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Enzymic Deactivation in Tender Coconut Water by Supercritical Carbon Dioxide

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Abstract: Polyphenol oxidase (PPO) and peroxidase (POD) are target enzymes in the processing of tender coconut water (TCW). This study primarily evaluated the combined effect of supercritical carbon dioxide (SC-CO2) and mild temperatures on the PPO and POD deactivation of TCW. A factorial design was performed to investigate the effect of temperature (in the range of 35 to 85 °C), pressure (75 to 370 bar), and holding time (13 to 47 min) on the enzymic deactivation, physicochemical parameters, and color of the TCW. The percentages of reduction in PPO activity ranged from 3.7 to 100%, and POD ranged from 43.4 to 100%. The pH values of the freshly extracted and processed TCW were 5.09 and 4.90, and the soluble solids content were 5.5 and 5.4 °Brix, respectively. The holding time (t) had a significant effect ($p \leq 0.1$) on the total color variation. As for the reduction of PPO activity, the temperature (T) and the interaction between pressure (P) and t had a significant effect. None of variables (P, T, or t) affected ($p > 0.1$) the POD reduction, pH, and soluble solids variation. The combination of SC-CO_2 and mild temperatures is a promising intervention in the enzymic stabilization of TCW.

Keywords: processing; hurdle technology; factorial design

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1. Introduction

Tender/green coconut water (TCW) is a low-calorie drink rich in electrolytes such as potassium, magnesium, and calcium. Due to its high rehydration potential, TCW is also known as "nature's isotonic" and is often used as an effective rehydration fluid. TCW is a relatively clear and colorless liquid, but its appearance can be affected by the degree of ripeness of the fruit and environmental exposure [1].

Coconut water is naturally sterile inside the fruit but quickly becomes susceptible to microbial contamination after it is extracted. A number of technologies (classic and emergent) have been explored by manufacturers to extend its shelf life and market it as a ready-to-drink beverage [2].

Once the coconut is opened, the water begins to lose its nutrients and flavor, and it also changes color, partly due to the activity of endogenous enzymes, especially peroxidase (POD) and polyphenol oxidase (PPO). These enzymes catalyze changes in the flavor profile, as well as objectionable discoloration, forming brown and pink pigments [1,3]. A number of studies [4–10] targeting the PPO and POD deactivation have been conducted, applying either thermal or non-thermal technologies.

Heat methods (pasteurization, sterilization in packaging, and UHT treatment) have been explored due to their positive effects, such as the destruction of pathogens and deterioration of microorganisms and enzymes, providing consumers with the benefit of a shelf-stable product that can be distributed over long distances and supplied to new markets [1]. Despite these advantages, the heat treatment commercially applied to the TCW affects its taste due to the formation of off-flavors resulting from the Maillard browning (cooked taste) and discoloration (browning and pinking) reactions, in which the TCW changes from colorless to brown or pink due to enzymic phenolic oxidation. Furthermore, the degradation of essential amino acids and vitamins may compromise the acceptability and marketability of the product [1,3,11]. These drawbacks highlight the importance of investigating non-thermal and/or gentle heat-assisted processing technologies for preserving the TCW.

The main mechanism of enzyme deactivation associated with emerging non-thermal technologies is the conformational change in the structure of the enzyme (protein denaturation), which loses its specificity and ability to bind to the substrate [12]. Emerging non-thermal processing techniques that have been studied and successfully used to preserve TCW include high hydrostatic pressure, ultraviolet radiation, ultrasound, high-pressure carbon dioxide, high-pressure homogenization, microfiltration, and the combination of these treatments with the assistance of with mild heating [3,11,13,14].

In high-pressure carbon dioxide (HPCD) processing, CO_2 is applied at pressures greater than 0.1 MPa (1 bar) at a mild temperature, i.e., lower than that used in thermal pasteurization [4,12] CO_2 has different thermodynamic states at different temperatures and pressures, and above critical conditions (73.8 bar and 31.1 °C), it is found as a supercritical fluid (SC-CO_2) exhibiting both gas and liquid properties [15].

Currently, there is a growing interest in the food industry in the application of supercritical fluids as an alternative non-thermal technology. These fluids are characterized by their safety, being ecologically friendly and economically viable. SC-CO_2 is the most widely used solvent in this context, being preferred in several areas, such as food, cosmetics, pharmaceuticals, and biomedicines, due to its versatility for a variety of applications [16–19].

Some studies [4,6–10] report the use of CO_2 at high pressures to preserve fruit and vegetable juices, conducted at temperatures ranging from 25 to 100 °C with pressures between 1 and 500 bar and holding times from 3 to 60 min. Also, the instability of the enzymes and their rapid degradation when exposed to the SC-CO_2 result, in some cases, in the total denaturation of proteins [20,21].

This technology has stood out for achieving microbial and enzymic stability and preserving the nutritional and sensory attributes of TCW, in addition to its negligible toxicity and the low cost of CO_2 [9,22,23]. The study herein primarily focused on employing the combination of SC-CO_2 and mild temperatures to deactivate PPO and POD in TCW. Specifically, the experimental assays were designed to optimize the CO_2 pressure temperature and holding time to achieve enzymic stabilization with minimal changes in pH, soluble solids, and color.

2. Material and Methods

2.1. Processing

The experimental trials were conducted on a weekly basis at the Laboratory of High Pressure Technology and Natural Products of the Food Engineering Department at the Faculty of Animal Science and Food Engineering of the University of São Paulo.

2.1.1. Extraction of Tender Coconut Water

Tender coconuts of 6–7 months old (green dwarf variety) were purchased from the local market in Pirassununga, SP, Brazil. The fruit was cleaned with running water and detergent and sanitized with 70%(v/v) ethanol. The coconut water was manually extracted and filtered through a culinary sieve to remove particles from the shell and the pulp of the fruit. Samples with an abnormal appearance, aroma, or taste were discarded. In each test, 200 mL of water was fractionated into two 100 mL parts and packed in glass jars with screw caps. One fraction was used as a control (fresh coconut water) and the second fraction was treated with SC-CO_2.

2.1.2. Treatment with $SC\text{-}CO_2$

The treatment of coconut water with direct injection of $SC\text{-}CO_2$ was conducted in the reactor of a supercritical fluid extractor (Thar SFE, Pittsburgh, PA, USA). The sample was transferred to the reactor (Figure 1a,b), and the parameters (P/T/t) were adjusted. The coconut water treated with $SC\text{-}CO_2$ was transferred to a depressurization chamber and collected in a sterilized glass vial (Figure 1c).

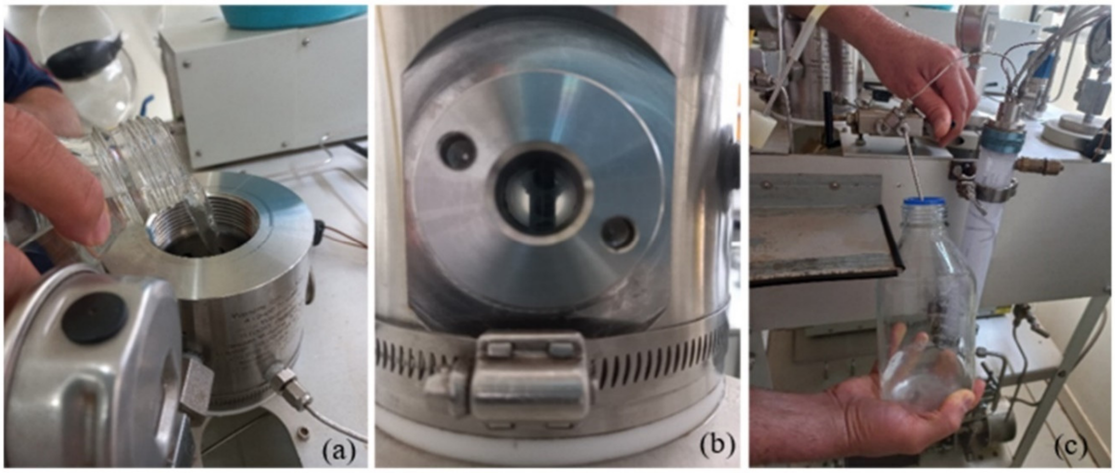

Figure 1. (**a–c**) Tender coconut water processing.

2.1.3. Factorial Design

Table 1 shows the independent variables (factors) and their respective levels (actual and coded), tested in the central composite rotational design (CCRD), as described in [24]. The temperature and pressure ranges were set within the CO_2 supercritical zone. The operational constraints of the equipment were taken into account when defining the upper levels. The complete CCRD matrix is exhibited in Table 2.

Table 1. Actual and coded levels tested in the treatment of tender coconut water with $SC\text{-}CO_2$.

Variable	Code	−1.68 (-α)	−1	0	+1	+1.68 (+α)
P (bar)	x_1	75	135	223	310	370
T (°C)	x_2	35	45	60	75	85
t (min)	x_3	13	20	30	40	47

(−1.68) lower axial point; (−1) lower level; (0) central point; (+1) upper level; (+1.68) upper axial point. $\alpha = (2^n)^{1/4} = 1.68$. n = number of independent variables (3).

Table 2. Physicochemical parameters of green coconut water treated with $SC\text{-}CO_2$.

Test	Treatment	pH	Soluble Solids (°Brix)
1	*Raw*	5.20 ± 0.03	5.7 ± 0.1
	135 bar/45 °C/20 min	4.96 ± 0.02	5.5 ± 0.1
	Δ	**−0.23**	**−0.2**
2	*Raw*	5.47 ± 0.01	6.6 ± 0.1
	310 bar/45 °C/20 min	5.17 ± 0.01	6.4 ± 0.1
	Δ	**−0.31**	**−0.2**
3	*Raw*	5.26 ± 0.02	5.1 ± 0.0
	135 bar/75 °C/20 min	4.99 ± 0.01	5.4 ± 0.1

	Δ	**−0.27**	**+0.3**
	Raw	6.11 ± 0.01	5.9 ± 0.0
4	310 bar/75 °C/20 min	5.41 ± 0.01	5.8 ± 0.00
	Δ	**−0.70**	**−0.1**
	Raw	4.54 ± 0.01	5.3 ± 0.1
5	135 bar/45 °C/40 min	4.49 ± 0.01	5.0 ± 0.1
	Δ	**−0.05**	**−0.3**
	Raw	4.96 ± 0.01	5.7 ± 0.0
6	310 bar/45 °C/40 min	4.82 ± 0.00	5.7 ± 0.0
	Δ	**−0.14**	**0.0**
	Raw	6.30 ± 0.01	4.0 ± 0.0
7	135 bar/75 °C/40 min	5.66 ± 0.01	4.0 ± 0.1
	Δ	**−0.64**	**0.0**
	Raw	4.68 ± 0.01	6.0 ± 0.0
8	310 bar/75 °C/40 min	4.60 ± 0.01	5.8 ± 0.1
	Δ	**−0.09**	**−0.2**
	Raw	4.89 ± 0.01	5.0 ± 0.0
9	75 bar/60 °C/30 min	4.84 ± 0.02	4.9 ± 0.1
	Δ	**−0.06**	**−0.1**
	Raw	4.69 ± 0.01	6.1 ± 0.1
10	370 bar/60 °C/30 min	4.64 ± 0.01	5.8 ± 0.0
	Δ	**−0.04**	**−0.3**
	Raw	4.46 ± 0.01	5.1 ± 0.1
11	223 bar/35 °C/30 min	4.40 ± 0.01	4.9 ± 0.1
	Δ	**−0.06**	**−0.2**
	Raw	4.49 ± 0.01	5.3 ± 0.1
12	223 bar/ 85 °C/ 30 min	4.46 ± 0.01	5.3 ± 0.1
	Δ	**−0.02**	**0.0**
	Raw	4.68 ± 0.02	5.3 ± 0.0
13	223 bar/60 °C/13 min	4.63 ± 0.01	5.3 ± 0.1
	Δ	**−0.05**	**0.0**
	Raw	5.62 ± 0.02	6.1 ± 0.1
14	223 bar/60 °C/47 min	5.25 ± 0.02	6.0 ± 0.0
	Δ	**−0.36**	**−0.1**
	Raw	5.41 ± 0.01	6.2 ± 0.1
15	223 bar/60 °C/30 min	5.24 ± 0.01	6.2 ± 0.1
	Δ	**−0.17**	**0.0**
	Raw	5.24 ± 0.01	5.6 ± 0.1
16	223 bar/60 °C/30 min	5.19 ± 0.01	5.5 ± 0.1
	Δ	**−0.05**	**−0.1**
	Raw	4.56 ± 0.02	4.8 ± 0.1
17	223 bar/60 °C/30 min	4.53 ± 0.02	4.6 ± 0.1
	Δ	**−0.03**	**−0.2**

Mean values of three replicates ± standard deviation. Δ = variation.

To obtain an approximate statistical inference, three trials were carried out at the central point of the experimental space, which provide information on the behavior of the responses between the levels assigned to the factors, and above all, demonstrate the repeatability of the process [25].

2.2. Physicochemical, Enzymic, and Color Tests

Both the freshly extracted and the processed coconut water fractions were subjected to the pH and soluble solids measurement, enzymic assays (POD and PPO activity) and color analysis, in triplicate.

2.2.1. Determination of pH and Soluble Solids

The pH was determined using an pHmeter Akso (São Leopoldo, RS, Brazil) and the soluble solids content (expressed in °Brix) was determined using a Reichert model AR 200 digital portable refractometer (Woonsocket, RI, USA).

2.2.2. Enzymic Tests

The determination of PPO and POD activity was carried out according to the methodology described by [26] using catechol as the phenolic substrate for PPO and guaiacol for POD. A sample without the coconut water was used as a blank. PPO and POD activities were expressed as U, with one unit (U) being equivalent to a 0.001 variation in absorbance per minute.

To analyze PPO activity (whose optimum activity is found at pH 6.0 at 25 °C), test tubes with lids containing 5.5 mL of phosphate buffer (0.2 M and pH 6.0) and 1.5 mL of a 0.2 M catechol solution were immersed in a water bath at 25 °C for 10 min. After the temperature stabilized, 1 mL of coconut water was transferred to a test tube, and the mixture was homogenized for 10 s in a Fisatom vortex tube shaker and incubated at 25 °C for 30 min. The absorbance was immediately read at 425 nm using a Jenway 7305 spectrophotometer.

To determine POD activity, test tubes containing 7 mL of phosphate buffer (0.2 M and pH 5.5) and 1 mL of coconut water were immersed in a water bath at 35 °C for 10 min. Once the temperature had stabilized, 1.5 mL of 0.05% guaiacol and 0.5 mL of 0.1% hydrogen peroxide were added to the tubes. The mixture was homogenized in a test tube shaker for 10 s and incubated in a water bath at 35 °C for 15 min. Then, the absorbance was read at 470 nm.

2.2.3. Instrumental Color Analysis

As the fresh coconut water is a translucent liquid (almost colorless), the color measurement was performed with the samples arranged in Petri dishes under a standardized white background. The color parameters were determined using the CIELab system in a HunterLab Aeros light reflection color spectrophotometer (Reston, VA, USA) with illuminant parameters of D65 and an observation angle of 10°. The parameters L*, a*, and b* were used to calculate the total color difference between the fresh and processed samples (TCD*, Equation (1)), chroma (C*, Equation (2)), and °hue (Equation (3)). The °hue represents the color classification (yellow, blue, green, red, etc.), and chroma denotes saturation (vivid or faded color) [27].

$$\mathrm{TCD}^* = \left(\Delta \mathrm{L}^{*2} + \Delta \mathrm{a}^{*2} + \Delta \mathrm{b}^{*2}\right)^{\frac{1}{2}} \tag{1}$$

$$\mathrm{C}^* = \left(\mathrm{a}^{*2} + \mathrm{b}^{*2}\right)^{\frac{1}{2}} \tag{2}$$

$$°\mathrm{hue} = \arctan\left(\frac{\mathrm{b}^*}{\mathrm{a}^*}\right) \tag{3}$$

where:

L*—lightness (0 to 100);

a*— red (+60)/green (−60) coordinate;
b*—yellow (+60)/blue (−60) coordinate;
ΔL*—lightness variation;

Δa*—red/green variation;
Δb*—yellow/blue variation.

The L*, a*, and b* parameters were entered into the EasyRGB color calculator (https://www.easyrgb.com/en/convert.php), accessed on 5 February 2024, to obtain the color of the samples.

2.2.4. Statistical Analysis of Data

Data were subjected to the analysis of effects to find the variables (P, T, and t) that had significant effect on the responses (PPO reduction, POD reduction, total color difference, pH and soluble solids variation), at 10% of significance. Protimiza Experimental Design (http://experimental-design.protimiza.com.br, accessed on 5 February 2024) software (Campinas, SP, Brazil) was used to carried out the statistical tests. All analytical measurements were conducted in triplicate.

3. Results and Discussion

3.1. pH and Soluble Solids

Table 2 points out the pH values and soluble solids determined in the fresh and processed samples.

The pH is a crucial physicochemical parameter to monitor in processed beverages due to its effect on physical and chemical stability, as well as sensory acceptance. Post-processing changes can affect these properties and, ultimately, the product's stability [28].

The pH values ranged from 4.46 to 6.30 for the fresh coconut water (5.09 on average) and from 4.40 to 5.66 for the processed water (4.90 on average), showing a reduction in this parameter. Test 4 (310 bar/75 °C/20 min) resulted in the greatest variation (ΔpH = −0.70) between fresh and processed coconut water, while the smallest variation (ΔpH = −0.02) was observed in test 12 (223 bar/ 85 °C/ 30 min). The pH values found in this study are in line with those of [26], who reported levels ranging from 4.70 to 6.40. The reduction in the pH associated with SC-CO_2 is expected—and also reported by [7,8,11]—as a result of the formation of carbonic acid when CO_2 is dissolved into the sample. This effect is enhanced by the supercritical state of the fluid. Another factor that influences the pH of coconut water, and which can explain the variations in this parameter, is the stage of ripeness of the fruit as the pH of coconut water increases with the stage of ripeness, ranging from 4.5–5.3 in young coconuts (7–9 months) to 5.3–5.8 in mature fruit (10–13 months) [1]. Despite these limits, pH values greater than 5.8 (as determined in trials 4 and 7) were reported by [8], who found an average pH of 6.13 for fresh coconut water.

With regard to the soluble solids content, values between 4.0 and 6.6 ºBrix were obtained for fresh coconut water (5.5 °Brix on average) and between 4.0 and 6.4 for processed coconut water (mean 5.4 °Brix on average). The variations caused by the treatments were between 0.0 and 0.3, with a slight reduction in this parameter. [26] found values at 20 °C between 4.46 and 7.02 °Brix in fresh tender coconut water, with an average of 5.34 °Brix; therefore, the data from this research are within the range reported in the literature. The variations in soluble solids between fresh and processed coconut water were minimal (Δ ≤ 0.3), as also reported by [9].

3.2. Enzymic Assays

The activities of the endogenous enzymes PPO and POD, as well as the percentages of reduction achieved by the different treatments, are gathered in Table 3.

Table 3. Polyphenol oxidase (PPO) and peroxidase (POD) activities (U) in green coconut water treated with SC-CO_2.

Trial	Treatment	PPO	POD
1	raw	2.9 ± 0.1	65 ± 4
	135 bar/45 °C/20 min	1.7 ± 0.1	4 ± 1
	red (%)	**41.8**	**93.5**
2	raw	2.3 ± 0.2	79 ± 3
	310 bar/45 °C/20 min	1.7 ± 0.2	8 ± 2
	red (%)	**25.8**	**89.7**
3	raw	4.0 ± 0.3	59 ± 1
	135 bar/75 °C/20 min	0.02 ± 0.04	0.8 ± 0.1
	red (%)	**99.4**	**98.7**
4	raw	2.20 ± 0.00	91 ± 3
	310 bar/75 °C/20 min	0.54 ± 0.02	0.90 ± 0.04
	red (%)	**75.6**	**99.0**
5	raw	2.7 ± 0.4	45 ± 1
	135 bar/45 °C/40 min	2.6 ± 0.5	13.5 ± 0.7
	red (%)	**3.7**	**69.8**
6	raw	2.7 ± 0.5	50 ± 3
	310 bar/45 °C/40 min	1.2 ± 0.5	3.1 ± 0.6
	red (%)	**55.6**	**93.9**
7	raw	3.2 ± 0.7	99 ± 4
	135 bar/75 °C/40 min	1.9 ± 0.1	4 ± 1
	red (%)	**40.1**	**96.3**
8	raw	2.4 ± 0.2	33 ± 4
	310 bar/75 °C/40 min	0.0 ± 0.0	0.3 ± 0.2
	red (%)	**100**	**99.2**
9	raw	2.5 ± 0.2	58 ± 1
	75 bar/60 °C/30 min	1.3 ± 0.1	33 ± 5
	red (%)	**46.2**	**43.4**
10	raw	1.7 ± 0.2	61.0 ± 0.5
	370 bar/60 °C/30 min	0.0 ± 0.0	0.0 ± 0.0
	red (%)	**100**	**100**
11	raw	2.6 ± 0.2	41 ± 2
	223 bar/35 °C/30 min	0.40 ± 0.06	3.58 ± 0.04
	red (%)	**84.5**	**91.2**
12	raw	2.0 ± 0.5	51 ± 3
	223 bar/ 85 °C/ 30 min	0.0 ± 0.0	0.0 ± 0.0
	red (%)	**100**	**100**
13	raw	1.3 ± 0.2	23 ± 34
	223 bar/60 °C/13 min	0.0 ± 0.0	1.0 ± 0.7
	red (%)	**100**	**95.7**
14	raw	6.6 ± 0.3	81.1 ± 0.7
	223 bar/60 °C/47 min	1.56 ± 0.08	14.5 ± 1.0
	red (%)	**76.3**	**82.2**
15	raw	1.7 ± 0.2	52 ± 2
	223 bar/60 °C/30 min	1.3 ± 0.1	13 ± 1

	red (%)	**20.8**	**75.1**
16	raw	0.8 ± 0.2	28 ± 3
	223 bar/60 °C/30 min	0.4 ± 0.2	12 ± 1
	red (%)	**50.0**	**56.2**
17	raw	3.2 ± 0.5	43 ± 1
	223 bar/60 °C/30 min	2.5 ± 0.3	20 ± 2
	red (%)	**21.9**	**53.6**

Mean values of three replicates ± standard deviation. Red (%) = percentage reduction.

The results in Table 3 demonstrate the potential of SC-CO_2 with mild temperature assistance to inactivate the endogenous enzymes responsible for undesirable changes in the color, taste, and nutritional value of coconut water. The percentages of reduction in PPO (3.7 to 100%) and POD (43.4 to 100%) activities varied widely and differently. In 11 of the 17 trials, PPO showed greater resistance than POD; in 4 of them, POD showed greater resistance; and in 2 trials, both showed the same resistance. Refs. [1,29] reported that PPO activity is higher than that of POD in tender coconut water, contrasting with the results of the present study. However, the authors observed that PPO exhibited greater thermoresistance compared to POD, which corroborates most of the results herein. For this reason, PPO is considered an indicator for heat treatment aimed at the enzymic stabilization of coconut water.

The data from trials 3 (135 bar/75 °C/20 min), 8 (310 bar/75 °C/40 min), 9 (75 bar/60 °C/30 min), and 13 (223 bar/60 °C/13 min) indicated a lower percentage reduction in POD, showing that it is more resistant to the action of SC-CO_2 and temperatures equivalent to 60 and 75 °C. Similar results were reported by [5] when analyzing the thermal inactivation of POD and PPO in tender coconut water. Higher decimal reduction times at 87 °C (D87 °C) were obtained for the thermolabile and thermoresistant fractions of POD as compared to PPO.

A study carried out by [16] examined pomegranate juice treated with SC-CO_2 (160 bar/45 °C/40 min) and ascorbic acid (0.1%). Ascorbic acid (AA) exhibited a strong inhibition of enzymes. SC-CO_2 treatment resulted in a significant reduction (69%) of POD activity but failed to completely inactivate it in the absence of AA.

Of particular relevance is that the trinomials applied in trials 10 (370 bar/60 °C/30 min) and 12 (223 bar/ 85 °C/30 min) achieved full enzyme inactivation. On the other hand, trial 5 (135 bar/45 °C/40 min) resulted in the lowest percentage (3.7%) of PPO reduction, and trial 9 (75 bar/60 °C/30 min) resulted in the lowest percentage (43.4%) of POD reduction. These data suggest, in both cases, that lower pressure and/or temperature, even for longer holding times, have a low effect in enzymes′ inactivation.

3.3. Instrumental Color Analysis

The instrumentally measured color parameters are shown in Table 4. Luminosity (*L), hue (°hue), and saturation (C*) are the three color attributes that, taken together, form a three-dimensional solid used to compare the color of samples [27].

Table 4. Color parameters determined in green coconut water treated with SC-CO_2.

Trial	Treatment	L*	a*	b*	Chroma	°hue	Color
1	raw	92.41 ± 0.02	−0.08 ± 0.00	−3.87 ± 0.02	3.87	268.82	
	135 bar/45 °C/20 min	91.12 ± 0.01	−0.02 ± 0.00	0.25 ± 0.01	0.25	274.51	
2	raw	93.16 ± 0.02	0.06 ± 0.00	−5.18 ± 0.01	5.18	270.66	
	310 bar/45 °C/20 min	93.03 ± 0.01	0.03 ± 0.00	−4.97 ± 0.01	4.97	270.35	
3	raw	93.34 ± 0.03	0.26 ± 0.01	−5.36 ± 0.02	5.37	272.74	
	135 bar/75 °C/20 min	91.8 ± 0.6	0.79 ± 0.01	−4.01 ± 0.03	4.09	281.19	

4	raw	93.62 ± 0.03	0.03 ± 0.01	−5.55 ± 0.01	5.55	270.28	
	310 bar/75 °C/20 min	92.86 ± 0.01	0.03 ± 0.00	−4.31 ± 0.01	4.31	270.40	
5	raw	93.59 ± 0.01	0.17 ± 0.01	−5.60 ± 0.01	5.60	271.77	
	135 bar/45 °C/40 min	93.49 ± 0.01	0.17 ± 0.00	−5.43 ± 0.00	5.43	271.79	
6	raw	93.5 ± 0.1	0.02 ± 0.00	−5.79 ± 0.01	5.79	270.20	
	310 bar/45 °C/40 min	93.43 ± 0.01	0.05 ± 0.00	−5.53 ± 0.01	5.53	270.52	
Trial	Treatment	L*	a*	b*	Chroma	°hue	Color
7	raw	91.62 ± 0.05	0.00 ± 0.01	−3.84 ± 0.02	3.84	269.95	
	135 bar/75 °C/40 min	91.57 ± 0.01	−0.04 ± 0.00	−3.55 ± 0.01	3.55	269.41	
8	raw	93.56 ± 0.00	0.04 ± 0.01	−5.61 ± 0.00	5.61	270.44	
	310 bar/75 °C/40 min	93.30 ± 0.01	−0.04 ± 0.01	−5.19 ± 0.00	5.19	269.56	
9	raw	93.31 ± 0.01	0.02 ± 0.00	−5.51 ± 0.01	5.51	270.21	
	75 bar/60 °C/30 min	93.22 ± 0.00	−0.01 ± 0.00	−5.37 ± 0.00	5.37	269.89	
10	raw	93.07 ± 0.01	0.06 ± 0.00	−5.43 ± 0.00	5.43	270.63	
	370 bar/60 °C/30 min	93.17 ± 0.00	0.02 ± 0.00	−5.28 ± 0.00	5.28	270.22	
11	raw	93.07 ± 0.01	0.02 ± 0.00	−5.47 ± 0.00	5.47	270.21	
	223 bar/35 °C/30 min	92.91 ± 0.01	0.06 ± 0.01	−5.23 ± 0.00	5.23	270.69	
12	raw	93.32 ± 0.01	−0.01 ± 0.00	−5.58 ± 0.00	5.58	269.90	
	223 bar/85 °C/30 min	93.33 ± 0.01	−0.14 ± 0.01	−5.21 ± 0.00	5.21	268.50	
Trial	Treatment	L*	a*	b*	Chroma	°hue	Color
13	raw	93.19 ± 0.01	−0.01 ± 0.00	−5.35 ± 0.00	5.35	269.89	
	223 bar/60 °C/13 min	93.00 ± 0.01	−0.01 ± 0.01	−5.56 ± 0.00	5.56	269.90	
14	raw	91.93 ± 0.01	0.27 ± 0.00	−1.95 ± 0.00	1.97	277.98	
	223 bar/60 °C/47 min	92.50 ± 0.01	0.03 ± 0.01	−4.06 ± 0.00	4.06	270.42	
15	raw	93.32 ± 0.01	−0.15 ± 0.00	−5.04 ± 0.00	5.05	268.30	
	223 bar/60 °C/30 min	92.84 ± 0.01	−0.16 ± 0.01	−4.95 ± 0.00	4.95	268.11	
16	raw	92.52 ± 0.01	0.11 ± 0.00	−6.40 ± 0.00	6.40	270.96	
	223 bar/60 °C/30 min	92.55 ± 0.01	0.00 ± 0.01	−6.17 ± 0.00	6.17	270.03	
17	raw	92.84 ± 0.01	0.09 ± 0.00	−5.77 ± 0.00	5.77	270.89	
	223 bar/60 °C/30 min	92.75 ± 0.01	0.03 ± 0.01	−5.57 ± 0.00	5.57	270.27	

L* (lightness) = 0 (black); 100 (white); +a* = red; −a* = green; +b* = yellow; −b* = blue. Mean values of 3 replicates ± standard deviation. $\text{Chroma} = (a^{*2} + b^{*2})^{\frac{1}{2}}$; $°\text{hue} = \arctan\left(\frac{b^*}{a^*}\right)$.

The L parameter varied very little between the fresh (91.62 to 93.62) and processed (91.12 to 93.49) samples, with averages of 93.02 and 92.76, respectively. Most of the treatments resulted in a decrease in the L* parameter, indicating that the fresh samples were slightly lighter than the processed ones. [9] also observed a slight reduction in the L* parameter in coconut water samples processed with SC-CO_2.

The chroma (C*) parameter, which denotes the saturation of the sample, showed a reduction with processing in 15 of the 17 trials, with averages equivalent to 5.14 and 4.75 for the fresh and processed samples, respectively. These results differ from those reported by [9] (0.52 and 1.03), indicating an increase in sample saturation with processing. The variety, the stage of ripeness of the fruit and the parameters used in processing may explain the differences observed between the studies.

With regard to °hue, which indicates the color of the samples, the average values for fresh (270.81°) and processed coconut water (270.93°) were very similar. The variations in this parameter for fresh coconut water was lower (268.30 to 277.98°) than the variation found in the processed sample (268.11 to 281.19°). Despite this, all trials triggered subtle changes in °hue after processing, indicating that there was no major change in this parameter of the fresh (raw) coconut water, with all the samples being positioned at the threshold between quadrants III and IV of the color circle, and all of them could be classified as bluish.

To better observe the effect of processing with SC-CO_2 on the color of coconut water, the total color difference (TCD) between the fresh and processed samples was calculated (Figure 2).

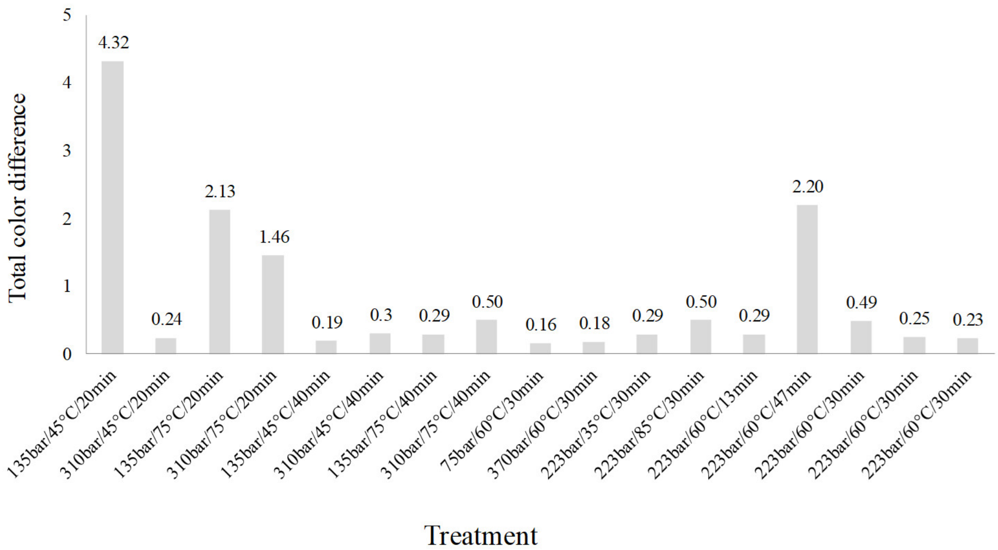

Figure 2. Total color difference between fresh and processed tender coconut water.

The highest (4.32) and lowest (0.16) TCD values were obtained in treatment 1 (135 bar/45 °C/20 min) and 9 (75 bar/60 °C/30 min), respectively. According to [30], if two objects are positioned side by side in a controlled environment, the smallest color difference detected by human observers is 1; however, [31] found that, under industrial conditions, the total color difference must approach 3 or more for the human eye to detect any difference. Given this, of the 17 trials carried out, 4 resulted in changes greater than 1, and only one was greater than 3.

3.4. Statistical Analysis

Figure 3 gathers the Pareto diagrams, indicating the variables (pressure/P/x1, temperature/T/x2, and holding time/t/x3) that had a significant effect ($p \leq 0.10$) on the responses (reduction in peroxidase/%redPOD, reduction in polyphenol oxidase/%redPPO, pH variation/ΔpH, soluble solids variation/ΔSS, and total color difference/TCD).

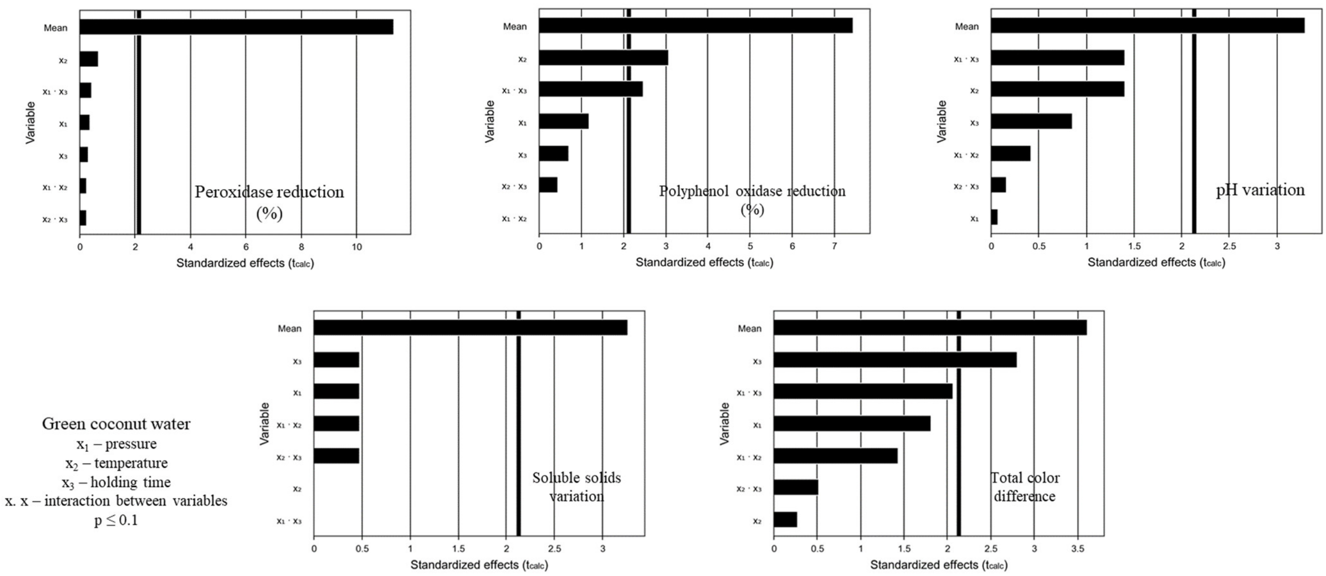

Figure 3. Diagrams of the effects for coconut water treated with SC-CO_2.

Figure 3 shows that no variable (P, T, or t), nor the interactions among them, had an effect ($p > 0.1$) on the POD reduction, pH variation, and soluble solids variation. As for the PPO reduction, T and the interaction between P and t had a significant effect. With regards to the TCD, only t was significant.

The analysis of variance (ANOVA) performed herein indicated that the re-parameterized mathematical models generated for the different responses (PPO reduction, POD reduction, total color difference, pH, and soluble solids variation) were not significant. For this reason, the response surfaces were not built. Notwithstanding, the combination of SC-CO_2 and mild temperatures could potentially be used to deactivate deteriorating endogenous enzymes in tender coconut water.

4. Discussion

The findings herein indicated the viability of supercritical carbon dioxide (SC-CO_2) treatment assisted with mild temperatures to deactivate PPO and POD in tender coconut water (TCW). Small variations in pH and soluble solids content were found between fresh and processed coconut water; however, they were not significant. Only the holding time (t) in the range studied played a significant effect in altering the TCW´s color. There was also a significant and positive effect of temperature and the interaction between CO_2 pressure and t in reducing the PPO activity. Further studies could be performed to evaluate the microbicidal effect of SC-CO_2 on TCW.

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