5

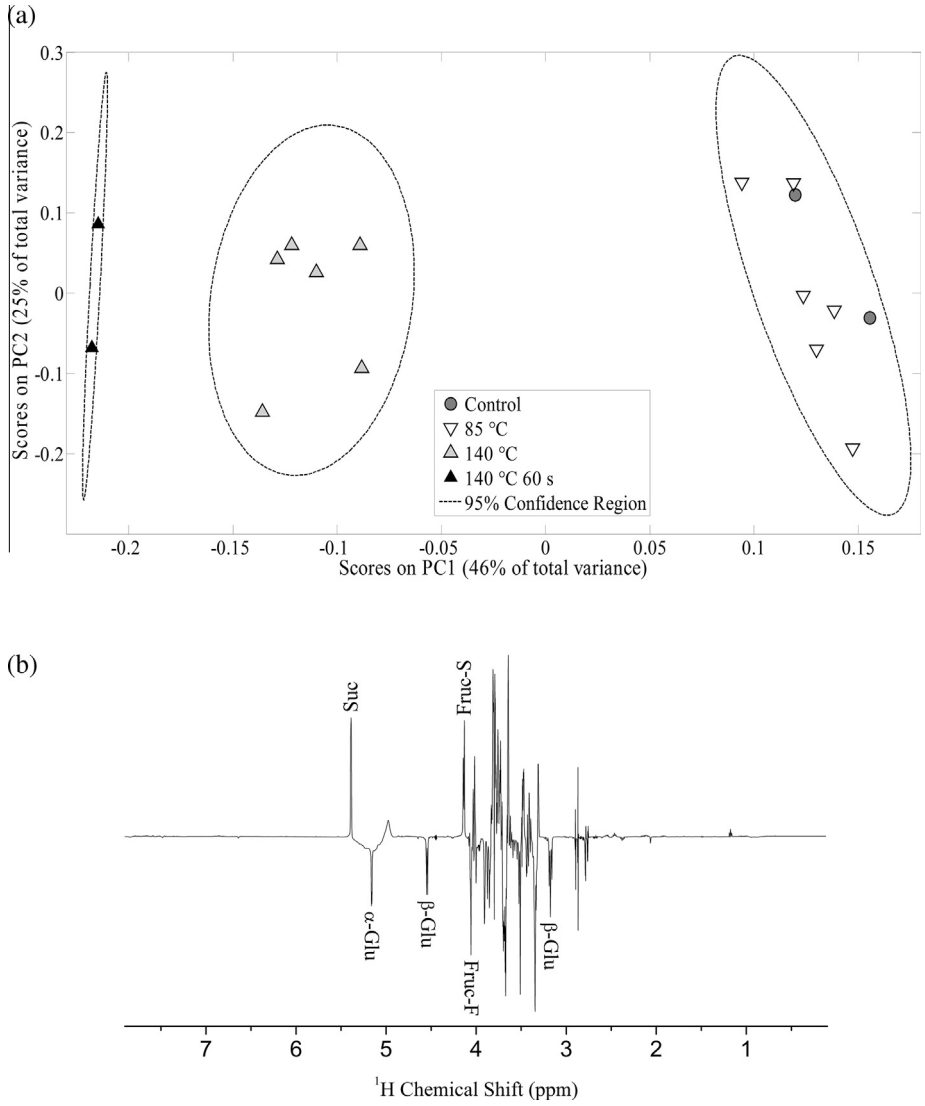

Fig. 2. a) PC1 *vs* PC2 score plot of passion fruit juice before (STR) and after the thermal treatments at 85 and 140 °C for 4; 15; 30; and 60 s. b) loadings plot of PC1. Suc: Sucrose; α-Gluc: α-glucose; β-Gluc: β-glucose; Fruc_S: fructose from sucrose; Fruc_F: Free fructose. The dashed ellipses are the 95% confidence region of each group.

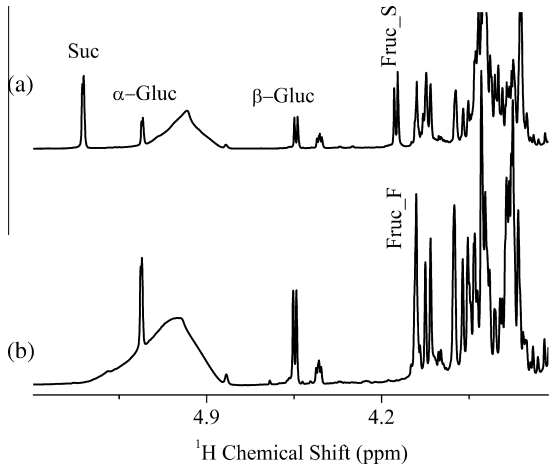

Fig. 3. Expansion of anomeric region of a) control passion fruit juice; b) passion fruit juice submitted to 140 °C for 60 s. Suc: Sucrose; α-Gluc: α-glucose; β-Gluc: β-glucose; Fruc_S: fructose from sucrose; Fruc_F: Free fructose.

to 9.7 ppm). The Fig. 4a shows the expansion of loadings plot of PC1 for the aliphatic region and the Fig. 4b shows the expansion of loadings plot of PC1 for the aromatic region. Thus, the samples located in positive scores of PC1 (control and 85 °C) showed a higher amount of amino acids proline (2.12 and 2.57 ppm) and GABA (1.9 and δ 2.45 ppm). Whereas the samples processed at 140 °C (regardless of the time), presented a lower amount of total amino acids and with a higher amount of acetic acid (2.06 ppm) (glucose degradation product) and an unknown compound (2.40 ppm). The amino acids degradation might induce the formation of amines, ammonium, pyrazines, pyridines, and pyrroles (Sohn & Ho, 1995), but these compounds were not detected.

In addition, the expansion of the aromatic region (Fig. 4b) revealed that the signals from HMF (6.62 ppm; 7.42 ppm; and 9.52 ppm) and an unknown compound (7.82 ppm and 8.24 ppm) with negative loadings are responsible for allocating the sample submitted to 140 °C at the negative scores of PC1. This showed that a strong thermal treatment results in the production of furfural (thermal-degradation product of glucose) and others thermally generated compounds. The HMF is produced by Maillard reaction, a non-enzymatic browning reaction between amino acids and reducing sugars (Teixidó, Núñez, Santos, & Galceran, 2011) commonly found in food. The monitoring of the formation of the HMF is used to evaluate the severity of heating during fruit-juice processing (Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2008; Burdurlu, Koca, & Karadeniz, 2006). Furthermore, the formation of HMF only occurs when the passion fruit juice is submitted to 140 °C for 60 s.

The presence of HMF in food and its derivatives such as 5-chloromethylfurfural (CMF) and 5-sulphooxymethylfurfural (SMF) have been of great concern, since they are cytotoxic, mutagenic, and carcinogenic (Monien, Frank, Seidel, & Glatt, 2009; Nässberger, 1990; Surh, Liem, Miller, & Tannenbaum, 1994). The European Union has adopted the HMF as a chemical marker of deterioration and heat-treatment, establishing the concentration of 50 mg/L as maximum HMF amount in apple juice (Directive 2001/110/EC, 2001) and the concentration of 40 mg/kg for honey. In the present case, the passion fruit juice treated at 140 °C for 60 s presented 44.6 mg/L of HMF. Despite the maximum international limit has not been exceeded, the thermally induced chemical alterations in the juice might compromise its nutritional properties since the presence of HMF has been related to toxicological (Janzowski, Glaab, Samimi, Schlatter, & Eisenbrand, 2000; Pereira, Albuquerque, Ferreira, Cacho, & Marques, 2011) and carcinogenic effects (Durling, Busk, & Hellman, 2009; Monien et al., 2009).

Therefore, after the definition of the main molecular markers of the thermal treatment by PCA (sucrose; β-glucose; citric acid; and HMF) the quantification of these markers was performed. The sucrose content (Fig. 5a) decrease with the 85 °C and 140 °C thermal treatments and a higher sucrose degradation effect was observed when 140 °C during 60 s was used. The amount of glucose (Fig. 5b) in the 140 °C thermal treatment, was higher than the control, and can be related to the sucrose degradation in this treatment. Additionally, it was observed that the temperature promoted a degradation of citric acid (Fig. 5c). On the other hand, the HMF was not formed at 85 °C (Fig. 5d), but at 140 °C, a heating for 4 s was enough to produce this compound and after 60 s at 140 °C its content was 20 times higher than in shorter treatment periods.

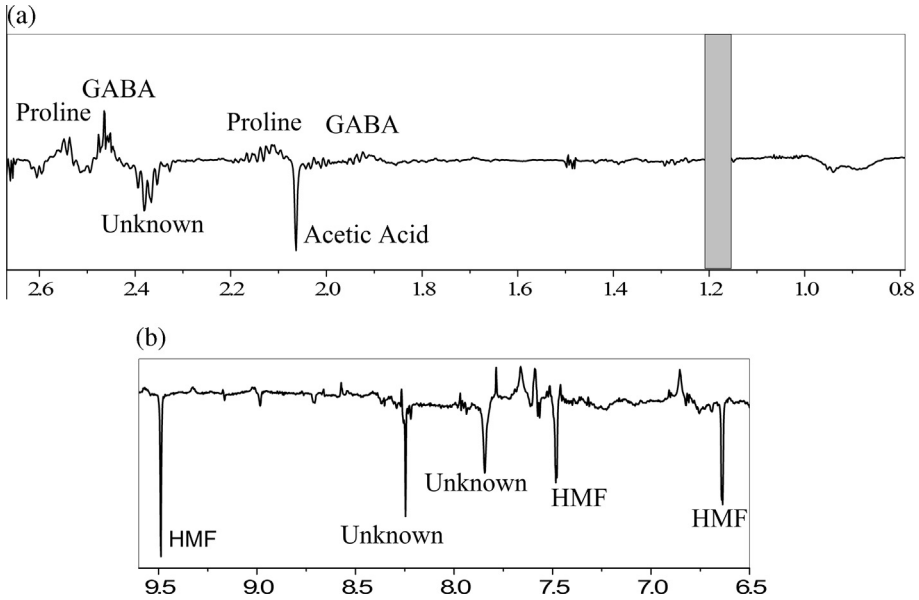

Fig. 4. Expansion of loadings plot of PC1 of a) aliphatic region and b) aromatic region. GABA: gama aminobutyric acid; HMF: 5-(hydroxymethyl)-2-furfural. The dark region was removed from the PCA analysis.

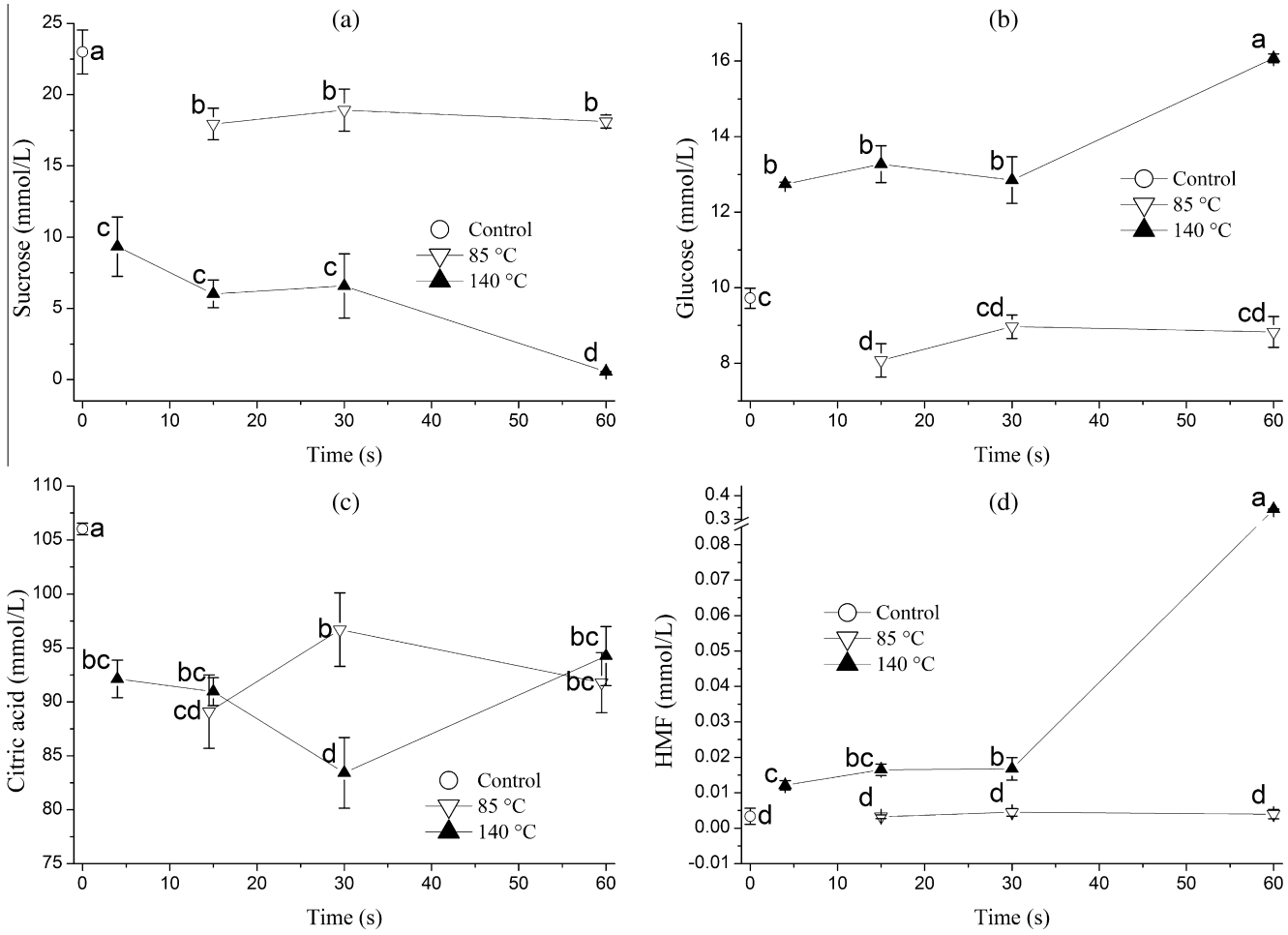

Fig. 5. Concentration of: a) sucrose; b) glucose; c) citric Acid; and d) HMF. HMF: 5-(hydroxymethyl)-2-furfural. Vertical bars denote 0.95 confidence intervals.

To observe the isolated temperature and time effects in the juice composition a PLS-DA was used. With two latent variables (LV) the model presented a prediction ability of 96% (calibration and validation R^2) (Fig. 6). An inspection of the scores of the LV permitted to verify that the LV1 describes the changes in the chemical composition caused by the temperature variation. On the other hand, the LV2 describes the chemical changes that occur by increasing the time of the thermal treatment. The loadings interpretation (data not shown) indicated that, besides orthogonal, both LV presented high positive loadings for glucose, free fructose and HMF, and high negative loadings for sucrose, however the disaccharide hydrolysis to monosaccharides is more important (highest loadings) for LV1 (temperature effect) while HMF formation is more important for LV2 (time effect). Thus, the conversion of sucrose to glucose and fructose was the major responsible for the classification of the data on the LV1, accounting for 82.2% of the total variance. In addition, the HMF formation also contributed to the data distribution on the LV1. However, the formation of the LV2 axis (14.5% total variance) is related mainly to the formation of the HMF, especially due to the highest HMF content in the samples treated at 140 °C during 60 s.

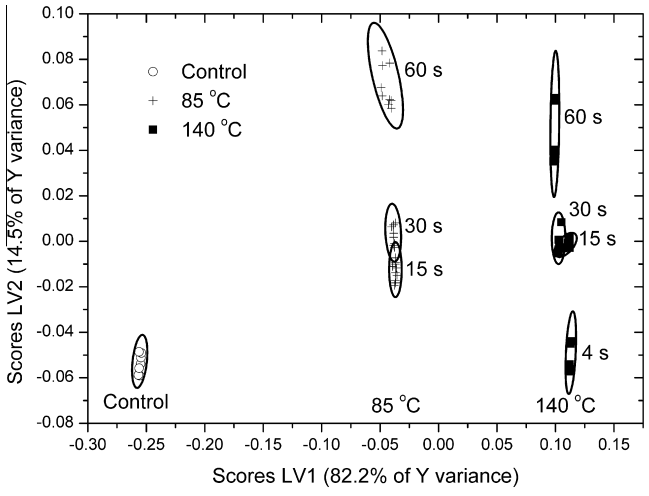

Fig. 6. LV1 *vs.* LV2 scores plot from PLS-DA for the passion fruit juice submitted to various thermal treatments.

4. Conclusions

Our results indicate that the ^{1}H NMR combined with multivariate data analysis is a practical and useful tool for tracking the thermal processing of fruit juices, since it provides rapidly comprehensive and quantitative information on the chemical composition of the thermally processed juice. Although no unexpected chemical change has been found or novel chemical markers has been identified, ^{1}H NMR was able to reveal individual and global variations in the contents of sugars, amino acids and organic acids in the thermally treated passion fruit juices. Furthermore, possible

overheat damages could be easily detected and measured through ^{1}H NMR profile, monitoring the fluctuations in the concentrations of sucrose and β-glucose and consequent formation of 5-(hydroxymethyl)-2-furfural (HMF).

Acknowledgments

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References

Aguiar, N. O., Novotny, E. H., Oliveira, A. L., Rumjanek, V. M., Olivares, F. L., & Canellas, L. P. (2013). Prediction of humic acids bioactivity using spectroscopy and multivariate analysis. *Journal of Geochemical Exploration, 129*, 95–102.

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Comparative study on color, viscosity and related enzymes of tomato juice treated by high-intensity pulsed electric fields or heat. *European Food Research and Technology, 227*(2), 599–606.

Alves Filho, E. G., Almeida, F. D. L., Cavalcante, R. S., de Brito, E. S., Cullen, P. J., Frias, J. M., ... Bourke, P. (2016). 1H NMR spectroscopy and chemometrics evaluation of non-thermal processing of orange juice. *Food Chemistry, 204*, 102–107.

Awuah, G. B., Ramaswamy, H. S., & Economides, A. (2007). Thermal processing and quality: Principles and overview. *Chemical Engineering and Processing: Process Intensification, 46*(6), 584–602.

Bellon, G., Faleiro, F. G., Junqueira, K. P., Junqueira, N. T. V., Santos, E. C. d., Braga, M. F., & Guimarães, C. T. (2007). Variabilidade genética de acessos silvestres e comerciais de Passiflora edulis Sims. com base em marcadores RAPD. *Revista Brasileira de Fruticultura, 29*, 124–127.

Biais, B., Allwood, J. W., Deborde, C., Xu, Y., Maucourt, M., Beauvoit, B., ... Dunn, W. B. (2009). 1H NMR, GC-EI-TOFMS, and data set correlation for fruit metabolomics: Application to spatial metabolite analysis in melon. *Analytical Chemistry, 81*(8), 2884–2894.

Burdurlu, H. S., Koca, N., & Karadeniz, F. (2006). Degradation of vitamin C in citrus juice concentrates during storage. *Journal of Food Engineering, 74*(2), 211–216.

Butz, P., & Tauscher, B. (2002). Emerging technologies: Chemical aspects. *Food Research International, 35*(2–3), 279–284.

de Oliveira, C. R., Carneiro, R. L., & Ferreira, A. G. (2014). Tracking the degradation of fresh orange juice and discrimination of orange varieties: An example of NMR in coordination with chemometrics analyses. *Food Chemistry, 164*, 446–453.

Dhawan, K., Dhawan, S., & Sharma, A. (2004). Passiflora: A review update. *Journal of Ethnopharmacology, 94*(1), 1–23.

Domingues, R. C. C., Ramos, A. A., Cardoso, V. L., & Reis, M. H. M. (2014). Microfiltration of passion fruit juice using hollow fibre membranes and evaluation of fouling mechanisms. *Journal of Food Engineering, 121*, 73–79.

Durling, L. J. K., Busk, L., & Hellman, B. E. (2009). Evaluation of the DNA damaging effect of the heat-induced food toxicant 5-hydroxymethylfurfural (HMF) in various cell lines with different activities of sulfotransferases. *Food and Chemical Toxicology, 47*(4), 880–884.

Granato, D., de Araújo Calado, V. M., & Jarvis, B. (2014). Observations on the use of statistical methods in Food Science and Technology. *Food Research International, 55*, 137–149.

Grandizoli, C. W. P. d. S., Campos, F. R., Simonelli, F., & Barison, A. (2014). Grape juice quality control by means of 1H nmr spectroscopy and chemometric analyses. *Química Nova, 37*, 1227–1232.

Janzowski, C., Glaab, V., Samimi, E., Schlatter, J., & Eisenbrand, G. (2000). 5-Hydroxymethylfurfural: Assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food and Chemical Toxicology, 38*(9), 801–809.

Le Gall, G., Puaud, M., & Colquhoun, I. J. (2001). Discrimination between orange juice and pulp wash by 1H nuclear magnetic resonance spectroscopy: Identification of marker compounds. *Journal of Agricultural and Food Chemistry, 49*(2), 580–588.

Monakhova, Y. B., Kuballa, T., & Lachenmeier, D. W. (2013). Chemometric methods in NMR spectroscopic analysis of food products. *Journal of Analytical Chemistry, 68*(9), 755–766.

Monien, B. H., Frank, H., Seidel, A., & Glatt, H. (2009). Conversion of the common food constituent 5-hydroxymethylfurfural into a mutagenic and carcinogenic sulfuric acid ester in the mouse in vivo. *Chemical Research in Toxicology, 22*(6), 1123–1128.

Nässberger, L. (1990). Influence of 5-hydroxymethylfurfural (5-HMF) on the overall metabolism of human blood cells. *Human & Experimental Toxicology, 9*(4), 211–214.

Pereira, V., Albuquerque, F. M., Ferreira, A. C., Cacho, J., & Marques, J. C. (2011). Evolution of 5-hydroxymethylfurfural (HMF) and furfural (F) in fortified wines submitted to overheating conditions. *Food Research International, 44*(1), 71–76.

Rusilowicz, M., O'Keefe, S., Wilson, J., & Charlton, A. (2006). Chemometrics Applied to NMR Analysis. In *Encyclopedia of Analytical Chemistry*. John Wiley & Sons, Ltd..

Silva, L. M. A., Alves Filho, E. G., Choze, R., Lião, L. M., & Alcantara, G. B. (2012). 1H HRMAS NMR spectroscopy and chemometrics for evaluation of metabolic changes in *Citrus sinensis* caused by *Xanthomonas axonopodis* pv. citri. *Journal of the Brazilian Chemical Society, 23*, 1054–1061.

Sohn, M., & Ho, C.-T. (1995). Ammonia generation during thermal degradation of amino acids. *Journal of Agricultural and Food Chemistry, 43*(12), 3001–3003.

Spraul, M., Schütz, B., Humpfer, E., Mörtter, M., Schäfer, H., Koswig, S., & Rinke, P. (2009). Mixture analysis by NMR as applied to fruit juice quality control. *Magnetic Resonance in Chemistry, 47*(S1), S130–S137.

Sun, D. W. (2012). *Thermal food processing: New technologies and quality issues* (2nd ed.). Taylor & Francis.

Surh, Y.-J., Liem, A., Miller, J. A., & Tannenbaum, S. R. (1994). 5-Sulfooxymethylfurfural as a possible ultimate mutagenic and carcinogenic metabolite of the Maillard reaction product, 5-hydroxymethylfurfural. *Carcinogenesis, 15*(10), 2375–2377.

Teixidó, E., Núñez, O., Santos, F. J., & Galceran, M. T. (2011). 5-Hydroxymethylfurfural content in foodstuffs determined by micellar electrokinetic chromatography. *Food Chemistry, 126*(4), 1902–1908.

Vera, E., Sandeaux, J., Persin, F., Pourcelly, G., Dornier, M., & Ruales, J. (2009). Deacidification of passion fruit juice by electrodialysis with bipolar membrane after different pretreatments. *Journal of Food Engineering, 90*(1), 67–73.

Wishart, D. S., Tzur, D., Knox, C., Eisner, R., Guo, A. C., Young, N., ... Querengesser, L. (2007). HMDB: The human metabolome database. *Nucleic Acids Research, 35* (Database issue), D521–D526.

Zeraik, M. L., Pereira, C. A. M., Zuin, V. G., & Yariwake, J. H. (2010). Maracujá: um alimento funcional? *Revista Brasileira de Farmacognosia, 20*, 459–471.