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A Novel Functional Fruit/Vegetable Beverage for the Elderly: Development and Evaluation of Different Preservation Processes on Functional and Enriched Components and Microorganisms

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Abstract

Despite lower energy intakes with age, elderly have higher requirements for several nutrients, making them vulnerable to deficiencies that further aggravate aging chronic conditions. To manage this problem, new food formulations are needed to address the nutritional needs of the worldwide growing aging population. In the present work, we develop an innovative functional fruit/vegetable beverage for the elderly using coconut water, orange juice and carrot juice. The beverage was enriched with vitamin A and C and supplemented with omega-3 fatty acids and inulin to provide important nutritional elements to the target consumers of this study. Osmolality classified the beverage as hypertonic. Heat (TT), supercritical carbon dioxide (SC-CO_2) and dimethyl dicarbonate (DMDC) treatments were applied as preservation methods, and the responses of the functional and enriched components and enzymes were evaluated. *Alicyclobacillus acidoterrestris* ATCC 49025 and a surrogate *Escherichia coli*, ATCC 25922, were inoculated in the beverage to induce spoilage and verify the effectiveness of inactivation processes on the cells. The DMDC process reduced *E. coli* to undetectable levels and exhibited a greater reduction of *A. acidoterrestris* vegetative cells, molds and yeasts. The conditions applied in the process with SC-CO_2 did not show satisfactory results in reducing juice microbiota. Loss of vitamin C and A was mainly associated with the SC-CO_2 treatment. The beverage received a positive acceptability evaluation among the tasters in the sensory analysis and may be introduced as a new vehicle for the consumption of functional compounds, especially by vegans and/or vegetarians and lactose-intolerant elderly.

Keywords: coconut water, dimethyl dicarbonate, microbiological challenge testing, non-dairy beverage, orange juice, supercritical carbon dioxide, carrot juice

1. Introduction

Consumers are looking for nutrition that goes beyond the status quo, either in terms of extra nutrition or condition-specific benefits. They desire ready-to-drink (RTD) beverages with “better for you” ingredients and simple “clean” labels, and they seek products with functional benefits and great taste. Beverage companies are meeting new consumer demands by introducing group-specific (*e.g.,* gluten-free, muscle recovery, bone health) beverages. Functional beverages can be specifically targeted to particular sectors of the population, focusing, *e.g.*, on women or the elderly. The increase in the elderly population is a general global trend. The leading worldwide cause of death among the elderly is associated with chronic disease, and there is great potential for the prevention of these diseases through diet (United States Department of Health and Human Services and World

Health Organization, 2011). Fruit/vegetable-based beverages represent an easy and convenient way of consuming important sources of compounds such as vitamins, phenolics and fibers, so necessary for this group of population.

In spite of the many nutritional properties and bioactive compounds described for coconut water (Yong, Ge, Ng, & Tan, 2009), orange juice (Agvan, Akyildiz, & Akdemir Evremdilek, 2014) and carrot juice (Corona et al., 2016), there are no reports on the state-of-the-art technological development of an orange/carrot functional beverage based on coconut water. Recently, Camargo Prado et al. (2015) reported a closely related topic on the development of an innovative functional beverage using coconut water as the main ingredient and cited its natural hydrating qualities, functional health properties and nutritional benefits.

To commercialize an RTD fruit/vegetable beverage, appropriate preservation process and subsequent storage methods are necessary to guarantee the stability of the nutritional and functional compounds, as fruits and vegetables are susceptible to oxidative, enzymatic or microbial spoilage. Heat treatments are the most widely used preservation method for coconut water (Tan, Cheng, Bhat, Rusul, & Easa, 2014) and fruit juices (Bilek & Bayran, 2015), as high temperatures lead to inactivation of microbial and enzymatic activities. However, excessively high process temperatures lead to loss of nutritional and healthy compounds as well as the sensory properties of food (Cappelletti et al., 2015).

Non-thermal food processing technologies for preservation and safety have gained widespread acceptance throughout the food industry (Rawson et al., 2011) but still need to be evaluated for effectiveness in different matrices that have not been wet-tested. Among these technologies, particular attention has been given to the use of antimicrobials such as dimethyl dicarbonate (DMDC) and supercritical carbon dioxide (SC-CO_2). DMDC, a dicarbonic acid ester approved by U.S. FDA (United States Food and Drug Administration, 2001a), is a microbial control agent inhibitor of yeasts used in beverages at a maximum limit of 250 mg/L. Numerous studies have demonstrated that DMDC treatment efficiently causes lethality in many pathogenic microorganisms in fruit juices and has few effects on sensory properties and nutritional value (Yu et al., 2013). High-pressure carbon dioxide (HPCD) process that applies SC-CO_2 is being used to inactivate enzymes and pathogenic microorganisms, resulting in minimal degradation of thermo-labile nutrients and bioactive compounds of foods and preserving sensory and nutritional characteristics (Zhou, Wang, Hu, Wu, & Liao, 2009). Many studies have demonstrated that HPCD treatment at moderate pressure and temperature can effectively inactivate microorganisms in foods (Damar & Balaban, 2006).

Microbiological challenge testing continues to be a useful tool for the validation of processes that are intended to deliver some degree of lethality against a target food microorganism. *Alicyclobacillus acidoterrestris* is an acidothermophilic spore-forming bacterium of great concern to the fruit beverage industry that cause spoilage by producing taint compounds responsible for off-flavors (*e.g.*, 2-methoxyphenol “guaiacol”) (Molva & Baysal, 2015). Thermal resistance studies have demonstrated the ability of this microorganism to survive pasteurization applied to acidic fruit juices (Oteiza, Soto, Alvarenga, Sant’Ana, & Gianuzzi, 2015). Additionally, pathogens from known foodborne outbreaks should be included in challenge tests to ensure that the formulation and process is sufficiently robust to inhibit those organisms. Pathogenic strains of *Escherichia coli* were involved in outbreaks linked to the consumption of contaminated unpasteurized fruit juices (Besser et al., 1993). Outbreaks have indicated that low pH (< 4.0) of fruit beverages cannot safeguard against the survival of *E. coli* O157:H7 (Leyer, Wang, & Johnson, 1995). Thus, an ideal surrogate *E. coli* strain applied in the challenge must have, for example, similar thermal and acidic inactivation kinetics as pathogenic *E. coli* strains (U.S. FDA, 2001b).

In this context, the present study was performed to develop an innovative functional fruit/vegetable beverage using coconut water as the main ingredient. Preservation processes were tested in order to correlate losses in nutritional ingredients with the treatments. The beverage was inoculated with *A. acidoterrestris* and nonpathogenic *E. coli* to induce spoilage and to verify the effectiveness of inactivation processes on the cells using microbial challenge testing criteria. Although the formulation of juices has been widely explored, the developed beverage can be introduced as a new nutritional vehicle for the consumption of functional and enriched components, especially by vegans and/or vegetarians and lactose-intolerant elderly.

2. Method

The fruit/vegetable beverage was produced for this study to evaluate the relationship between its chemical, physicochemical, functional composition and its sensory acceptance after three different preservation processing technologies. To evaluate the effectiveness of the treatments, the beverage was microbiologically challenged.

2.1 Extraction of Raw Materials and Beverage Formulation

Green coconuts (*Cocos nucifera* L. var. *nana*) between 6 and 8 months of age and oranges (*Citrus x sinensis* var. *bahia*) and carrots (*Daucus carota* subsp. *sativus*) at commercial maturity were purchased at the local market in Florianópolis (SC, Brazil). The coconut water, orange juice and carrot juice used in this work were extracted after the fruits were brushed and washed with water containing 100 ppm active chlorine. The coconut water and fresh-squeezed juices were homogenized, filtered under vacuum using filter paper (Whatman, UK) and maintained at -20 °C to prevent any microbial or enzymatic activity.

The fruit/vegetable beverage formulation was previously developed after pre-trials using not structured sensory tests (data not shown), and production was carried out at the Federal University of Santa Catarina (UFSC), Food Science and Technology Department Pilot Plant. The formulation was composed of 48% (w/v) coconut water, 40% (w/v) orange juice and 10% (w/v) carrot juice. Enriched and functional ingredients (vitamins A and C, omega-3 fatty acids and inulin) were added in the amount necessary to achieve, in the final formulation, 100% of the RDI (Recommended Daily Intake for adults) in Brazil (Brasil, 2005; Brasil, 2008). In total, forty-five mg of vitamin C as ascorbic acid (SweetMix, São Paulo, Brazil), 600 µg of vitamin A as retinyl acetate (M. Cassab, São Paulo, Brazil), 1.5 g of an omega-3 mixture from natural fish oil concentrate with EPA (10%) and DHA (8%) (Vana-Sana EPA/DHA 10/8 ES, FrieslandCampina Kievit, Meppel, the Netherlands) and 1.5 g of inulin as soluble fiber from the chicory root (Cargill, São Paulo, Brazil) were added to compose a dose. After weighing and mixing the components in a tank under agitation (600 rpm) for 10 min, the pH of the beverage was adjusted to 3.50 (HI9321 pH meter, Hanna Instruments, São Paulo, Brazil) with citric acid (Cargill, São Paulo, Brazil), and total soluble solid content was determined directly using a digital refractometer (PR-101, ATAGO, Tokyo, Japan) at 25 °C, resulting in 8.30 °brix. The beverage was degassed under vacuum (-0.65 mbar) and bottled in 250-ml glass bottles previously sanitized by immersion in peracetic acid solution (1800 ppm) for 10 min. After filling, the headspace was flushed with N_2 flux at 1 bar, and the bottles were sealed with sanitized metallic caps and stored at 4 °C in total darkness.

2.2 Preservation Processing Technologies

The analyses performed in this work were carried out on fresh untreated matrix (F) and thermally (TT), supercritical carbon dioxide (SC-CO_2)- and dimethyl dicarbonate (DMDC)-treated beverages.

2.2.1 Thermal Treatment (TT)

Heat pasteurization equipment consisted of a water bath (Dubnoff Bath TE-0532, Tecnal, Piracicaba, Brazil) with agitation into which 250-mL bottles of beverage were placed. The pasteurization was performed at 85 °C for 5 min. The process conditions were chosen based on Cappelletti et al. (2015). After treatment, the bottles were cooled and exhausted in an ice water bath and subsequently stored at 4 °C.

2.2.2 Supercritical Carbon Dioxide Treatment (SC-CO_2)

Treatment was conducted employing the static-synthetic method in a high-pressure variable-volume reactor as described by Silva et al. (2013). The experimental setup consisted of a variable-volume reactor with a maximum internal volume of 27 mL, two sapphire windows for visual observation, an absolute pressure transducer (Smar LD301) with a precision of ~0.03 MPa, a portable programmer (Smar HT201) for pressure data acquisition and a syringe pump (ISCO 260D). The reactor contained a movable piston that permits pressure control inside the cell without allowing exchange of fluids.

Initially, an amount of 15 mL of beverage was loaded into the cell with help of a sterile syringe. The juice/CO_2 mass ratio was 1:08. The charge of CO_2 was performed with the help of the syringe pump. Then, the cell content was kept at continuous agitation with the help of a magnetic stirrer and a Teflon-coated stirring bar. A metallic jacket surrounds the cell and water from a thermostatic bath was used as heating fluid, which flows through the jacket, so that the cell was kept at the temperature of 33 °C. The pressure system was increased at pressurization rate of 10 MPa/min using CO_2 as pressurizing fluid from its vapor pressure at room temperature (~5 MPa) up to 8 MPa, which was considered the initial working pressure. At this point the system was hold for a short period (~1 min) to allow system stabilization, and then the pressure was increased until 20 MPa. After the procedure, the system pressure was reduced to 8 MPa at the rate of depressurization pre-established. At the end of this process the pressure was manually reduced from 8 MPa to atmosphere pressure. All runs were conducted using one pressure cycle for 120 min (Fig. 1).

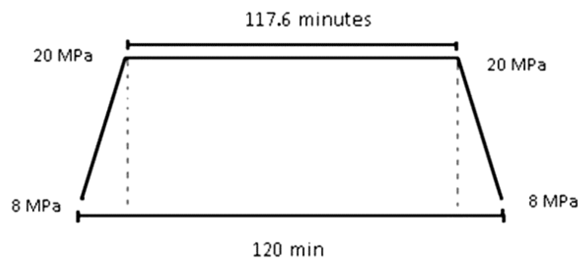

Figure 1. Supercritical carbon dioxide (SC-CO_2) treatment conditions adopted, shown in terms of time and pressure

2.2.3 Dimethyl Dicarbonate Treatment (DMDC)

The beverage was vigorously mixed in bottles by inversion for 2 min after the addition of 200 μL/L DMDC (Velcorin, Lanxess, Cologne, Germany) and kept for 4 h at 25 ℃. The bottles were cooled and maintained at 4 ℃ until analysis. The final concentration of DMDC added to the beverage was 250 mg/L.

2.3 Assessment of Composition and Physicochemical Properties of the F Matrix and TT Beverage

The following analyses were performed to characterize the F matrix and TT beverage.

2.3.1 Physicochemical

The pH was measured directly using a model HI9321 pH meter (Hanna Instruments, São Paulo, Brazil). Acidity was measured by titrimetric analysis using the TT beverage diluted 1:50 (v/v) in distilled water. The diluted solution was neutralized with 0.1 M NaOH (Merck, Germany) using an alcoholic solution of phenolphthalein (1 % v/v) as an indicator. Total soluble solids content was determined directly using a digital refractometer (PR-101, ATAGO, Tokyo, Japan) at 25 ℃. The concentration of reducing sugars was measured using the 3, 5-dinitrosalicylic acid (DNS) method (Miller, 1959), and total sugar content was measured using acid hydrolysis (Association of Official Analytical Chemistry, 2000). The protein content was determined according to Lowry, Rosebrough, Farr and Randall (1951).

2.3.2 Antioxidant Activity

The F and TT samples were analyzed for antioxidant activity (free radical scavenging capacity), which was examined by the reduction of the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) as described by Ramadan, Kroh and Moersel (2003) with modifications. Five mL of a 20 mg/mL DPPH solution in methanol were added to 5 mL of a methanolic solution of the beverage (1:10, v/v). Absorbance was determined spectrophotometrically (UV-1601PC Spectrophotometer, Shimadzu, Japan) at 517 nm after 30 min, and scavenging activity was calculated as the percent radical reduction. The percent inhibition was defined as [(A517 blank − A517 sample) A517 blank−1]·100−1 (%). Ascorbic acid was used as a reference component. The analysis was performed in triplicate, and the standard deviation of the mean values was calculated.

2.3.3 Total Phenolic Content

The Folin-Ciocalteu method (Singleton & Rossi, 1965) was used with some modifications to quantify total phenolic compounds using a gallic acid calibration curve (0 to 500 mg/L). A total of three hundred µl of diluted F matrix and TT beverage in a ratio of 1:100 with methanol:water (6:4) was mixed with 1.5 mL of 10-fold-diluted Folin-Ciocalteu's phenol reagent and 1.2 mL of 0.1 M sodium carbonate solution. The mixture was maintained in the dark for 90 min at 25 ℃ before absorbance was measured at 760 nm using a UV-1601PC spectrophotometer (Shimadzu, Japan). Dilutions were performed in triplicate, and the results are expressed as mg of gallic acid equivalents (GAE)/L of sample.

2.3.4 Omega 3 Fatty Acid

The total lipid content of the F matrix and TT beverage was determined by extracting with organic solvent in a Soxhlet extractor (Tecnal Equip., Piracicaba, Brazil) (AOAC, 2005). The samples had been previously homogenized and subjected to acid hydrolysis with 8 M HCl. From the extract obtained in the determination of total lipids, esterification of fatty acids was performed according to a procedure developed by Hartman and Lago (1973). The methyl esters of the fatty acids were determined by gas chromatography with a flame ionization detector (GC-FID) (GC-2014, Shimadzu, Kyoto, Japan) using an Rtx-2330 fused silica capillary column (105 m,

0.25 mm i.d., 0.20 μm film thick, Restek Corp., Bellefonte, PA, USA). The operating conditions were: initial temperature of the column, 140 ℃ for 5 min; increasing to 240 ℃ at a rate of 2.5 ℃/min; and remaining at 240 ℃ for 15 min. The detector was isothermally maintained at 260 ℃. The fatty acids were identified using GC Solution software (Shimadzu, Kyoto, Japan) by comparing retention times with those of standards (F.A.M.E. Mix C4-C24, Supelco Analytical, Bellefonte, PA, USA). Nitrogen was used as the carrier gas at a flow rate of 26 mL/min with a split ratio of 20:1.

2.3.5 Osmolality

The osmolality of the TT beverage was determined according to Henriques and Rosado (1999). The freezing point of the sample was measured using a cryoscope (MK 540L, ITR, Esteio, RS, Brazil) and the following equation was applied to determine the osmolality: molal concentration of the sample=Δtc/Kc, where Δtc is the cryoscopic decrease, and Kc is the cryoscopic constant of water (1.86 ℃/mol/kg). One osmole is equal to 1 mole for osmotic effects, which is the osmolality of the solution at the freezing point.

2.3.6 Microbiological Analysis

Microbiological analysis of TT beverages was based on Brazilian criteria (Resolution RDC 12) that establish presumptive tests for total and thermo-tolerant coliform Most Probable Number (MPN) and the absence of *Salmonella* sp. in a 25-mL sample (Brasil, 2001). For the MPN of total and thermo-tolerant coliforms and the determination of presence or absence of *Salmonella* sp., the APHA (American Public Health Association, 1992) recommendations were followed. Suspected *Salmonella* colonies were submitted to standard biochemical and serological tests (agglutination test performed with polyvalent flagellar antiserum) (Probac, São Paulo, Brazil).

2.3.7 Sensory Evaluation

Sensory evaluation was approved by the Human Research Ethics Committee (CEPSH) at UFSC, human ethics approval number 1076963. Sensory evaluation of the TT beverage was performed after 15 days of storage at 4 ℃. Sensory characteristics of the TT beverage were compared with those of the leading commercial pasteurized orange and carrot juices. Sensory hedonic testing of the beverages was performed according to Ferrari Pereira Lima, De Dea Lindner, Thomaz Soccol, Parada and Soccol (2012) by a group of 57 non-trained testers (elderly participants greater than 60 years old) from the Sector of Studies of Elderly (NETI) from UFSC. The participants were regular fruit juice consumers who judged the color, odor, taste and overall acceptability using a hedonic rating scale from 1 to 9 (1: extremely dislike; 2: dislike very much; 3: moderately dislike; 4: slightly dislike; 5: neither like nor dislike; 6: slightly like; 7: moderately like; 8: like very much; 9: extremely like) (Meilgaard, Civille, & Carr, 2007). Sensory tests were performed in individual booths under white light in the morning (9:00-11:30 a.m.). Samples were served refrigerated at 5 ℃ in transparent glass cups. The data obtained were analyzed by ANOVA and Tukey's test according to Monteiro (1994) using Assistat version 7.5 software (Assistat, Brazil). To verify the acceptability of the tested beverages, an acceptability factor (*AF*) (Dutcosky, 1996) using standardized criteria was calculated to evaluate each sensory analyzed attribute: $AF = A \cdot 100/B$, where A is the mean value obtained for each attribute, and B is the maximum mean value ascribed to each attribute.

2.4 Comparison of Preservation Process Technologies and Stabilization of the Beverage Components

2.4.1 Vitamin A

An LC10AT high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) equipped with a diode array detector (DAD) was used to measure vitamin A in the samples tested. Chromatographic separations were performed using an Eclipse XDB-C_{18} column - 150 mm x 4.6 mm, 5 μm (Agilent Technologies, Palo Alto, CA, USA). The mobile phase was composed of 10% acetonitrile and 90% methanol/water 98/2, and the device was operated in the isocratic mode with a flow rate of 1 mL/min. A total of 20 μL of sample was injected, and the analyte was monitored at 330 nm. The analyte was confirmed by enrichment of sample using a pharmaceutical standard of retinol and its UV-spectra. The retention time of the analyte was 4.4 min under the experimental conditions. For this assay, the peak area of vitamin A observed for the F matrix was considered 100 % concentration; the percentage of the analyte presented in the treated beverages could thus be estimated.

Before injection into the HPLC system, all samples were prepared as follows: 0.5 mL of chloroform was added to 2 mL of sample in a Falcon tube, and the mixture was vortexed for 1 min and centrifuged for 10 min. Thereafter, 200 μL of the organic phase was transferred to a microtube and dried using an N_2 flow. The sample was resuspended using 100 μL of acetonitrile and injected into the chromatographic system.

2.4.2 Vitamin C

A 7100 capillary electrophoresis (CE) system (Agilent Technologies, Palo Alto, CA, USA) equipped with a DAD was used in this assay. For all analyses, a fused silica capillary with 32 cm total length (23 cm of effective length) x 75 µm internal diameter x 375 µm outer diameter was used. Samples were injected hydrodynamically by applying 50 mbar for 3 s, and the analyte was monitored at 266 nm. The separation voltage was 25 kV under positive polarity at the injection side, and the temperature was maintained at 25 ˚C. To minimize instrumental errors, sorbic acid was used as internal standard. The background electrolyte (BGE) was composed of 40 mM tris(hydroxymethyl)aminomethane (TRIS) and 20 mM 2-(N-morpholino)ethanesulfonic acid (MES), buffered at pH 8.2.

To quantify the concentration of vitamin C in the samples, a calibration curve was constructed using an analytical standard of ascorbic acid from 10.1-40.6 mg/L using 7 concentration levels. The limit of detection and quantification of the method were 0.4 mg/L and 1.35 mg/L, respectively, calculated by the signal-to-noise ratio of 3:1 and 10:1, respectively.

Before injection into the CE system, 1 mL of sample was centrifuged, and the supernatant was collected and properly diluted to 1 mL of deionized water. Both the calibration curve and samples were prepared in duplicate, with a final internal standard concentration of 25 mg/L.

2.4.3 Enzymatic Activity

Peroxidase (POD, EC 1.11.1.7) activity was determined according to the method described by Abreu and Faria (2007) and Campos, Souza, Coelho and Glória (1996), with modifications. A total of 7 mL of 0.2 M buffer solution of monobasic sodium phosphate (pH 5.8), 1.5 mL 0.05% guaiacol (phenolic substrate) and 0.5 mL 0.1% hydrogen peroxide were added to a test tube maintained in a water bath at 35 ℃. After stabilization of the temperature, 1 mL of the treated beverage samples was added to the tube. The mixture was homogenized, and changes in absorbance at 470 nm were measured in a U-1800 UV/VIS Spectrophotometer (Hitachi, Berkshire, United Kingdom).

Polyphenol oxidase (PPO, EC 1.10.3.1) activity was determined according to the method described by Abreu and Faria (2007) and Campos et al. (1996), with modifications. Volumes of 5.5 mL of 0.2 M buffer solution of monobasic sodium phosphate (pH 6.0) and 1.5 mL of 0.2 M pyrocatechol solution (phenolic substrate) were added to a test tube maintained in a water bath at 25 ℃. After stabilization of the temperature, 1 mL of the treated beverage samples was added to the tube. The mixture was homogenized, and changes in absorbance at 425 nm were measured in a U-1800 UV/VIS Spectrophotometer.

The absorbance values were acquired after 10 min for both POD and PPO enzymes. The blank for analysis of both enzymes was a mixture of all the reactants, but replacing the beverage sample with distilled water. The enzyme activity was expressed in units/mL. One unit is equivalent to a change in absorbance of 0.001/min/mL of sample. The following equation was used: Enzymatic activity=$(A10_{sample}-A0_{sample})-(A10_{blank}-A0_{blank})/0.01$, where A10 is the absorbance after 10 min of the reaction, and A0 is the initial absorbance.

2.4.4 Microbiological Challenge Testing

To evaluate the effectiveness of the preservation processes used to deliver some degree of lethality against target potential pathogenic and/or spoilage microorganisms, a surrogate *E. coli*, ATCC 25922, and *A. acidoterrestris* ATCC 49025 were separately inoculated into the F matrix before treatment.

The *E. coli* stock culture was maintained on Brain-Heart Infusion agar (BHI) (Acumedia, Lansing, Michigan, USA) slants at 4 ℃. The cultures for the experiments were subcultured twice from the stock culture in nutrient broth (NB) (Oxoid, Basingstoke, Hampshire, England) incubated at 35 ℃ for 18 h. The culture was inoculated at a concentration of 1.5×10^6 CFU/mL in the F matrix. To evaluate the effect of the treatments on microbial inactivation, the enumeration of viable *E. coli* cells, after serial dilutions of the treated beverages in buffered peptone water, was obtained using violet red bile agar with 4-methylumbelliferyl-β-D-glucuronide (VRBA MUG) (Acumedia, Lansing, Michigan, USA) at 45 ℃/24 h. Replicate counts in CFU were converted to log values and expressed as averaged log numbers.

A. acidoterrestris was cultivated in yeast glucose starch (YGS) medium pH 3.7 at 45 ℃ for 5 days according the methodology described by Alberice, Funes-Huacca, Barreto Guterres and Carrilho (2012). The spores obtained from the culture were spread onto YGS agar and incubated at 45 ℃ for 5 days. After reaching more than 90% of sporulation, as confirmed by microscopy following staining with malachite green, spores were collected with a sterile swab and resuspended in sterile distilled water. The spores collected were centrifuged at 4.500*g* for 15 min at 4 ℃, washed two times with sterile distilled water, resuspended in acid sterile distilled water (pH 3.7) and

stored at −20 °C until use. The bacterial spore suspensions were activated by heat-shock at 80 °C for 10 min immediately prior to inoculation at a concentration of approximately $4x10^4$ CFU/mL in the F matrix. The number of viable *A. acidoterrestris* cells was evaluated from serial dilutions of the treated beverages in YGS agar pH 3.7 (45 °C/5 days). Counts in CFU were obtained in the same manner as described above for *E. coli.*

Plate count agar (PCA) (Himedia, Mumbai, India) at 30 °C for 48 h and dichloran rose bengal chlortetracycline (DRBC) (Acumedia, Lansing, Michigan, USA) at 25 °C for 5 days were used to enumerate the presence of uninoculated (naturally present in the F matrix) viable mesophilic aerobic bacteria and yeasts and molds, respectively, in the untreated and treated samples for each preservation process. Counts for CFU were performed in duplicate, and the mean values were calculated.

3. Results and Discussion

3.1 Characterization of the Beverage

3.1.1 Physicochemical and Composition Characterization

The characterization of the TT beverage, presented in Table 1, is consistent with those reported for coconut water and orange juices. The obtained pH values classified the beverage as low-acid, and the high carbohydrate content could contribute to the development of food-borne pathogen contamination (Walter, Kabuki, Esper, Sant'Anan, & Kuaye, 2009). A beverage pH below 3.8 is significant for determining microbiological stability (Battey & Schaffner, 2001) and is directly correlated with the product's taste. However, the thermo-acidophilic spore-forming bacteria *Alicyclobacillus sp*. (TAB) can not only survive the normal pasteurization procedure applied to fruit juices and beverages but also germinate and proliferate in acidic products (Smit, Cameron, Venter, & Witthuhn, 2011).

Table 1. Characteristics of the TT beverage

Parameter	Mean ± SD
pH	3.50 ± 0.0
Acidity*	7.30 ± 0.01
Soluble solids*	8.33 ± 0.58
Reducing sugars*	42.90 ± 0.05
Total sugars*	66.00 ± 0.11
Proteins*	0.66 ± 0.07

Note. SD= standard deviation; *g/L.

The sugars that are known to contribute to the sweet taste in coconut water and orange juice are fructose, glucose and sucrose (Bilek & Bayram, 2015; Camargo Prado et al., 2015). Trace amounts of protein were found in the beverage. The presence of free amino acids and reducing sugars may result in the occurrence of Maillard reactions during thermal processing, which would leads to nutritional and sensory changes in the beverage (Cappelletti et al., 2015).

3.1.2 Vitamin C, Antioxidant Activity and Total Phenolic Content

The concentrations of vitamin C as ascorbic acid in the F and TT beverage are shown in Table 2. Of this total, 180 mg/L was added to the beverage during formulation as a technological antioxidant and vitamin enrichment component. With this amount, the TT beverage achieves 162% of the RDI recommended in Brazil. Both vitamin C concentration and total phenolic content are strongly correlated with antioxidant capacity, as determined by the reduction of DPPH (Gardner, White, McPhail, & Duthie, 2000). The DPPH assay has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. Santos et al. (2013) detected and quantified ascorbic acid in several varieties of coconut water (25.8 mg/L). In cell culture, the green dwarf variety of coconut water was efficient in protecting against oxidative damage induced by hydrogen peroxide. Vitamin C is highly bioavailable and is consequently one of the most important water-soluble antioxidants in cells (Halliwell, 1996).

Table 2. Ascorbic acid content, antioxidant activity, total phenol content, omega 3 fatty acid content and osmolality of F (fresh untreated matrix) and TT (thermally treated beverage)

	F	TT
Ascorbic acid (mg/L)*	291.73 ±0.68[a]	285.23 ±1.45[b]
Antioxidant activity (% inhibition of DPPH)*	63.48 ±2.41[a]	68.48 ±2.00[b]
Total phenol content (mg/L)*	952.75 ±5.69[a]	962.50 ±2.06[b]
Omega 3 fatty acids (mg/L)	516.40	530.10
Osmolality (mOsmol/L)	ND	545.00

Note. *Mean ±standard deviation. Values with different capital letters in the same line are significantly different ($P < 0.05$). ND= not determined.

The F matrix and TT beverage exhibited high ascorbic acid content due to the high ascorbic acid content in the orange juice (403.15 mg/L) and coconut water (23.10 mg/L), which were added to 40% and 48%, respectively, in the beverage formulation. The consumption of fruit juice-based beverages contributes to overall antioxidant intake, with potentially beneficial biological effects for human health. Coconut water and orange juice were shown to have antioxidant activity, which was positively correlated with the concentration of flavanones and kinetin in orange juice and coconut water, respectively (Sánchez-Moreno et al., 2005; Yong et al., 2009). The F and TT beverage showed different results for antioxidant activity (Table 2). Comparison of the beverage with that in the study conducted by Ferrari Pereira Lima et al. (2012) showed that the anti-radical performance towards DPPH radicals was just below that of a fermented herbal mate beverage rich in phenolic compounds. According to Qin, Jin and Park (2010), individual phenolic compounds with high antioxidant activity might be produced during the fermentation process. Compared with other antioxidant beverages (Ramadan-Hassanien, 2008), especially those rich in caffeine, the TT beverage has high antioxidant capacity, slightly less than that of tea with lemon, green tea, black tea and soluble coffee.

The total phenolic content in the TT beverage was slightly above that of F (Table 2). The major contribution to phenolics in the beverage came from the orange juice. In a study conducted by Gardner and White (2000), the level of phenolic compounds in orange juice was 755 mg GAE/L. As mentioned above, the addition of 40% of orange juice, increased the total phenolic content of the beverage. The use of orange juice in the formulation is thus important as a source of phenolic compounds. The coconut water, present in the formulation in the range of 46%, did not contribute significantly to the total phenolic content; Santos et al. (2013) and Tan et al. (20140 found a low content of phenolics in coconut water (99.40 and 54.00 mg/L, respectively).

3.1.3 Omega 3 Fatty Acids

The concentration of omega-3s (EPA+DHA) in the F and TT beverage is shown in Table 2. In this amount, a dose of 250 mL of beverage achieves 51.3% of the FAO minimal recommendation per day. Decreases in DHA status are associated with cognitive decline in the elderly and those with Alzheimer's (Abubakari, Naderali, & Naderali, 2014). Human studies suggest that an adequate dietary intake of omega-3s could decrease age-related cognitive decline, may protect against senile dementia and provide better cognitive performance (Denis, Potier, Vancassel, & Lavialle, 2013; D'Ascoli et al., 2016). García-Alonso, Jorge-Vidal, Ros and Periago (2012) compared the effects of consumption of omega-3-enriched tomato juice on serum lipid profiles and levels of biomarkers related to antioxidant status and cardiovascular disease (CVD) risk in healthy women. Stronger positive amelioration of CVD risk factors was observed following the intake of enriched juice, suggesting a possible synergistic action between omega-3s and tomato antioxidants (362 mg/L). In comparison, the TT beverage had 2.65-fold more total phenolics in almost the same amount of omega-3s. Hawthorne, Abrams and Heird (2009) evaluated the effects of providing a supplement of micro-encapsulated algal DHA in orange juice. They demonstrated that DHA supplementation of juice at either 50 mg/day or 100 mg/day for 6 weeks was effective in increasing the plasma phospholipid DHA contents of children.

3.1.4 Osmolality

The osmolality of the beverage, expressed in mOsmol/L, depends on its osmotic pressure. Osmolality is a crucial determinant of the physiological acceptance of the beverage. The desired osmolality should be < 700 mOsm (Klang, McLymont, & Ng, 2013). An appropriate osmolar load is necessary for the beverage be tolerated by a eutrophic elderly consumer, ensuring the successful assimilation of the formulation components. The osmolar concentration found in the TT beverage (Table 2) was 545.00 mOsm/L, classifying the product as a hypertonic

beverage (> 330 mOsmol/L). Fruit juices are considered typical hypertonic beverages, with osmolality values in the range of 600-700 mOsmol/L (Henriques & Rosado, 1999).

3.1.5 Microbiological Analysis and Sensory Evaluation

Microbial evaluation of total, thermo-tolerant coliforms and *Salmonella* sp. was performed (data not shown) in accordance with Brazilian criteria to ensure safe consumption of the TT beverage, indicating that efficient heat treatment occurred after a satisfactory hygienic process. Then, a panel of 57 non-trained elderly volunteers, each greater than 60 years old and a regular fruit juice consumer, participated in the evaluation. The acceptance levels for the TT beverage and the leading commercial pasteurized orange/carrot juice are presented in Table 3. No significant differences in global evaluation, taste, color or aroma were obtained.

Table 3. Acceptance (average values ±SD) and acceptability factors (AFs) for TT (thermally treated beverage) and commercial pasteurized orange/carrot juice

Attributes (AF)	TT beverage	Commercial pasteurized juice
Global evaluation	6.89 ±1.50[a] (100)	5.61 ±2.20[a] (81)
Taste	6.81 ±1.70[a] (100)	5.19 ±2.30[a] (76)
Color	7.12 ±1.80[a] (100)	5.47 ±2.10[a] (77)
Aroma	6.70 ±1.70[a] (100)	5.42 ±2.10[a] (81)

Note. [a] Means ±standard deviation with the same letter in the same line are not significantly different ($P > 0.05$).

These findings were in accordance with Camargo Prado et al. (2015), who evaluated a fermented coconut-based beverage supplemented with sucrose and artificial coconut flavor. Figure 2 shows that the TT beverage received the best overall rating from the panel. For the overall evaluation, the average values for the sensory analysis and acceptability factors were kept within the acceptance range. According to Dutcosky (1996), AF ≥ 70% represented good acceptability for the attribute analyzed in a sensory analysis. Therefore, product acceptance was good and was comparable to that of similar commercial beverages available at the local market.

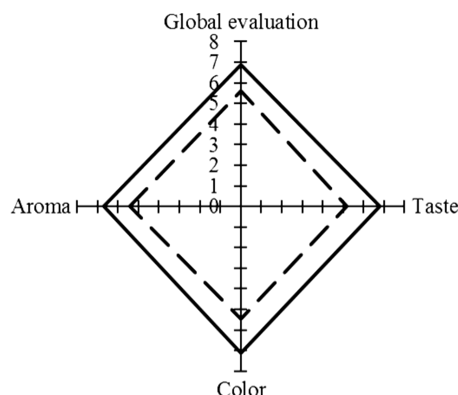

Figure 2. Characteristics of the acceptance profile of the TT beverage (thermally treated beverage) (solid line) and the leading commercial pasteurized orange/carrot juice (dashed line)

3.2 Comparison of Preservation Process Technologies and Stabilization of the Beverage Components

Thermal processes induce undesirable changes in beverages, such as micronutrient losses, through chemical reactions and leads to losses in perceived freshness (Rodríguez-Roque et al., 2015). Non-thermal beverage preservation technologies such as SC-CO_2 and DMDC have been developed as an alternative. Previous studies have reported that both technologies inactivate microorganisms without compromising the nutritional and sensory attributes of food (Yu, Xiao, Xu, Wu, & Wen, 2014; Cappelletti et al., 2015).

3.2.1 Vitamins A and C and Enzymatic Activity

The analyses used to measure the reduction of vitamin content and enzymatic activity during the processes are described in table 4.

Table 4. Vitamin A and ascorbic acid levels and peroxidase and polyphenol oxidase activities of coconut water (CW), fresh untreated matrix (F), thermally treated beverage (TT), supercritical carbon dioxide-treated beverage ($SC-CO_2$) and dimethyl dicarbonate-treated beverage (DMDC)

			Preservation process			
	Orange juice	Coconut water	F	TT	$SC-CO_2$	DMDC
Vitamin A (%)	n.d.	n.d.	100	144	92	106
Ascorbic acid (mg/L)*	403.15±0.78	23.10±3.0	291.73±3.68^a	285.23±1.45^b	245.69±0.52^c	279.30±8.86^{ab}
Peroxidase (U/mL)	7.40	66.30	3.90	ND	ND	ND
Polyphenol oxidase (U/mL)	ND	14.7	ND	ND	ND	ND

Note. *Means ± standard deviation. n.d.= not determined, ND= not detectable. ^{a,b,c} Values with different capital letters are significantly different ($P < 0.05$).

The TT beverage showed higher vitamin A values (increased 44% in relation to F). This result was in agreement with a report by Sánchez-Moreno, Plaza, De Ancos and Cano (2003) that showed an increase in extractable carotenoids due to thermal treatment of orange juice. According to Schieber and Carle (2005), the availability of carotenoids may increase due to rupture of the food matrix by heat or mechanical treatment. Thermal processes increase the chemical extractability of carotenoids and other substances.

According to Zhou et al. (2009), who investigated the effect of $SC-CO_2$ on the quality of carrot juice, the $SC-CO_2$ treatment resulted in an 8% decrease in the vitamin A concentration, and they observed that carotenoids in the treated juices were stable. Carotenoids are bound to protein and membrane lipids. Processes that apply high pressure are known to affect food macromolecular structures. Sánchez-Moreno et al. (2005) found that in orange juice, high pressure processes increased the release of carotenoids from the suspended pulp particles, making them more accessible to extraction and leading to increased carotenoid release and vitamin A values. Our result, in accordance to Johannsen and Brunner (1997), may be related to the solubility of fat-soluble vitamins after supercritical CO_2 interaction that is correlated with differences in the temperature and density of CO_2 applied.

Compared to F, the DMDC treatment showed a 6% increase in the vitamin A concentration. There is little known about the interaction between this molecule and vitamin A. More research could clarify the mechanism of interaction between DMDC treatment and vitamins.

The content of ascorbic acid from the treatments analyzed is reported in Table 4. Before the addition of ascorbic acid to the beverage formulation, ascorbic acid was measured as 23.10 and 403.15 mg/L for coconut water and orange juice, respectively. Significant differences among the preservation processes were detected. In contrast, Rodríguez-Roque et al. (2015) and Sánchez-Moreno et al. (2005) showed that the greatest losses of this bioactive compound were found in TT fruit juice beverages (by 31% and 9%, respectively, compared with untreated matrixes) subjected to different preservation technologies. Vitamin C is a heat-sensitive nutrient and is vulnerable to enzyme-catalyzed oxidation by oxidoreductases (*e.g.,* ascorbate oxidase and POD) (Davey et al., 2000). In our work, the treatments applied may have partially eliminated some of the enzymes responsible for vitamin C loss. Thermal treatments with higher temperature tended to result in a higher decrease in the content of vitamin C, but in our case, the binomial time-temperature applied in the TT process demonstrated no significant difference from non-thermal treatments.

There was no significant degradation of ascorbic acid after treatment with DMDC (Table 4). Yu et al. (2014) treated fermented litchi juice with DMDC and observed, contrary to our results, a 58.1% reduction in the ascorbic acid content relative to the fresh sample. Leong and Oey (2012) reported that ascorbic acid was minimally reactive with DMDC and that the loss of the compound could be attributed to ascorbic acid oxidases in the juice during DMDC treatment. In our case, depleting dissolved oxygen in the deaeration process and filling the bottle headspace with N_2 both inhibited the oxidation of ascorbic acid.

$SC-CO_2$ treatment using 20 MPa showed the greatest decrease in ascorbic acid content, a more than 15% reduction compared with F (Table 4). According to Fabroni, Amenta, Timpanaro and Rapisarda (2010), the addition of CO_2 to orange juice is probably beneficial for ascorbic acid retention due to the displacement of dissolved oxygen from the liquid matrix. Fabroni et al. (2010) applied CO_2 over 23 MPa for 15 min, and 6% ascorbic acid in the juice was degraded, but at lower pressures (13 MPa), the concentration remained unchanged. They concluded that lower operative pressures are likely to be beneficial for the retention of ascorbic acid, and

the change in CO_2 concentration did not appear to have an influence.

Higher concentrations of POD and PPO were found in coconut water (Table 4), the major component of the beverage. In orange juice and F, the high concentration of ascorbic acid and the pH of 3.5, respectively, were sufficient to inhibit enzyme activities. These results are in accordance with Abreu and Faria (2007), who reported that the addition of ascorbic acid was effective in the inactivation of PPO in coconut water before heat treatment. Campos et al. (1996) showed that 20 mg of ascorbic acid per 100 mL of coconut water was sufficient to reduce the activity of both oxidoreductases. Cultivar differences and component matrix variations during fruit maturity are key elements in the variations in enzymatic value.

No enzyme activity was detected in the TT, SC-CO_2 or DMDC beverages (Table 4), which could be related to the synergic effect of the treatments, the presence of ascorbic acid in the formulation and/or the low pH of the beverage. Campos et al. (1996) and Tan et al. (2014) reported that thermal treatment had a pronounced effect on the activities of POD and PPO in coconut water, with a drastic decrease observed at higher heating temperatures. The results showed that complete inactivation of POD was achieved with heat treatment at 90 °C and a holding time of 2.5 min. The cited works also reported a similar result; PPO was more heat-resistant than POD.

Yu et al. (2014) used DMDC in litchi juice and observed that the content of ascorbic acid increased in *Lactobacillus casei*-fermented juice; this result was attributed to the deoxidization of oxidized ascorbic acid. During a four-week period, no large variations in color were observed, demonstrating the stability of the enzyme activities in the DMDC-treated juice. According to Wang et al. (2013), DMDC can inhibit the activity of PPO and POD, contributing to the maintenance of ascorbic acid content.

Depending on the processing parameters (*e.g.*, temperature, pressure and depressurization rate), SC-CO_2 may negatively affect the enzyme activity due to conformational changes caused by gas in the secondary and tertiary structure. Gui et al. (2007) observed that cloudy apple juice, when exposed to SC-CO_2 at 30 MPa (55 °C for 60 min), presented a reduction in PPO activity of over 60%, which was greater than that observed under atmospheric conditions at the same temperature (27.9% reduction) and indicated a combined effect of pressure, temperature and time after SC-CO_2 treatment. Four years later, Xu et al. (2011) studied the SC-CO_2-mediated inactivation of enzymes in the same matrix. PPO was completely inactivated after 10 min of treatment at 22 MPa and 60 °C. Liu, Hub, Zhaoc and Song (2012) studied the effect of SC-CO_2 on watermelon juice and obtained 95.8% reductions in PPO activity and 57.9% in PDO when 30 MPa pressure was applied at 50 °C for 50 min. The cited works reported that the major effect of SC-CO_2 on enzymes occurred during the first minutes of treatment, in accordance with our results.

3.2.2 Microbiological Challenge Testing

The inoculum level used in a microbiological challenge depends on the objective of the study. In our case, the level was determined for validation of process lethality, and 10^6 CFU/mL for *E. coli* was used to demonstrate the extent of reduction in the challenge. In the USA, juice processors are required to demonstrate a 5 log reduction in relevant hazardous microorganisms in their products (5 D performance standard) (U.S. FDA, 2016).

Several pathogenic bacteria may resist the inherent acidity of fruit juice and develop adaptive mechanisms that enhance their survival and sometimes even their ability to grow in acidic environments. Analysis of the data (Table 5) showed that the TT and DMDC processes could deliver the required level of lethality according to the pre-determined performance standard of a 5 log reduction for juices (U.S. FDA, 2016). The SC-CO_2 treatment demonstrated a 3.38 log reduction. Our results are in agreement with those of (Basaran-Akgul, Churey, Basaran, & Worobo, 2009), who challenged apple cider (pH 4.0) by inoculating 10^6-10^7 CFU of three different strains of *E. coli* O157:H7 per mL of juice. A greater than 5 log reduction was achieved at room temperature with 250 ppm DMDC after incubation for 6 h. Treatment with DMDC may offer a viable alternative to TT for the production of safe juice.

Table 5. Viable cell counts (log orders CFU/mL) performed in duplicate. The results are presented as the mean values for *E. coli* ATCC 25922, *A. acidoterrestris* ATCC 49025, mesophilic aerobic bacteria, yeasts and molds in the fresh untreated matrix (F), thermally treated beverage (TT), supercritical carbon dioxide-treated beverage (SC-CO2) and dimethyl dicarbonate-treated beverage (DMDC)

		Preservation process			
	Medium	F	TT	SC-CO_2	DMDC
E. coli ATCC 25922	VRBA MUG	6.08	ND	2.70	ND
A. acidoterrestris ATCC 49025	YGS	4.60	2.60	2.78	2.18
Mesophilic aerobic bacteria*	PCA	2.60	ND	1.70	2.18
Yeasts and molds*	DRBC	3.48	2.70	3.30	2.30

Note. ND= colonies not detected - counts below the detection limit (1 CFU/mL). *Enumeration of uninoculated microorganisms (naturally present in the matrix).

E. coli ATCC 25922 demonstrated some level of resistance to the conditions applied in the SC-CO_2 treatment (Table 5). Silva et al. (2013) used SC-CO_2 to build an inactivation curve for the same ATCC 25922 strain. The researchers obtained considerable log reductions using pressures of 10 MPa/25 min and found that microbial inactivation increases with increasing supercritical CO_2 pressure cycles and system pressure. The food matrix can cause variations in the ability of pressurized CO_2 to physicochemically act (four mechanisms described by Spilimbergo and Bertucco, 2003) to lead to loss of cell viability and the resistance and recovery of the microorganism (Debs-Louka, Louka, Abraham, Chabot, & Allaf, 1999).

Detectable taint production in fruit juice is generally reported when the levels of *A. acidoterrestris* reach approximately 4 to 5 log CFU/mL (Molva & Baysal, 2015); using this information, we chose to inoculate 4 log. TT reduced the concentration of *A. acidoterrestris* in the beverage by 2 log (Table 5). The heat resistance of *A. acidoterrestris* was reported in several studies. According to Bevilacqua, Sinigaglia and Corbo (2008), it is necessary to correlate heat with pH and soluble solids concentration to achieve a significant reduction in thermoacidophilic bacteria (TAB) because the resistance of endospores is strain-dependent. Alberice et al. (2012) investigated heat treatment and incubation time in orange juice inoculated with 4 log CFU/mL of *A. acidoterrestris* spores to evaluate the best temperature for inactivation. At 87 ℃, counts of cell viability decreased slowly within the first 50 min of incubation. The best inactivation was obtained with a one and two minutes at 99 ℃.

Viable *A. acidoterrestris* cells demonstrated resistance to inactivation with DMDC treatment (Table 5). The reduction observed was only 2.18 log. According to Chen, Harte, Davison and Golden (2013), DMDC (250 ppm) reduced the initial vegetative cell population by 2 log CFU/mL in *Bacillus acidoterrestris* thermophilic broth and significantly increased the time to reach stationary phase. During the hydrolysis period, DMDC demonstrated activity, achieving some level of reduction for vegetative cells and spores, but after hydrolysis occurred, considerable growth of cells was detected. Therefore, the hydrolysis time was not sufficient to affect spores structured to resist environmental stresses. Although DMDC treatment may help control the vegetative cells of *A. acidoterrestris*, it may not provide adequate overall control for spores. Based on these results, the use of other antimicrobial agents for long shelf-life products is recommended.

The SC-CO2 treatment also failed to achieve the expected effectiveness for *A. acidoterrestris*; the reduction observed was only 1.82 log (Table 5). This technique combines many variables, such as pressure, pressurization/depressurization rate and cycles, temperature and process time, which are necessary to apply an experimental design to evaluate the behavior of conditions with the juice matrix. According to Garcia-Gonzalez et al. (2009), both Gram-positive and Gram-negative bacteria can be sensitized by 10 MPa SC-CO_2 at 35 ℃ for 20 min, but yeasts and the vegetative cells of *A. acidoterrestris* in apple juice show higher resistance (reduction of 2.0 and 0.3 log, respectively).

In the present study, negative results were recorded for *A. acidoterrestris* in all treatments tested even though viable spores may be present in the beverage. Consequently, defective batches of juice may enter the filling line after treatment. Therefore, the fate of *A. acidoterrestris* spores in our beverage is worthy of investigation. No shelf life tests were conducted to further explore the prevalence of the strain with time after treatment.

Viable mesophilic aerobic bacteria, yeasts and molds were tested in terms of resistance to inactivation by the applied treatments (Table 5). Thermal treatment was able to reduce the total cultivable mesophilic aerobic bacteria naturally present in the matrix, in accordance with the results of Cappelletti et al. (2015). SC-CO_2 and DMDC were

not able to act similarly. Furthermore, our results demonstrate that the applied treatments were not adequate to reduce naturally present yeasts and molds. Many fungi have the ability to produce ascospores that survive heat treatment, germinating within the packaging and causing deterioration. Salomão, Muller, Couto do Amparo and Falcão de Aragão (2014) evaluated molds and yeasts in the apple juice heat concentration process and identified heat-resistant molds such as *Byssochlamys fulva*. Consistent with our results, Yu et al. (2014) achieved a small reduction of 3.5 and 1.6 log for yeast and molds, respectively, in fermented litchi juice by applying DMDC (250 mg/L) at 30 ℃. Generally, molds are more resistant to DMDC treatment than are yeasts and bacteria.

In conclusion, preventive interventions through dietary modification are attractive strategies for promoting the health of the elderly. RTD beverages are a great vehicle for healthful ingredients for elderly consumers' dietary needs, vulnerabilities, preferences and restrictions because of their convenience and availability. The developed beverage can contribute to vitamin C, antioxidant, phenolic, omega-3 and fiber intake. The osmolality tests indicate that the beverage is hypertonic and appropriate for tolerance by eutrophic elderly consumers, ensuring the assimilation of the formulation components. The study demonstrated that TT was more effective than the SC-CO_2 and DMDC treatments in preserving bioactive compounds in the fresh beverage, but the alternative non-thermal preservation methods represented a promising alternative to thermal processing for microbiologically stabilizing beverages without altering their quality attributes. The beneficial effects of the beverage as a healthy product should be explored in further studies, including trials with the elderly. Such research will provide insights into the use of the beverage as a non-dairy substrate and its introduction as a new vehicle for the consumption of functional beverages, especially by vegans and/or vegetarians and lactose-intolerant elderly.

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