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High Pressure Carbon Dioxide Pasteurization of Coconut Water: A sport drink with high nutritional and sensory quality

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ABSTRACT

High Pressure Carbon Dioxide (HPCD) treatment was applied to the pasteurization of coconut water in order to guarantee both its microbial stability and preserve its nutritional and sensory attributes. It was demonstrated that 120 bar, 40°C, 30 min were the optimal process conditions to induce a 5 Log(CFU/ml) reduction of mesophilic microorganisms, lactic acid bacteria, yeasts and molds and a 7 Log reduction of the total coliforms. The effect of HPCD on the quality traits of coconut water were investigated by means of physical-chemical and sensory analyses and compared to the Heat Pasteurized (HP, 90°C, 1 min) and Fresh Untreated (FU) product. No differences in the basic chemical composition, vitamins and amino acids, were detected between HPCD and FU products. However, differences in the volatile compounds present in the three products were clearly distinguishable; HPCD resulted in a reduction of most of the volatile fractions while HP induced the formation of compounds with a toasted and malty aroma. Nevertheless, few sensory differences were perceived between the FU and the HPCD coconut water, and both were clearly differentiated from the HP product.

KEYWORDS: Coconut water, high pressure carbon dioxide, pasteurization, natural microbial flora, sensory properties, volatile compounds.

HIGHLIGHTS

- HPCD treatment guaranteed the inactivation of the natural microbial flora of coconut water.
- HPCD preserved the nutritional and sensory qualities of coconut water better than thermal processing.
- HPCD could contribute to the commercialization of coconut water as a natural and healthy drink.

1. INTRODUCTION

The hydration and absorption of liquids with a high content of mineral salts, vitamins, sugars and nutritional substances are fundamental for a correct diet of active people. Decades of research clearly demonstrate the benefits derived from the consumption of drinks during and after physical activity (Sawka et al., 2007). Coconut water, the clear liquid inside young green coconuts, is becoming more and more popular among the athletes as a healthy drink rich in vitamin C, magnesium, calcium, potassium, vitamin B, arginine, alanine, lysine, glutamic acid, enzymes with anti-inflammatory properties, minerals and antioxidants (Reddy, 1995). Its claims as a natural alternative in the sports drink market are supported by its low calorie content (about 17.4 kcal per 100 g), and by its delicate aroma, taste, nutritional and functional characteristics (FAO, 2000).

Currently, coconut water is processed by heat treatments, which destroy the natural microbial flora occurring in the product, prolonging its shelf life for 2-3 months (Reddy, 1995). The high process temperatures of heat pasteurization (HP) grossly alter the product's sensory quality and change its nutritional contents. Different preservation techniques like filtration, increasing the sugar and total solid content, pH adjustment, ultrasonic treatments, concentration by reverse osmosis, spray drying, the addition of preservatives, etc., have been investigated either alone or in various combinations (Reddy, 1995; Bergonia et al., 1982; Magda, 1992; Reddy et al., 2005). High Pressure Carbon Dioxide (HPCD) is an emerging non-thermal treatment processes, in addition to Pulsed Electric Fields (Zhao et al. 2012) and High Hydrostatic Pressures (Rendueles et al., 2011). Since the 1980s, HPCD has been increasingly considered as a technique able to induce the inactivation of the natural microbial flora and pathogens occurring in solid and liquid matrices (Arreola et al., 1991; Zhou et al., 2009; Spilimbergo and Ciola, 2010a; Ferrentino and Spilimbergo, 2011). The CO_2 used in this process is relatively inert, inexpensive, non-toxic, non-flammable, recyclable and readily available in high purity, and leaves no residues when removed after the treatment process. Furthermore, it is

considered a Generally Recognized as Safe (GRAS) substance, meaning it can be used safely on food products.

Several studies have addressed the effect of HPCD treatment on the physical-chemical parameters of fruit juices and beverages (Gui et al., 2005; Ferrentino et al., 2009). Zhou et al. (2009) treated carrot juice with HPCD showing that the browning degree and pH of the treated juice decreased, the cloud and the titratable acidity increased, polyphenol oxidase was inactivated, and that the soluble solids and carotenoids were stable. Gasperi et al. (2009) also tested the efficiency of the process on fresh apple juice. They confirmed that CO_2 was able to inactivate microorganisms naturally present in the juice with a 10 min treatment at 10 MPa and 36°C. The process did not change the composition of the juice in terms of sugars, amino acid content acidity, and polyphenol concentration. However, analysis performed with Solid Phase Microextraction Gas Chromatography - Mass Spectrometry (SPME GC-MS) and Proton Transfer Reaction - Mass Spectrometry (PTR-MS) indicated that the treatment induced a reduction in the concentration of many volatile compounds (esters and aldehydes) responsible for the observed changes in the odor and flavor of the treated juice. Damar et al. (2009) applied the HPCD process to coconut water for the first time, using a continuous system with the objective to evaluate the microbial inactivation, physical-chemical characteristics, and the consumer acceptability of the coconut water after the treatment. In this study, the experiments were carried out on an acidified (with the addition of malic acid to lower the pH to 4.20), sweetened (with the addition of a chemically modified form of sucrose in concentration of 0.7% w/w, °Brix equal to 6.0) and carbonated (with the addition of CO_2 at 4°C and 0.18 MPa) coconut water. The results were promising, with a decrease of total aerobic bacteria by more than 5 Log(CFU/ml), (logarithm of colony forming unit per ml of sample) and with a good product likeability, despite the pH decrease and the sugar increase.

In this context, the present study aimed to investigate the possibility of applying HPCD treatment to the pasteurization of natural coconut water in order to guarantee both its microbial stability and the

retention of its quality attributes, without the addition of acidifiers or sweeteners. The feasibility of the process was determined by the inactivation of the natural microbial flora (mesophilic microorganisms, lactic acid bacteria, total coliforms, yeasts and molds) as a function of pressure, temperature and time.

The impact of the process on the quality traits of the coconut water was also verified through a deep physical-chemical and nutritional characterization (pH, soluble solids, mineral salts, sugars, vitamins, amino acids, and volatile compounds) in order to investigate the effects of the treatment on the composition, paying particular attention to the compounds with nutritional importance or sensory impact. Despite sensory quality is one of the key factors for consumer acceptance, only a few papers considered this aspect for coconut water (Assa et al., 2013). These studies investigated the acceptability of coconut water treated with different processes, but basic sensory analysis principles were not rigorously respected (Damar et al., 2006; Silva do Amaral et al., 2012). In the present study, a descriptive sensory analysis performed by a trained panel was carried out with the aim to verify whether HPCD treatment induced potentially perceptible sensory modifications, compared to fresh untreated (FU) and heat pasteurized (HP) coconut water.

2. MATERIALS AND METHODS

2.1 Coconut water

2.1.1 Extraction and filtration

Seventy young green coconuts (*Cocos nucifera cv Nam Hom*) from Thailand were bought and sent to Trento where they were aseptically opened, the water extracted and accumulated in a 20 liter plastic pail and placed in an ice bath. A flow of inert gas (Argon) was used to create an oxygen free atmosphere above the collected coconut water to avoid any possible oxidation. After the extraction process, coconut water was homogenized, portioned in sterilized glass jars of 200 or 400 ml and immediately frozen at -20°C to prevent any microbial or enzymatic activity. All samples for further trials were prepared by thawing the coconut water glass jars at 4°C for 12 h.

2.1.2 Coconut water contamination for HPCD and HP

The initial microbial load of coconut water was measured after the extraction process. The product showed 4 Log(CFU/ml) of mesophilic microorganisms and 2 Log (CFU/ml) of lactic acid bacteria, total coliforms and yeasts and molds, by standard plate count. To evaluate the effect of the processes, the coconut water was aged at 30°C for 18 h to increase the initial microbial load. The resulting microbial load was about 8.5 Log(CFU/ml) of mesophilic microorganisms, lactic acid bacteria, and total coliforms and about 6 Log(CFU/ml) of yeasts and molds.

2.2 Set up of the optimal stabilization conditions

2.2.1 HPCD treatment

HPCD treatments were carried out in a multi-batch apparatus. The system consisted of 10 identical reactors with an internal volume of 15 ml connected in parallel, so that each experimental run provided a set of experimental data taken in identical process conditions but at different treatment times. Each reactor was connected to an on-off valve that could be used to depressurize it

independently from the others. The 10 reactors were submerged in a single temperature-controlled water bath. Liquid CO_2 (Messer, Carbon dioxide 4.0, purity 99.990%) was fed into the reactors by a volumetric pump (Lewa, mod. LCD1/M910s) that increased the pressure to the desired processing levels, at a rate of about 6 MPa/min. The apparatus was provided with a transducer (Hendress+Houser GmbH, Maulburg, Germany) to control the pressure values while one cover lid of the 10 reactors was equipped with a fixed thermocouple (Pt 100 Ω) to measure the product temperature. At the end of the process, two micrometric valves and one on-off valve were used to depressurize and release CO_2 from the apparatus over approximately 1 min. The processed samples were collected in sterile containers and cooled down immediately at 4°C until further use.

The operating parameters (temperature, pressure and time) were continuously recorded by a real time data acquisition system (National Instruments, field point FP-1000 RS 232/RS 485) and monitored by a custom program (LabView™ 5.0). The process conditions tested were: 8 and 12 MPa; 22, 30, 35, 40 and 45°C; with treatment times ranging from 5 to 60 min, and were chosen based on previous findings (Spilimbergo and Ciola, 2010a). For the qualitative characterizations, the same process conditions were applied to a larger volume (about 100 ml) of coconut water based on previous findings (Gasperi et al., 2009).

2.2.2 Heat treatment

Heat pasteurization (HP) equipment consisted of a water bath (Dubnoff Bath-BSD/D, International PBI, Milano, Italy) with an agitated platform where 200 ml jars of coconut water where placed. The pasteurization of the coconut water was performed at 72°C for 5 and 10 min and 90°C for 1, 3, 5, 10 and 20 min. The process conditions were chosen based on literature findings (Reddy, 1995). After the treatments, the samples were cooled down in a water bath placed in a refrigerated chamber, in order to speed up the cooling process and decrease the time they were exposed to high

temperatures, trying to minimize the temperature's impact on their quality attributes. Subsequently the samples were stored at 4°C until microbial analysis was performed.

2.2.3 Microbial analysis

The microbial analyses were performed using the plate count method. The sample was serially diluted in a phosphate buffer solution (PBS, 0.01 M, pH 7.4) and plated in duplicate onto selective media. Mesophilic microorganisms, total coliforms, yeasts and molds, and lactic acid bacteria were plated onto Plate Count Agar (Liofilchem, TE, Italy), Chromatic Coli/coliform Agar (Liofilchem, TE, Italy), Malt Extract Agar (Liofilchem, TE, Italy), and MRS Agar (de Man, Rogosa and Sharpe, Oxoid, MI, Italy), respectively. The incubation temperatures and times were: 30 °C for 48 h for mesophilic microorganisms; 30 °C for 24 h for total coliforms; 25 °C for 4 d for yeasts and molds; and 35 °C for 48 h for lactic acid bacteria. At the end of the incubation periods, the number of colonies was counted and the inactivation level was determined by evaluating the Log(CFU/ml) of the microorganisms before and after the treatments. The results were means based on data from at least three experimental runs. Standard deviations were shown by error bars.

2.3 Quality control

The physical-chemical and sensory analyses were performed on UF, HPCD and HP coconut water samples stored at –20 °C. The day of the analysis, the samples were thawed at 4 °C (overnight) and processed without incubation. Microbiological analyses were performed to verify the inactivation of the natural microbial flora to undetectable levels.

2.3.1 Physical-chemical analysis

The color of the coconut water was determined using a portable colorimeter (Chroma Meter CR400, Minolta, Japan) measuring the *L*a*b** color space (CIE, 1986) in reflectance mode. The

colorimeter was calibrated using the illuminant D65, and measurements were made through a 10-mm optical path. Chroma (C) and hue angle (h°) were calculated from numerical values of L^* (lightness), a^* (redness) and b^* (yellowness) according to Eqs. (1) and (2), respectively:

$$C = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$h° = tan^{-1}(b^*/a^*) \quad (2)$$

Total color difference (ΔE^*) was calculated between FU (reference), HPCD and HP coconut water, respectively, using the Eq. (3):

$$\Delta E^* = \sqrt{(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)} \quad (3)$$

The pH of the samples was measured with a digital pH meter (Inolab pH level 1, WTW GmbH, Weilheim). Water content was determined by weight loss during heating at 70 °C for 96 h and dry matter by the resulting weight difference. Soluble solids, expressed as °Brix, were measured using a digital portable refractometer (DBR95, Singapore).

Sugars (sucrose, glucose, and fructose) were quantified by High performance liquid chromatography (HPLC) coupled to a refractive index detector (Agilent 1200) using a Rezex RCM-Monosaccharide Ca^{2+} (8%) column (300 mm × 7.8 mm) supplied by Phenomenex (Castel Maggiore, Bologna, Italy). Minerals (Mg, K, Ca, Fe, Na) were measured by inductively coupled plasma optical emission spectrometry (Optima 8300, Perkin Elmer) after sample mineralization by concentrated HNO_3 at 100 °C for 4 h. Water-soluble vitamins were analyzed by High-performance liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD-MS/MS) (Acquity/Xevo TQ; Waters) using a Acquity UPLC HSST3 (1.8µm; 2.1x10mm) column. Free

aminoacids were measured after derivatization with *o*-phthalaldehyde by ultra-high performance liquid chromatography with postcolumn fluorescence derivatization (UHPLC-FLD) (UltiMate™ 3000 UHPLC system, Thermo Scientific).

2.3.1.1 Volatile compounds

A 5 ml aliquot of coconut water was transferred to a 20 ml vial, and 2 g of NaCl were added together with 100 µl of an internal standard (2-octanol, 0.25 mg/l). The vial was crimp-closed with a Teflon-lined silicacap (Supelco) and equilibrated at 50°C for 10 min under constant stirring. Solid Phase Microextraction fiber (HS-SPME, DBV/CAR/PDMS, Supelco, Bellefonte, PA, USA) was exposed for 60 min in the vial headspace. The compounds adsorbed by HS-SPME were analysed on a GC interfaced with a mass detector which operates in electronionisation mode (EI, internal ionisation source; 70 eV) with a scan range from m/z 35 to 300 (GC Clarus 500, PerkinElmer, Norwalk CT,USA). Separation was achieved on a HP-Innowax fused-silica capillarycolumn (30 m, 0.32 mm ID, 0.5 µm film thickness; Agilent Technologies, Palo Alto, CA, USA). The GC oven temperature programme started at 40°C and arrived to 150°C at 2°C min^{-1}, and then increased at 6°C min^{-1} to 250 °C, which was maintained for 15 min. Helium was used as the carrier gas with a constant column flow rate of 1.5 ml min^{-1}. Samples were analysed in triplicate. Semi-quantitative data were expressed as µg/l equivalent of internal standard. Compound identification was based on mass spectra matching using the NIST-98/Wiley library and linear retention indices (LRI) compared with literature. LRI were determined after the injection of a C7–C30 n-alkane series (Supelco) under the same chromatographic conditions.

2.3.2 Sensory analysis

A comparative descriptive flash profiling (Delarue and Sifferman, 2004) was applied by a panel of 16 trained judges to establish how the different stabilization treatments affected sensory properties.

A vocabulary list was developed by consensus between the judges consisting of 14 mandatory descriptors chosen to emphasize the differences among the products: 2 tastes (sweetness, acidity), 6 odors (butter, cardboard, cooked, floral, hazelnut, toasted bread) and the relative 6 flavours (i.e. odor sensation by retro-olfactive evaluation). During the analysis, the panelists had the possibility to add other descriptors to their descriptor list. To avoid any expectation effect due to the sight sense, no evaluation on sample color was performed and samples were presented under red light conditions. The intensity of each descriptor was rated using a linear scale anchored to 0 (minimum intensity) and 100 (maximum intensity). The samples were presented to judges in the same quantities and at the same temperature, in 80 ml plastic glasses, closed with a lid, coded with random numbers in a balanced order following a latin square design and analyzed in triplicate over three consecutive days.

All tests were conducted in the sensory lab of FEM (Fondazione Edmund Mach, Italy), equipped with 22 booths for individual computer assisted evaluation. The implementation of the test, the recording of the judge responses and the data analysis were performed with FIZZ software 2.46A (Biosystemes, France).

2.3.3 Statistical analysis

Principal Component Analysis (PCA) was used for exploring VOC data by Simca P+ v.12 (Umetrics, Sweden). A three-way ANOVA, using the product and replicate as fixed factors and the judge as a random factor, was used to evaluate the performance of the descriptive panel and to identify the sensory attributes to discriminate the products (Statistica v.9, Statsoft Italia srl).

3. RESULTS AND DISCUSSION

3.1 Microbial inactivation kinetics

The inactivation kinetics of the natural microbial flora of coconut water as a function of temperature and time are reported in Figures 1-4 for each microbial strain and two treatment pressures, 8 MPa (a) and 12 MPa (b). The effect of the process parameters on the inactivation kinetics was evident. The results indicated that the increase of temperature induced a higher microbial reduction both at 8 and at 12 MPa. For mesophilic microorganisms, at 8 MPa about 4 Log reductions were achieved in 30 min at 45°C while 60 min were needed at 35°C (Figure 1(a)). A similar behavior was also observed for the other microbial strains (Figures 1-4 (a) and (b)).

In addition to the impact that thermal treatment itself had on the inactivation of microorganisms, the effect of the increase of temperature on microbial inactivation kinetics was closely related to CO_2 mass transfer properties. More specifically, higher temperatures stimulated the diffusion of CO_2 and increased the fluidity of cell membranes to facilitate CO_2 penetration, causing several metabolic alterations that were responsible for cellular death (Lin et al., 1993; Erkmen, 2000; Garcia-Gonzalez et al., 2007). However, some studies demonstrated that this behavior was not always observed, because CO_2 solubility decreased as temperature increased limiting the amount dissolved in the liquid phase, thus the amount in contact with the microorganisms (Dodds et al., 1956). Hong and Pyun (1999) observed that under a constant pressure of 6.8 MPa, microbial inactivation of *Lactobacillus plantarum* increased by 1 Log as temperature decreased from 40°C to 30°C.

The increase of pressure from 8 to 12 MPa enhanced microbial inactivation. However, the effect was less evident than the one induced by the increase of temperature and the results were highly dependent on the resistance of the microbial strains to HPCD. For mesophilic microorganisms at a fixed temperature of 45°C (Figure 1 (a) and (b)), a treatment time of 45 min was needed to achieve inactivation to undetectable levels at both 8 and 12 MPa. A different behavior was observed for total coliform inactivation kinetics; at a fixed temperature of 45°C, an increase of pressure from 8 to

12 MPa resulted in the reduction of the treatment time from 45 to 30 min to achieve inactivation to undetectable levels. Generally, several studies have reported the beneficial effect of pressure during HPCD treatments (Lin et al., 1994; Hong et al., 1997; Damar et al., 2009). It has been demonstrated that pressure controlled both the solubilization rate and the solubility of CO_2. Further, the pressure increase was beneficial on microbial inactivation due to the dramatic increase in density and solvation power of CO_2 that promoted its contact with the cells, inducing the removal of vital constituents from cells or cell membranes. However, this increase was limited by the saturation solubility of CO_2 in the treatment medium. Therefore, once the treatment medium was saturated with CO_2, the bactericide effect of HPCD did not change significantly. This effect was demonstrated by Sims and Estigarribia (2002). They showed that 7.5 MPa was nearly as effective as 15 MPa, and room temperature was as effective as 31°C in inactivating microbial cells, once the treatment medium was saturated with CO_2. Similarly, The results of the study of Damar et al. (2009) performed on coconut water processed with a continuous HPCD system also showed that pressure, changing from 13.8 to 34.5 MPa, was not significant in microbial reduction whereas temperature and % CO_2 were significant.

The results of our study also showed that microbial kinetics were highly dependent on the type of microorganisms; total coliforms and yeasts and molds (Figures 3 and 4) were inactivated faster than mesophilic and lactic acid bacteria (Figures 1 and 2). Similar considerations were often reported in other research studies although comparisons were difficult to make, because they were performed using different equipment, treatment media, microorganism strains and test conditions. In general, Gram-positives microorganisms were expected to be more resistant than Gram-negatives due to the composition of their thicker cell wall (Dillow et al., 1999; Garcia-Gonzalez et al., 2007).

Based on the results of the inactivation kinetics, the optimal conditions of pressure, temperature and time, determined with the multifactor ANOVA analysis, were: 12 MPa, 40°C, 30 min. At these conditions, HPCD was able to induce 5 Log reductions of mesophilic microorganisms, lactic acid

bacteria, yeasts and molds and about 7 Log reductions of total coliforms, as required by the Food and Drug Administration (FDA).

3.2 Heat pasteurization

Microbial analysis performed on HP coconut water demonstrated that 90°C for 1 min was sufficient to inactivate the natural microbial flora to undetectable levels.

3.3 Quality traits

3.3.1 Physical-chemical and nutritional characterization

Both of the stabilization processes (HPCD and HP) induced a modification of the color (Table 1). Lightness and hue angle values decreased with the treatments while Chroma increased. In other words, treated products were less bright than FU coconut water. These changes were evaluated calculating ΔE* (Eq. 3) resulting equal to 5.1 and 8.1 for HPCD and HP, respectively. An absolute threshold value for human color discrimination has only been determined for few specific products (Martínez et al 2001), nevertheless, $\Delta E > 4$ is usually considered a clearly distinguishable color difference to the average person. Although coconut water was rich in free aminoacids and reducing sugars (as fructose and glucose, commonly present in an acidic environment and thus prone to Maillard Reaction), the temperatures used were not enough to bring the formation of colored melanoidins (Martins et al. 2000). For this reason, we attributed the color change to the destabilization of emulsions and protein precipitation (Tangsuphoom & Coupland 2005, 2008).

HPCD resulted in a product with a pH lower than the FU or the HP ones. A similiar pH decrease has been reported in several studies because of dissolved CO_2 in liquid foods (Kincal et al., 2006; Lim et al., 2006). Both HP and HPCD had no significant effect on dry matter and soluble solid content of coconut water (Table 1) while only HP did not significantly affect the sugar content (Table 2). Minerals (Mg, K, Ca, Fe and Na) were lower in HP while higher in HPCD coconut water.

These differences were attributed to lipids that, even in a small amount, were present in coconut water as emulsions (Campos et al. 1996; Jackson et al., 2004). Variation in pH and heat treatment destabilized lipid emulsion in coconut water causing emulsion coalescence and flocculation, which could interfere with sample mineralization prior metal analysis (Tangsuphoom & Coupland 2005, 2008).

Literature studies published the effect of HPCD on particle size distribution of juices and drinks although the formation of emulsion coalescence and flocculation was not obvious (Park et al., 2002). Zhou et al. (2009) showed an increase of particle size distribution of carrot juice for treatment times lower than 45 min, possibly due to acid induced protein coagulation caused by pH shift characteristic of proteins (Reiter et al., 2003). In the same study, the increase of the treatment time to 60 min decreased particle size distribution of carrot juice probably due to HPCD induced homogenization effect that caused smaller juice particle (Arreola et al., 1991; Kincal et al., 2006). They concluded that the longer the treatment time or the higher the pressure of the process was, the more intensive the homogenization effect was and that the alteration of the particle size for HPCD treated juices was an interaction between the acid induced protein coagulation and the HPCD induced homogenization effect.

Only three water-soluble vitamins (B1, B2, B5) were found in the fresh coconut water (Table 2). HP brought to a slight decrease of all three vitamins, as expected, while HPCD slightly reduced only vitamin B2 showing a lower impact on the nutritional values of the final product.

FU coconut water was rich in free aminoacids (Table 2). Alanine, γ-aminobutyric acid, glutamic acid, glutamine, serine and sum of arginine+cysteine were the most abundant free amino acids found. Both HPCD and HP induced a slight increase of the free aminoacids, probably due to proteins hydrolysis or the hydrolysis of bonded free amino acids (Yong et al., 2009).

3.3.2 Volatile compounds

The three products were clearly distinguishable based on their volatile compound content. In Table 3, the 38 most abundant chromatographic peaks are reported. In total 14 alcohols, 7 aldehydes, 3 ketons, 3 lactones, 4 esters, 5 acids, 1 monoterpene, 1 pyrrole and 1,1-diethoxyethane were identified. HPCD depleted most of the volatile compounds in the headspace of coconut water due to its extractive power during the decompression of the system, similar to what has previously observed on HPCD treated apple juice (Gasperi et al., 2009). Only alcohols, more polar compounds, were slightly higher compared to the FU. The HP caused a considerable increase in 2-Acetyl-1-Pyrroline (6x) and ketones (3x) and a slight increase in alcohols (1.4x), esters (1.5x) and aldehydes (1.4x). Only the total concentration of acids and the α-terpineol remained the same. Among the aldehydes, 3,4-dimethyl benzaldehyde (the main benzaldehyde derivative found in fresh coconut water headspace) was strongly depleted (0.1x) after the HP process and when compared to the FU product. To better understand the effect of the treatments on the volatile profiles, Figure 5 was drawn reporting the score-plot of the first 2 components of the PCA data were the 3 theses are clearly separated.

3.3.3 Sensory evaluations

The sensory data were checked to verify the ability of judges to detect differences between samples and to repeat their evaluations. Statistical analysis showed both high discriminant capacity and reproducibility. Seven attributes, among those used by the panel, discriminated between samples (attributes with asterisks in Figure 6) and the replication factor was only significant for the sweet taste attribute. Figure 6 shows the averaged profiles of each product in comparison with the others; HPCD and FU products were very similar for all the sensory attributes, except for “Cardboard” flavor that was less present in HPCD samples. The HP product seemed to be characterized by more intense odors and flavors of “Hazelnut” and “Toasted bread” (induced by aldehydes and ketones that were higher in the HP product). These odors and flavors were probably responsible for the

higher perceived sweetness observed thanks to the cross-modal interaction between aroma and taste although no differences were detected in the sugar contents (see Table 1). This observation was also compatible with the increased amount of 2-Acetyl-1-Pyrroline found (Table 3). The 2-Acetyl-1-Pyrroline was found at very low concentration in the headspace of FU and HPCD coconut water and increased significantly in the HP product. This compound has a sensory impact described as "toasted", "popcorn-like", and "malty" (Burdack-Freitag & Schieberle, 2010).

These findings were in accordance with Damar et al. (2009) who asked 50 consumers to evaluate global liking and off-flavor intensity on untreated, carbon dioxide -treated, and heat-treated coconut water. They found that carbon dioxide treated samples were liked similarly to untreated, whereas heat-treated samples were liked significantly less and had a significantly higher off-flavor score in comparison with the other two. Silva do Amaral et al. (2012) also asked to 40 consumers to evaluate their liking focusing on taste, flavor, appearance and color of fresh and pasteurized coconut water, finding that pasteurized samples were always less appreciated for all the attributes except for flavor.

3.3.4 Conclusions

This study indicated the feasibility of HPCD treatment as low temperature pasteurization of fresh coconut water. The ideal process conditions were determined to be 12 MPa, 40°C, 30 min and induced about 5 Log reductions of mesophilic microorganisms, lactic acid bacteria, yeasts and molds and about 7 Log reductions of total coliforms. Few differences in the physical-chemical and nutritional attributes were induced by HPCD treatment. No differences were observed in dry matter, soluble solids, sugars and vitamins content although a reduction of its volatile fraction was detected. Nevertheless, FU and HPCD treated coconut water were both clearly distinguished from the HP one.

Overall, the study demonstrated that HPCD technology represented a promising alternative to thermal processing for microbiologically stabilizing liquid foodstuffs without altering their quality attributes.

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Table 1. Physical-chemical parameters of fresh untreated (FU), high pressure carbon dioxide (HPCD) and heat pasteurized (HP) coconut water

	FU		HPCD		HP	
	mean	**RSD%**	**Mean**	**RSD%**	**mean**	**RSD%**
pH	6.3	<0.1	5.9	0.23	6.4	<0.1
Water (%)	93.5	<0.1	93.4	<0.1	93.3	<0.1
Dry matter (%)	6.5	<0.1	6.6	0.16	6.7	0.5
S.S. (°Brix)	6.8	1.5	6.9	0.8	7	0.8
Color:						
L*	99.59	<0.1	98.82	<0.1	98.35	<0.1
a*	0.003	17.3	0.07	1.4	0.11	1.4
b*	0.52	<0.1	1.02	0.1	1.36	<0.1
C*	0.52	0.2	1.03	0.2	1.36	0.1
h°	89.87	0.1	86.11	0.1	85.37	0.1

Table 1. Nutritional composition of fresh untreated (FU), high pressure carbon dioxide (HPCD) and heat pasteurized (HP) coconut water

	FU		HPCD		HP	
	mean	**RSD%**	**mean**	**RSD%**	**mean**	**RSD%**
Sugars (g/l)						
glucose	21.4	<3.0	21.5	<3.0	21.7	<3.0
fructose	19.8	<3.0	20.1	<3.0	20.2	<3.0
Sucrose	13.1	<3.0	13.3	<3.0	13.3	<3.0
Minerals (mg/l)						
Mg	155	<0.6	182	<0.6	111	<0.6
K	2197	<0.6	2577	<0.6	1593	<0.6
Ca	145	<0.6	170	<0.6	100	<0.6
Fe	0.12	<0.6	0.17	<0.6	0.07	<0.6
Na	144	<0.6	171	<0.6	104	<0.6
Vitamins (µg/l)						
B1	134.9	<10	136.3	<10	121.4	<10
B2	72.6	<10	67.9	<10	67.1	<10
B5	42.1	<10	42.2	<10	36.3	<10
Aminoacids (mg/l)						
Alanine	476	<10	510	<10	525	<10
Arginine+ Cysteine	216	<10	230	<10	237	<10
Asparagine	43	<10	50	<10	50	<10
Aspartic acid	38	<10	40	<10	44	<10
γ-Aminobutyric acid	218	<10	233	<10	237	<10
Glutamine	141	<10	150	<10	147	<10
Glutamic acid	113	<10	122	<10	123	<10
Glycine	32	<10	33	<10	34	<10
Histidine	16	<10	19	<10	19	<10

Isoleucine	37	<10	40	<10	41	<10
Leucine	58	<10	62	<10	71	<10
Lysine	64	<10	70	<10	70	<10
Methionine	14	<10	16	<10	16	<10
Ornithine	7	<10	8	<10	9	<10
Phenylalanine	27	<10	30	<10	39	<10
Serine	114	<10	125	<10	130	<10
Threonine	48	<10	51	<10	53	<10
Tryptophan	11	<10	9	<10	11	<10
Tyrosine	23	<10	24	<10	26	<10
Valine	71	<10	76	<10	95	<10

Table 3. Volatile compounds in the headspace of fresh untreated (FU), high pressure carbon dioxide (HPCD) and heat pasteurized (HP) coconut water.

Compound	**LRI**	**LRI[1]**	**FU**		**HPCD**		**HP**	
			mean	**RSD%**	**mean**	**RSD%**	**mean**	**RSD%**
1-Butanol	1159		438	2.3	446	1.4	431	3.0
2-Methyl-1-Butanol	1219	1216	798	2.3	502	3.2	739	3.5
3-Methyl-1-Butanol	1219	1216	1445	3.1	931	2.7	1293	4.4
3-Methyl-2-Buten-1-ol	1329	1337	214	0.5	144	3.1	198	2.0
Hexanol	1360	1370	2439	0.7	1423	1.0	2160	4.6
1-octen-3-ol	1457	1471	287	3.4	159	2.3	305	6.1
1-Heptanol	1461	1478	746	3.4	439	1.3	766	1.9
2-ethyl-1-hexanol	1496	1513	3199	4.7	3074	1.3	1652	1.4
1-Octanol	1563	1586	1813	2.0	8382	3.9	7792	2.1
1-Nonanol	1664		457	3.3	321	6.1	445	2.0
(Z)-3-Nonen-1-ol	1686	1694	253	4.3	149	1.3	228	3.1
1-Decanol	1766	1815	1303	8.2	2471	6.9	4127	3.1
2-phenyl ethanol	1905		646	3.5	476	4.1	674	1.8
total alcohols			14038		18917		20810	
Ethanal	708	721	4571	1.7	2587	1.5	4228	2.5
2-Butenal	1052	1035	813	3.0	1140	1.4	2815	2.0
Hexanal	1100	1093	611	2.7	294	3.8	534	5.3
Heptanal	1197		206	5.9	98	13.6	238	6.3
Nonanal	1396		1157	4.3	371	4.0	1170	11.1
Benzaldehyde	1519	1538	11680	8.2	11688	13.4	14511	1.4
3,4-Dimethyl Benzaldehyde	1798	1790*	32160	5.6	28852	3.3	3437	10.2
total aldehydes			19038		45030		26933	

3-Penten-2-one	1139	1148	109	11.2	655	2.8	9688	2.3
3-Hydroxy-2-Butanone	1291	1292	1282	2.6	1230	0.4	1289	1.0
Acetophenone	1643		300	6.2	260	3.7	632	0.7
δ-Octalactone	1952	2038	1846	4.0	1211	1.5	1808	1.3
δ-nonalactone	2188	2110	1190	3.9	793	0.7	1140	1.5
δ-decalactone	2311	2288	127	6.7	84	2.7	105	5.5
total ketones			4854		4232		14661	
Ethyl hexanoate	1243	1226	407	1.9	102	8.9	457	1.2
Ethyl octanoate	1439	1448	1262	6.9	409	2.8	1871	3.1
Ethyl decanoate	1641	1647	1508	20.7	614	8.6	4718	7.0
Ethyl dodecanoate	1844		2580	7.8	664	3.0	1630	13.6
total esters			5758		1789		8677	
Hexanoic Acid	2125	1891	2244	1.9	2313	3.2	2394	0.8
Octanoic acid	2238	2218	72480	4.1	62163	1.3	78621	2.6
Nonanoic acid	2319	2259	491	2.3	1086	6.9	913	1.0
Decanoic acid	2329	2375	17431	4.5	13825	2.1	15951	7.1
Dodecanoic Acid	2360		17571	4.9	10495	5.4	14187	2.2
total acids			110218		89880		112066	
2-Acetyl-1-Pyrroline	1339		379	6.8	398	1.5	2273	2.4
α-Terpineol	1694	1731	662	1.5	459	10.6	660	1.9
1,1-Diethoxyethane	901		1940	6.1	1347	2.3	1997	7.4

Data are expressed as µg/l of 2 octanol; LRI: calculated linear retention; LRI^{l}: linear retention indexes from literature (from Prades et al., 2012 but for* from Chung, 1999)

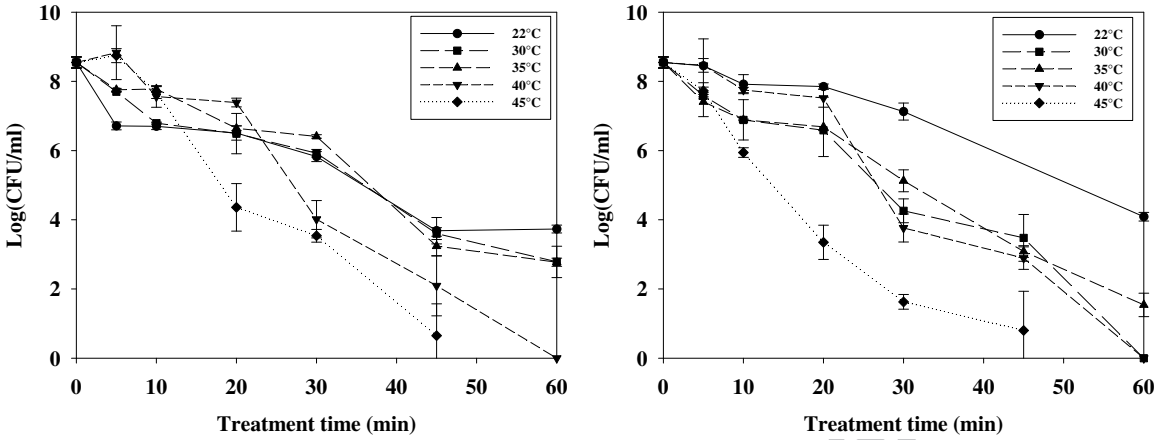

Figure 1. Mesophilic microorganisms inactivation kinetics as function of temperature and treatment time at (a) 8 and (b) 12 MPa.

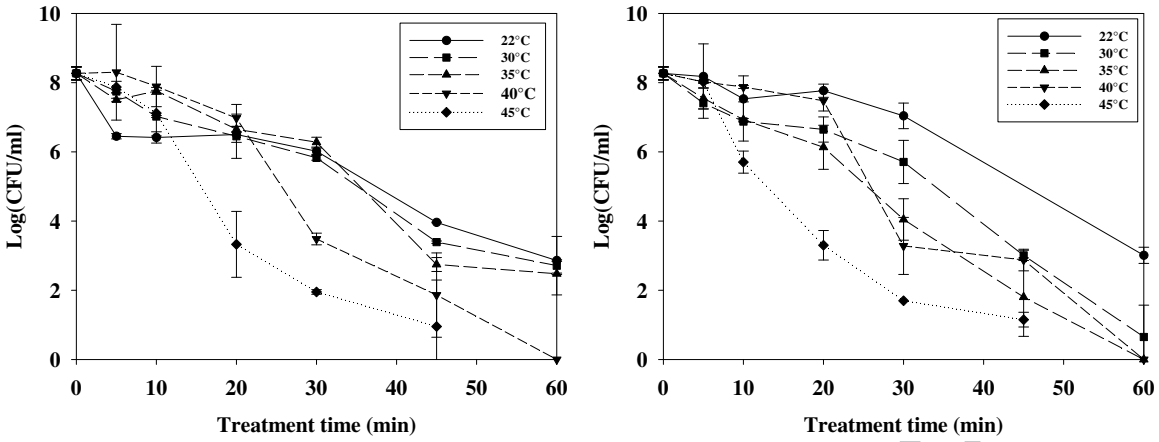

Figure 2. Lactic acid bacteria inactivation kinetics as function of temperature and treatment time at (a) 8 and (b) 12 MPa.

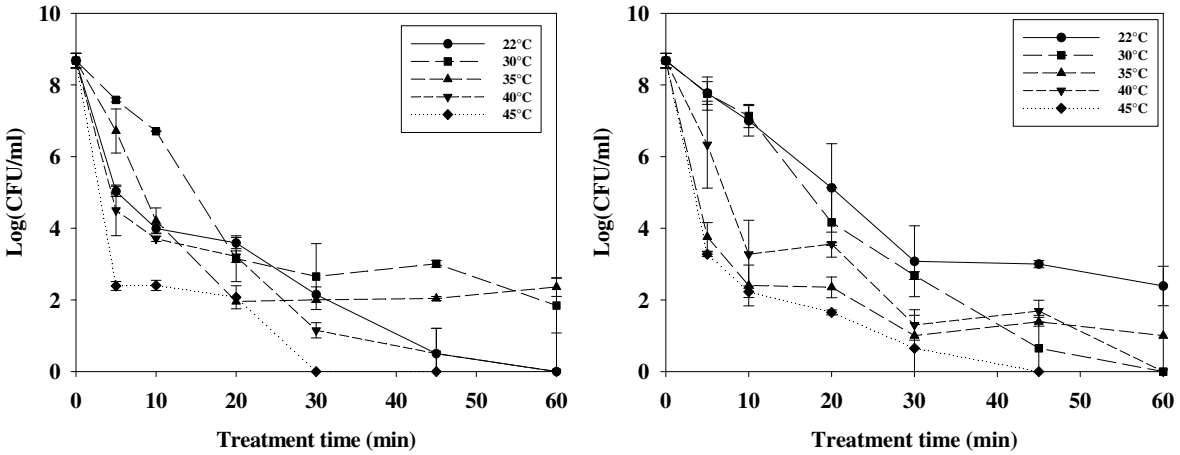

Figure 3. Total coliforms inactivation kinetics as a function of temperature and treatment time at (a) 8 and (b) 12 MPa.

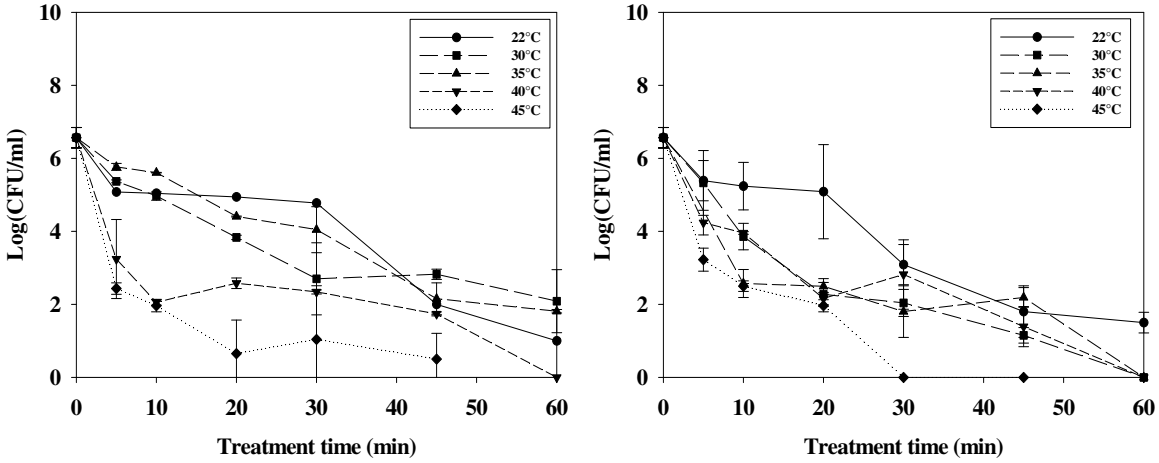

Figure 4. Yeasts and molds inactivation kinetics as function of temperature and treatment time at (a) 8 and (b) 12 MPa.

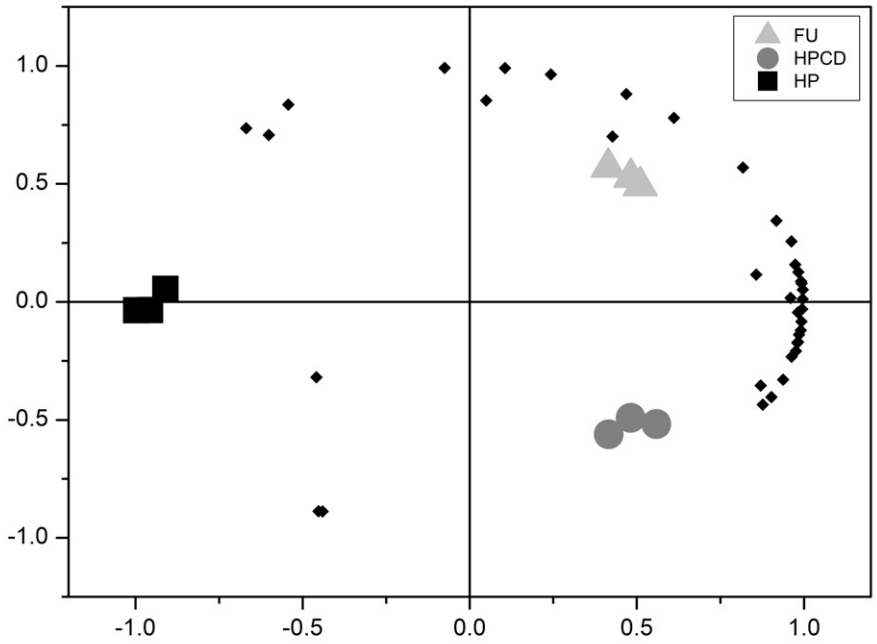

Figure 5. Plot of the first 2 components of the PCA explaining 93% of sample variance using the volatile compounds.

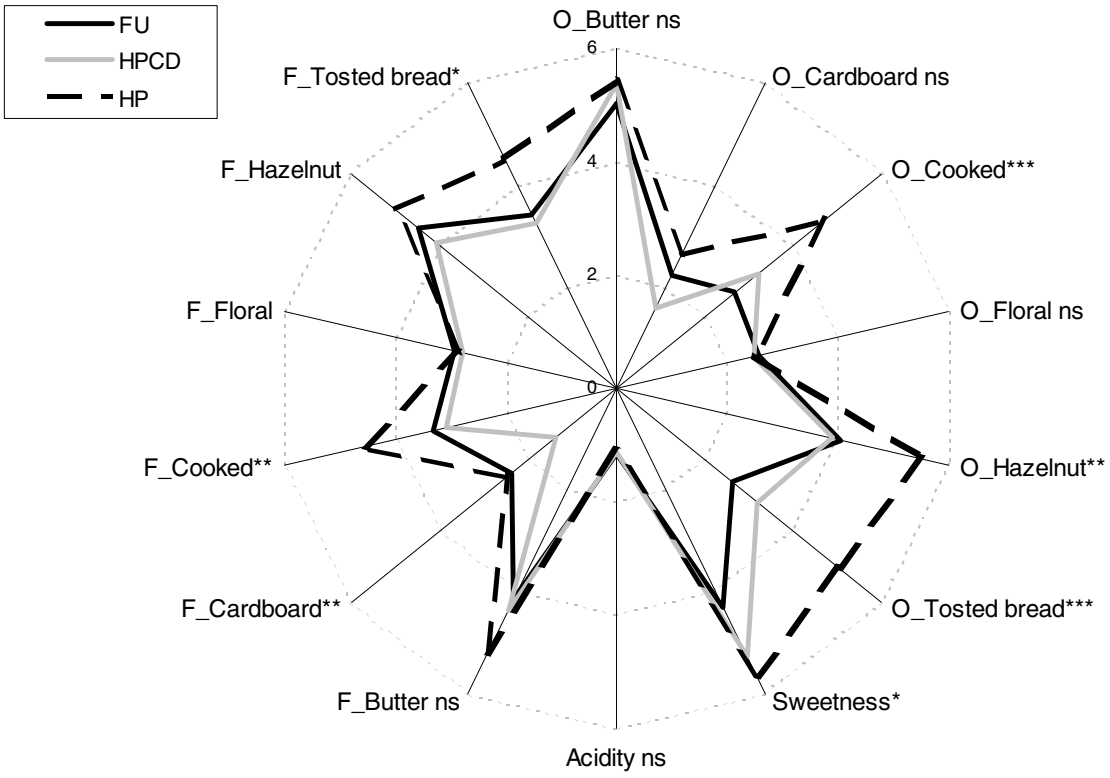

Figure 6. Spider plot of the average profile of the three coconut water samples (FU: fresh untreated, HPCD: high pressure carbon dioxide, HP: heat pasteurized). For each sensory attribute the name (O: odor; F: flavor) and its significance are reported (ns: not significant at 5%; *: 5%; **: 1%; ***: 0.1%).

HIGHLIGHTS

- HPCD treatment guarantees the inactivation of natural microbial flora of coconut water
- HPCD preserves better than thermal processing the nutritional and sensory qualities of coconut water
- HPCD can contribute to commercialize coconut water as a highly natural energy-drink