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Effect of Sulphite and UHT Sterilization Parameters in the Coconut Water Quality

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Coconut water (*Cocus nucifera* L.) has been widely exploited in recent years, with the UHT sterilization process (Ultra High Temperature) as the greatest economic impact for this sector. In a long period of storage, the color and quality of the product may change, impacting the visual aspect and influencing consumer acceptance.The aim of this study was to optimize the processing conditions, including temperature, retention time and sulphite content added. A central composite design (CCD) was performed, with temperature (128 °C to 142 °C), retention time (4 to 14 seconds) and sulphite addition (up to 50 mg.L^{-1}) as independent variables, and the chemical and physical analysis as dependent variables. Hunter color, enzyme activity and turbidity were performed in 24 h and after 45 days storage at 25 ºC. The results after one day storage, showed residual sulphite correlated only with the initial amount added, lowering around 20 %, allowing generate a response surface model. After 45 days it was found that the b* value and sulphite showed a significant effect with respect to the temperature level and added sulphite, respectively. Coconut water before processing presented polyphenoloxidase activity and no peroxidase activity, being inactivated in the UHT processing trials. In conclusion, all the levels used allow evaluate the processing effects in coconut water quality parameters, being effective in the polyphenoloxidase inactivation during storage.

1. Introduction

Coconut water is marketed as fresh fruit and some problems are related to transportation and storage, making it difficult to export (Matsui, 2006). Coconut water should be consumed as a beverage at the opening of the fruit because in contact with oxygen, and exposition to the action of microorganisms in the environment, their peroxidase (POD) and polyphenoloxidase (PPO) cause undesirable changes that affect the color and flavor (Murasaki-Aliberti et al., 2009). The pink color can also occur during storage, reducing the acceptability by consumers.

The UHT (Ultra High Temperature) sterilization processing is effective in the microbiological and enzymatic reduction, however, sensory and nutritional changes are usually presented, which compromises the quality and acceptance of the final product (Campos et al., 1996; Tan et al., 2014).

Sterilization associated with the use of sulphite has been adopted by the industry to increase the shelf life of the product. The sulphite is widely used to prevent browning caused by enzymatic or oxidative reactions, but due to its adverse effects on health, the World Health Organization (WHO) limits the use of sulfur dioxide (SO_2) in processed food products to a daily dose maximum of 0.7 mg.kg^{-1} body weight (Queiroz et al., 2008) and the Brazilian legislation through the RDC 08/2013 establishes 0.005 g of residual SO_2 limit in 100 mL coconut water (Brasil, 2013).

The objective of this study was to evaluate the effect of UHT sterilization parameters (temperature and retention time) and the added sulphite in the quality of coconut water after process and storage for 45 days.

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2. Materials and Methods

Green coconuts were used (*Cocus nucifera L.*), Jequi cultivar, in the stage six of maturity (harvested between sixth and seventh months of maturity). Coconuts were cleaned in a chlorine solution, cut for water extraction, filtered and frozen at -17 ± 2 °C for later use in the experiments. In each experiment, an amount of coconut water was thawed and added with sodium metabisulphite (Vetec) to reach sulphite content up to 50 mg L^{-1}. The UHT sterilization process was performed according to the experimental design, using temperatures between 128 °C and 142 °C, and flow controlled to a retention time from 4 to 14 seconds using an Armfield tubular heat exchanger, model FT74, and water cooling recirculation system (chiller) Armfield FT63 and filling under aseptic conditions.

2.1 Experimental Design

It was used a central composite design (CCD) with three independent variables (2^3) and α = 1.68. The levels used where x_1 = temperature (128; 131; 135; 139 and 142 ºC); x_2 = retention time (4; 6; 9; 12 and 14 seconds); x_3 = added sulphite (0; 10; 25; 40 and 50 mg.L^{-1}) and ten dependent variables (pH, SST, acidity, color (L*, a*, b*), PPO, POD, residual SO_2, turbidity), totaling 17 experiments. The retention time and temperature limits were based on preliminary tests and lethality rate (F_o value) in the UHT processing, while the sulphite amounts limit follow a maximum of 50 mg.L^{-1} (Brasil, 2013). The results were analyzed using the Statistica 7.0 software for calculation of the regression coefficients, analysis of variance (ANOVA) and surface responses, and was set the significance level of 5 %.

2.2 Analysis of coconut water

The pH was measured at 25 °C, according to AOAC (1995); Titratable acidity and total solids in accordance with the analytical standards of the Instituto Adolfo Lutz (2008); The color measurements were performed in a Konica Minolta CR - 400 colorimeter using the CIELAB color system (L*, a* and b* parameters). The polyphenol oxidase (PPO) and peroxidase (POD) activities were performed according to a method adapted by Campos et al. (1996) using catechol as a substrate for PPO and guaiacol for POD activity. A UV/visible spectrophotometer (Biospectro) was used for the measures and expressed as enzyme activity unit (U) per mL of solution. The sulphite analyze was performed according to the AOAC official method 990.28 (AOAC, 1995) and the turbidity was measured using a digital turbidimeter Tecnopon TB-1000.

3. Results and Discussion

The results for *in natura* coconut water showed pH 5.01, 6.5 °Brix for soluble solids and titratable acidity of 0.13 g.100 mL^{-1} expressed as citric acid. With these results, it was not necessary a correction of soluble solids (6.0 or higher) and pH (5.0 or less) according to values from Abreu (2005). The PPO activity was 4.23 U.mL^{-1}, and was observed absence of POD activity. Following are presented and discussed the CCD results after UHT processing and storage of bottled coconut water for 1 to 45 days at room temperature (25 - 30 °C).

3.1 Effect of the UHT processing in the coconut water after 24-h storage

After one day of storage at room temperature, in the results for the CCD treatments, it was observed that the pH and soluble solids showed no differences (Table 1) as well as the PPO and POD enzyme activity, which remained close to zero in the most assays. In the raw coconut water was quantified enzyme activity, and similar behavior was observed by Abreu and Faria (2007), who found 2.33 U.mL^{-1} for the PPO. Thus, it can be concluded that the all UHT treatments were effective in PPO inactivation, with or without the sulphite addition.

The CCD results showed a significant effect only for the added sulphite as independent variable ($p < 0.05$ and $R^2 = 0.95$). The average values of residual sulphite were proportional only to the initial content, with around 25 % reduction compared to the amount originally added, while the temperature and UHT retention time were not significant. An analysis of variance (ANOVA) for the residual sulphite content (residual SO_2) was carried out eliminating non-significant parameters ($p > 0.05$) (Table 2). The F value is highly significant and the percentage of variance explained by the model was about 91 %, and we can conclude that the model fits well to the experimental data, allowing the construction of response surface (Figure 1). The model where x_3 variable represents the added sulphite in mg L^{-1} as a function of retention time and sulphite added to the coconut water in the studied range, is as follows:

$$\text{Residual } SO_2 = 0.74x_3 + 0.82 \tag{1}$$

The color parameters (L*, a*, b*), turbidity, POD and PPO indicated no significant effect ($p > 0.05$) or had determination coefficient (R^2) lower than 0.8. Thus, the statistical models cannot be constructed, however, can be verified through the analysis of individual data, a tendency towards browning of the sample (lower L*) with increasing retention time; and lower a* values with increasing process temperature; as well as higher values of the b* parameter with increasing sulphite content. The analysis of the results and visual observations indicated no visible changes except for the turbidity in the fourth test samples that showed a higher turbidity, 33.6 NTU, but could not be related to UHT treatment parameters or added sulphite. The turbidity values ranged from 17 to 33 NTU, comparable to those obtained in study of Pereira et al. (2013), who observed average values of 33.88 for turbidity, marginally higher than those found in this study.

Table1: CCD results after one day of the UHT processing

Trials	x_1	x_2	x_3	pH	SS	Acidity	L*	a*	b*	PPO	POD	SO_2	TB**
1	131	6	10	5.61	6.4	0.15	45.15	0.10	1.33	0.0	0.0	6.4	18.6
2	139	6	10	5.71	5.9	0.13	45.06	-0.03	0.60	0.0	0.0	8.0	18.5
3	131	12	10	5.13	5.9	0.21	44.21	0.15	0.62	0.0	0.0	8.0	22.8
4	139	12	10	5.14	5.9	0.17	43.75	0.17	0.83	0,0	0.0	8.0	33.6
5	131	6	40	5.64	6.5	0.19	45.28	0.03	1.89	0.0	0.0	21.3	18.8
6	139	6	40	5.63	6.3	0.21	44.67	0.05	1.19	0.0	0.0	33.1	18.1
7	131	12	40	5.12	5.9	0.25	43.78	-0.03	1.23	0.0	0.0	32.0	22.6
8	139	12	40	5.11	5.9	0.27	43.97	0.08	0.88	0.0	0.1	29.9	22.9
9	128	9	25	5.70	6.0	0.17	44.07	-0.08	1.71	0.0	0.0	18.1	17.0
10	142	9	25	5.68	6.4	0.17	44.53	-0.02	1.42	0.0	0.1	13.9	17.9
11	135	4	25	5.16	6.4	0.23	44.70	0.27	0.70	0.0	0.1	20.3	23.9
12	135	14	25	5.12	5.9	0.21	43.79	0.12	0.87	0.0	0.0	18.1	23.7
13	135	9	0	5.68	5.8	0.13	44.30	-0.01	0.70	0.0	0.0	0. 0	22.0
14	135	9	50	5.61	5.8	0.17	45.02	-0.03	1.06	0.0	0.0	34.7	20.7
15	135	9	25	5.61	6.0	0.13	45.23	0.02	0.27	0.0	0.0	16.5	21.2
16	135	9	25	5.13	6.5	0.25	44.80	0.22	0.86	0.0	0.0	22.4	22.4
17	135	9	25	5.13	6.5	0.26	44.80	0.22	0.78	0.0	0.0	22.9	22.0
Control: *in natura* coconut water				5.01	6.5	0.13	44.63	0.12	-0.16	4.23	0.00	0.0	22.1

Note: the results are expressed in mean (n = 3). (SO_2) residual sulphite; (**) Turbidity (NTU);
The independent variables and level (α) used were: x_1= temperature (128; 131; 135; 139 and 142 ºC); x_2= retention time (4; 6; 9; 12 and 14 seconds); x_3= added sulphite (0; 10; 25; 40 and 50 mg.L^{-1})

Table 2: Analysis of variance (ANOVA) for the residual sulphite content in coconut water

Factor	SS	DF	MS	$F_{calc.}$	F_{tab}
Regression	1522.26	1	1522.26	148.27	4.54
Error	153.99	15	10.27		
Total SS	1676.26	16			

R^2 = 0.95; $R^2_{adjusted}$ = 0.92.

3.2 Effect of the UHT processing in the coconut water after 45 days of storage

After 45 days of storage at room temperature, the UHT processed coconut water showed little change, and the results are shown in Table 3, being significant only for the b* color parameter and residual sulphite. In these results, it was observed that turbidity increased in the storage period from 17 - 33 NTU up to 74 NTU, without significant relation to UHT process parameters. The same behavior was observed in the luminosity (L*), which decreased in some of the tests, with a tendency for slight darkening. Regarding the PPO and POD enzymes, only in some trials they were active, but still less than 1.2 U.mL^{-1}. This does not indicate a reactivation of the enzymes, and comparing with that found by Pereira et al. (2013) by analyzing the carbonated coconut water added with sulphite and potassium sorbate at different concentrations, showing that the thermal process applied to the samples was enough to inactivate the enzymes. Murasaki-Aliberti et al. (2009) evaluated the thermal inactivation of PPO and POD, in temperatures from 75 up to 85 °C, in holding times up to 8 min, which promoted 90 % enzymatic inactivation.

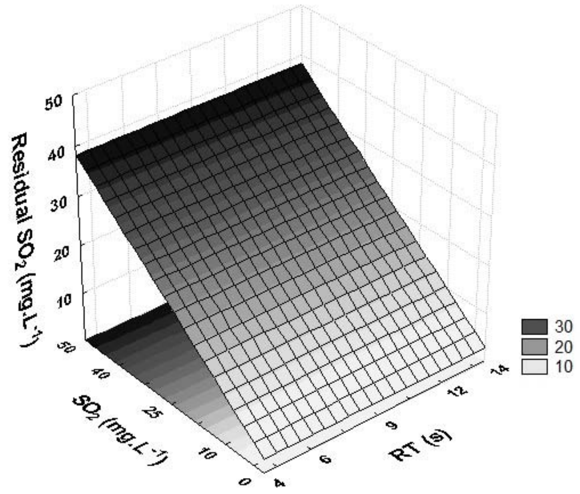

Figure 1 : Response surface for the residual sulphite, where RT is the Retention time

As the values of the b* parameter and SO_2 were significant ($p < 0.05$) and high determination coefficient (0.85 and 0.86, respectively), it was possible to prepare the analysis of variance (ANOVA) shown in Table 4, with the incorporation of non-significant ($p > 0.05$) factors to the residue. The F value is five and six times higher than minimum for the parameters b* and residual SO_2, respectively, being possible to generate the statistical model according to equations 2 and 3, where x_1 and x_3 are the variables of the UHT process, temperature and the initial amount of added sulphite, respectively.

$$b^* = 0.05\, x_1^2 - 13.5\, x_1 + 912.05 \qquad (2)$$

$$\text{Residual } SO_2 = 5.68\, x_3 + 12.68 \qquad (3)$$

Table 3: CCD results after 45 days of the UHT processing

Trials	x_1	x_2	x_3	L*	a*	b*	PPO	POD	SO_2	TB**
1	131	6	10	47.22	-0.11	2.57	0.4	0.0	4.8	55.7
2	139	6	10	25.71	-0.14	0.59	0.1	0.0	5.3	24.2
3	131	12	10	45.39	-0.31	0.95	0.0	0.1	8.5	52.2
4	139	12	10	45.81	-0.37	1.13	0.0	0.7	9.0	46.7
5	131	6	40	46.54	0.08	2.59	0.0	0.0	13.8	23.4
6	139	6	40	46.35	-0.27	2.30	0.0	0.0	10.6	30.4
7	131	12	40	44.83	-0.09	0.87	0.0	0.0	29.9	37.4
8	139	12	40	44.85	-0.39	1.65	0.3	0.0	14.9	41.2
9	128	9	25	45.12	-0.17	3.47	0.0	0.0	14.9	31.0
10	142	9	25	46.84	-0.06	2.73	0.0	0.0	13.9	74.3
11	135	4	25	43.67	0.13	0.47	0.0	1.0	14.9	43.9
12	135	14	25	44.33	-0.29	1.09	0.5	0.0	13.3	34.4
13	135	9	0	25.61	-0.09	0.56	1.2	0.0	0.00	25.8
14	135	9	50	25.60	-0.09	0.57	0.0	0.0	21.3	29.0
15	135	9	25	25.61	-0.07	0.57	0.0	0.2	16.0	33.6
16	135	9	25	44.40	-0.04	0.98	0.0	0.0	11.2	38.7
17	135	9	25	43.07	-0.11	1.46	0.0	0.0	12.8	39.7

Note: the results are expressed in mean (n = 3). (*) residual sulphite; (**) Turbidity (NTU);
The independent variables and level (α) used were: x_1= temperature (128; 131; 135; 139 and 142 ºC); x_2= retention time (4; 6; 9; 12 and 14 seconds); x_3= added sulphite (0; 10; 25; 40 and 50 $mg.L^{-1}$)

The residual sulphite correlated only with the level of added sulphite, similar to the behavior observed at 24-hour storage, with an average 45 % decrease in the residual content after 45 days of storage. The linear term of added sulphite was significant ($p < 0.05$), being possible to establish a predictive first order model, described in Equation 3, and the construction of the response surface shown in Figure 2a, where this parameter increases with higher amounts of sulphite added to the coconut water before UHT processing. Sulphite has high reactivity and large losses occur during storage (Luck and Jager, 1997). Sulphite can be removed from the liquid phase by several mechanisms such as volatilization, stripping and biodegradation (Beghi et al., 2012).The UHT process temperature was significant ($p < 0.05$) in the b* color measurements, as shown in Figure 2b, where at higher temperatures the coconut water show higher values of b*, tending to yellow. This result is consistent with the one indicated by Remacha et al. (1992), indicating that samples exposed to high temperatures are susceptible to browning during storage. The other variables L*, a*, PPO, POD, and turbidity (TB), were not significant ($p > 0.05$) and R^2 determination coefficient lower than 0.80.

Table 4: Analysis of variance (ANOVA) for color (b) and residual sulphite after 45 days storage*

Factor	SS		DF	QM		F_{calc}		F_{tab}
	b*	SO_2		b*	SO_2	b*	SO_2	
Regression	8.40	440.21	1	8.40	440.21	22.07	24.09	4.54
Error	5.71	274.00	15	0.38	18.26			
Total	14.11	714.21	16					
R^2	0.85	0.86						
$R^2_{adjusted.}$	0.59	0.62						

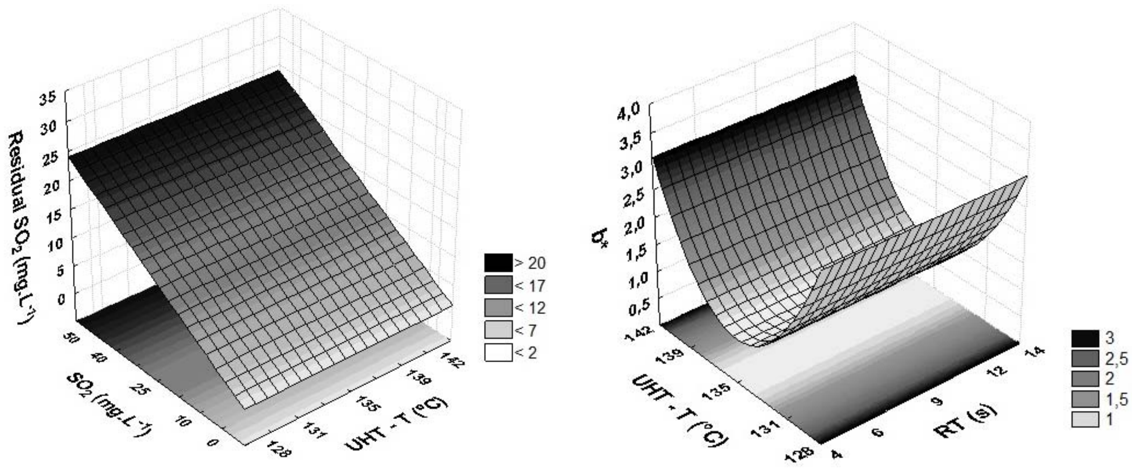

Figure 2: Response surface for residual sulphite (SO_2) (2a) and color - b (2b), where UHT – T is process temperature and RT is the retention time*

4. Conclusions

In conclusion, our present data indicate that 24 h after thermal processing the sulphite content added to the coconut water is the only variable that has influence on the residual sulphite. After 45 days storage, there was a significant effect on the color, dependent variable b*, and residual sulphite content. There was no optimization of the UHT process, but this was effective in reducing the polyphenoloxidase activity during storage.

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