NMR spectroscopy and chemometrics to evaluate different processing of coconut water

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ABSTRACT

NMR and chemometrics was applied to understand the variations in chemical composition of coconut water under different processing. Six processing treatments were applied to coconut water and analyzed: two control (with and without sulphite), and four samples thermally processed at 110 °C and 136 °C (with and without sulphite). Samples processed at lower temperature and without sulphite presented pink color under storage. According to chemometrics, samples processed at higher temperature exhibited lower levels of glucose and malic acid. Samples with sulphite processed at 136 °C presented lower amount of sucrose, suggesting the degradation of the carbohydrates after harshest thermal treatment. Samples with sulphite and processed at lower temperature showed higher concentration of ethanol. However, no significant changes were verified in coconut water composition as a whole. Sulphite addition and the temperature processing to 136 °C were effective to prevent the pinking and to maintain the levels of main organic compounds.

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1. Introduction

Coconut water is a popular isotonic beverage due to its refreshing, nutritional, and potential therapeutic properties (Tan, Cheng, Bhat, Rusul, & Easa, 2014), and moreover, is flavorful, sweet, slightly acidic, rich in phosphorus and potassium. It also contains proteins, fats, minerals, carbohydrates (glucose, fructose, and sucrose), and organic acids such as tartaric, citric and malic acids (Campbell-Falck, Thomas, Falck, Tutuo, & Clem, 2000; Santoso, Kubo, Ota, Tadokoro, & Maekawa, 1996). However, the water extracted from the nut spoils within a day after the exposure to air due to oxidation induced by the naturally presents enzymes as polyphenol oxidase (PPO) and peroxidase (POD) (Murasaki-Aliberti, Da Silva, Gut, & Tadini, 2009), as well as microbiological contamination (Reddy, Das, & Das, 2005). Those enzymes are thermophilic and induce the formation of yellow, brown and pink color of coconut water during the storage (Damar, Balaban, & Sims, 2009; Prades, Dornier, Diop, & Pain, 2012). Therefore, preservation processes are necessary to increase the shelf life of the product to enable long-term commercialization.

The UHT (Ultra High Temperature) sterilization processing is effective in the microbiological and enzymatic control (Awuah,

Ramaswamy, & Economides, 2007). In general, the sterilization have to be planned in order to reach microbiological stability and safety with a minimum recommended process lethality (F_o) of 3 min (Holdsworth, 1997). However, sensory and nutritional changes are usually present, which compromises the quality and acceptance of the final product (Campos, Souza, Coelho, & Glória, 1996; Tan et al., 2014). Sterilization associated with the use of sulphite has also been adopted by the industries to increase the shelf life of the product. Sulphur dioxide, sulphites and meta-bisulphites is widely used to prevent browning caused by enzymatic or oxidative reactions on foodstuffs (Pereira, Faria, & Pinto, 2013). In addition, the sulphite species exhibit antiseptic properties (Martins et al., 2011) and help to stabilize the product color, while improve flavor and appearance of several products (Ruiz-Capillas & Jiménez-Colmenero, 2009). According to Damar and co-workers (Damar et al., 2009), the appearance of pink color in coconut water thermally processed and stored is possibly due to aeration and heat treatment, and the addition of ascorbic acid or sulphite stabilize the color. The appearance of this color due microbial or enzymatic activity is unlikely, since even the boiling did not avoid the pinking.

Nuclear magnetic resonance (NMR) spectroscopy is an important analytical tool for complex mixture analysis, such as in the quality control of food (Choze et al., 2013; Silva, Alves Filho, Choze, Lião, & Alcantara, 2012). Nevertheless, considering the fact that NMR data might generate highly complex matrices with a large inherent spectral similarity, the visual analysis may be

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unfeasible (Spraul et al., 2009). Together to NMR, chemometrics is adopted to employ mathematical tools in the chemical data (Alves Filho et al., 2012; Larsen, van den Berg, & Engelsen, 2006). Purkayastha et al. (2012) studied the effects of addition of L-ascorbic acid on the quality of micro-filtered coconut water by using ^{1}H NMR and observed that on average, signals from α-rhamnopyranosyl ainomeric proton, free sugars or sugar alcohols were mostly present (Purkayastha et al., 2012). Jagannathan, Govindaraju, and Raghunathan (1995) studied the mature coconut water through magnetic resonance imaging (MRI) and verified sugar regions (δ 5.1–5.5) (Jagannathan et al., 1995).

The aim of this work was the evaluation of the chemical composition of coconut water subjected to sulphite adding and/or ultra high temperature sterilization by using ^{1}H NMR and chemometrics in order to detect possible chemical changes from different processing methods.

2. Experimental

2.1. Sample and UHT sterilization process

Green coconuts (*Cocos nucifera*, L.) with maturation ages between 6 and 7 months were harvested in Ceará state, Brazil. The coconuts were initially rinsed in tap water, followed by 15 min sanitization in chlorinated water (100 mg.L^{-1} of sodium hypochlorite) and then, cut for water extraction, filtered, and frozen at -17 ± 2 °C before the processing.

Six treatments were obtained with different heating temperatures and addition of sulphite, as described: control; control with sulphite; 110 °C (sample with pink color); 110 °C with sulphite; 136 °C; 136 °C with sulphite. These temperatures were chosen in a randomized experimental design and also based on previously sterilization studies in order to verity the appearance of pink color at 110 °C without sulphite addition. The thermal treatment of the samples was performed with retention time of 8 s using an Armfield tubular heat exchanger (model FT74), cooling with chiller Armfield FT63, filled under aseptic conditions in 210 mL glass bottles and closed with plastic screw cap. The packages were sterilized with 0.5% peracetic acid solution and rinsed with sterile water before filling. The sulphite (Vetec™) addition at 40 mg.L^{-1} was performed to prevent browning caused by enzymatic or oxidative reactions in the processed coconut water, and to compare the possible composition changes. The control samples were kept frozen until the NMR analysis. Samples submitted to heat treatment were stored at room temperature until the occurrence of the color changes (same time of storage for all processed samples) and then, aliquots were also frozen before the NMR analysis. All these processing were performed in triplicate.

2.2. NMR spectroscopy and molecular identification

An aliquot of 3.0 mL of the resultant samples was transferred to tubes and centrifuged at 605g for 15 min. Then, 130 µL of the samples were transferred to vials and mixed with 14 mM of EDTA in 350 µL of CD$_3$OD-d4 (tetradeutered methanol 98%) containing 1% of sodium-3-trimethylsilylpropionate (TMSP-2,2,3,3-d4 98% purity), and transferred to 5 mm NMR tubes. The EDTA was added to minimize the ionic strength effect on frequency shifts in the NMR spectra.

The NMR experiments were performed on Agilent 600-MHz spectrometer equipped with 5 mm (H-F/^{15}N-^{31}P) inverse detection One Probe™ and actively shielded Z-gradient. The NMR probe was frequency tuned and impedance matched before each acquisition. The ^{1}H NMR spectra were acquired in quadruplicate using the PRE-SAT pulse sequence for the water suppression (δ 4.98), since this

pulse program presented less effect in the surrounding region according to the saturation profile of the non-deuterated water signal. The data were acquired under quantitative conditions using 90° hard pulse (providing maximum signals intensity) determined by the annulment of the most ^{1}H signals after 360° pulse (90° = ¼ × 360°) (Alves Filho et al., 2015). A total of 64 scans were acquired with 64 k of time domain points for a spectral window of 12 ppm, and receiver gain adjusted to 16 for all ^{1}H NMR measurements. The acquisition time of 5.0 s and relaxation delay of 10.0 s used were more than 5 times the longest T$_1$ observed in the signals. The temperature was controlled at 298 K and the TMSP-d4 was used as an internal standard (0.0 ppm). The spectra were processed applying an exponential multiplication of the FIDs by a factor of 0.3 Hz before Fourier transformation of 64 k points. Phase corrections were performed manually and the baseline corrections were applied over the entire spectral range. The manual mode was used also for the signals integration process choosing the same width for each compound (Winning, Larsen, Bro, & Engelsen, 2008).

Two-dimensional (2D) NMR experiments were acquired using the standard spectrometer library pulse sequences. ^{1}H-^{1}H COSY experiments were obtained with spectral width of 18,028.1 Hz in both dimensions; 1442 × 200 data matrix; 32 scans per t1 increment and relaxation delay of 1.0 s. One-bond ^{1}H-^{13}C HSQC experiments were acquired with an evolution delay of 1.7 ms for an average ^1J(C,H) of 145 Hz; 1442 × 200 data matrix; 80 scans per t1 increment; spectral widths of 9615.4 Hz in f2 and 30,165.9 Hz in f1, and relaxation delay of 1.0 s. The ^{1}H-^{13}C HMBC experiments were recorded with an evolution delay of 50.0 ms for ^{LR}J(C,H) of 10 Hz; 1442 × 200 data matrix; 180 scans per t1 increment; spectral widths of 9615.4 Hz in f2 and 30,165.9 Hz in f1, and relaxation delay of 1.0 s.

The identification of the constituents within the coconut water samples was performed 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 data in open access databases and literature reports (see Supplementary Information).

2.3. Chemometric analysis

The ^{1}H NMR spectra were utilized as input data for Amix™ program to Principal Component Analysis (PCA) in order to create an overview, showing grouping trends and outliers in the data with confidence level of 95% (Hotelling, 1933). Chemometric analyses were performed using the quadruplicate of the six treatments of coconut water, as described in item 2.1. PCA analyses were performed using two regions of the ^{1}H NMR spectra: whole spectra – δ 0.84 to δ 8.54; and aliphatic region – δ 0.84 to δ 3.02.

For the PCA, each spectrum was divided into 0.04 ppm wide buckets, using simple rectangular bucket, sum of intensities in integration mode and scaled to total intensity in scaling process. The spectra were divided into 145 buckets for PCA using total spectra, and into 44 buckets using only the aliphatic region. The area influenced by water suppression according to the saturation profile (δ 4.62 to δ 5.15) was excluded of the bucketing process. The bucket tables were pre-processed by mean-centered, with the mean value of each column subtracted from individual elements since this pretreatment provided better differences between the samples and it did not allow that noises affect negatively the distribution (Beebe, Pell, & Seasholtz, 1998). The chemical shift values in the loadings plots refer to the center position of each bucket.

2.4. Quantification analysis

The compounds that stood out in the multivariate evaluation were quantified in order to corroborate the chemometric results. Therefore, sucrose, fructose, glucose, ethanol, and malic acid were