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Effect of UHT Processing Conditions on Color Changes in Sterilized Coconut Water

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Green coconut water preserved by UHT sterilization can be rejected due to a pinkish color forming during storage. This work aimed to evaluate the UHT temperature effect on pinkish color formation and if it is related to polyphenol oxidase (PPO) or peroxidase (POD) enzyme activities. Experiments were carried out with UHT sterilization followed by aseptic filling and storage at room temperature. With the use of UHT temperatures (142, 138, 120 and 110°C), the heat treatment inactivated the PPO and POD enzymes and did not show immediate changes in color, but after 24 h storage, only sub-processed samples (120 and 110ºC) presented pink coloration. The subprocessing treatment at 110 ° C / 8s and without the addition of sulfite, showed samples with a pink color after 24 h of processing. The pink color was not related to the enzymatic activity, and the sodium metabisulfite was effective in avoiding the pink color in the under-processing condition (110 ° C / 8s). Results of another experiment at 136°C and 110°C, a pink coloration was only observed in the treatment performed at 110°C, and no regeneration of POD or PPO activities were observed. The pink color in the UHT sub-processing treatments was not related to the enzymatic activity.

1. Introduction

Green coconut water processed by ultra-high temperature (UHT) sterilization and followed by aseptic packaging has faced problems of enzymatic or microbiological origin, which change the sensory characteristics. The main problems of conserving the coconut water are the color, turbidity, formation of a pinkish or yellowish coloration and flavor alteration.

The appearance of a pinkish color during storage is one of the problems faced by the industry, and Cabral (2002) related it to the enzymatic activity or non-enzymatic reactions, in addition to oxygen being incorporated during processing. Garcia et al. (2007) stated that the pink color in coconut water is due to enzymatic reactions caused by PPO enzyme activity. According to Damar (2006), the pink color formed during coconut water storage is possible due to aeration and accelerated heating and the author also observed that ascorbic acid and sulphite stabilize the color, thus avoiding the pink color appearing, and which is unlikely to be caused due to microbial or enzymatic action, as boiling coconut water did not prevent the pink color formation.

Therefore, the objective of this study was to evaluate the effect of the UHT sterilization severity (temperature and retention time), relating it to the enzyme activity and color changes of the heat-treated samples, and to evaluate possible enzymatic activity regeneration in storing processed coconut water with or without sulphite addition.

2. Material and methods

Green coconuts (*Cocus nucifera*, L.) were purchased in Paraipaba, Ceará State, Brasil, harvested between six to seven months of maturity. The packages used were glass or polyethylene terephthalate (PET) bottles, 210 mL, and 28 mm polypropylene (PP) screw caps.

Several preliminary tests were performed, and the appearance of pink coloration in coconut water was observed at temperatures from 80 to 120 °C (sub-processed samples in the UHT sterilization). It was then

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decided to conduct experiments using sub-processing temperatures (110 °C and 120 °C) and proper sterilization (138 °C and 142° C) to observe the pink color appearance and its association with enzymatic activity and/or the severity of the heat treatment. Two experiments were carried out: Experiment 1: Evaluation of the temperature effect on enzymatic activity and color change in coconut water; Experiment 2: Effect of thermal processing and sulphite use to inactivate the enzymatic activity and control the pinkish color during processed coconut water storage.

UHT sterilization process of coconut water was carried out using an Armfield model FT74 tubular heat exchanger, connected to a chiller and aseptic filling chamber, following the previous system´s sterilization procedures. The packages were sterilized in 1 % peracetic acid solution, and rinsed with sterile water. The heating process severity (temperature and retention time) planned for each treatment was made by adjusting the set point for automatic temperature control and the retention time by controlling the coconut water flow through the feed pump.

2.1 Experiment 1: Evaluation of the temperature effect on enzymatic activity and color change

Four different treatment severities (Table 1, Experiment 1) were performed, of which two treatments (138 and 142 °C) are suitable for commercial sterilization, with F_0 (process lethality) > 3 minutes at 121.1 °C, and two treatments considered as sub processing (110 and 120 °C). The process lethality was calculated according to Smith (2003), with 121.1 ºC being the reference temperature and considering z = 10 ºC as temperature spore resistance of *Clostridium botulinum*. Processing temperatures of 110 °C and 120 °C were tested to evaluate color changes and enzymatic activity in samples and did not reach sufficient lethality for commercial sterilization, as F_0 is less than 3 min.

After sterilization and cooling, the coconut water was bottled under aseptic conditions in PET packages, closed with plastic lids, and then stored at room temperature (28 ± 2 °C) and protected from light, to observe the color changes during storage. Analyses were performed 24 h after UHT processing.

The experiments were performed in triplicate, with intervals of approximately one month between each repetition, and the coconuts were harvested four days before the experiments. A completely randomized block design was used, with analysis of variance and Tukey's test between means ($p < 0.05$).

2.2 Experiment 2: Effect of thermal processing and sulphite use to inactivate enzymes and control the pinkish color during storage of processed coconut water

In this experiment, four treatments, with two process severities (process temperature x retention time) of 136 °C / 8s and 110 °C / 8 s, with and without sulphite addition, were carried out in order to verify enzymatic activity inactivation and its possible regeneration, and relation to the color change during storage. The treatments were named A, B, C and D, according to Table 1 (Experiment 2).

Table 1 : *Coconut water UHT sterilization processing conditions.*

Experiment 1			Experiment 2:		
Treatments	Severity T (ºC) / t (seconds)	Process lethality (F_0) (min)	Treatments	Severity T (ºC) / t (seconds)	Sulphite ($mg.L^{-1}$)
A	110 °C/4,1 s	0,005	A	136 °C/8 s	0
B	120 °C/ 4,1 s	0,05	B	136 °C/8 s	40
C	138 °C/ 5,7 s	4,6	C	110 °C/8 s	0
D	142 °C/ 4,1 s	8,4	D	110 °C/8 s	40

The temperature of 136 °C / 8s was chosen because it most approached the central point defined in previous planning (Sucupira *et al*., 2015) and the sulphite concentration (40 $mg.L^{-1}$) was applied because it is close to the amount allowed by current legislation (50 $mg.L^{-1}$). The UHT processed coconut water was cooled and filled under aseptic conditions in 210 mL glass bottles, closed with plastic caps and stored at room temperature (28 ± 2 °C), protected from light. Treatment samples (A, B, C and D) were evaluated on 1, 7, 14 and 28 days after processing, with analyses of pH, soluble solids, color (L*, a*, b*), PPO and POD enzymatic activities, residual sulphite and turbidity.

Coconuts were opened to provide coconut water for three experiment replicates. The water was extracted and then homogenized, packed in 5 liter plastic bags, and frozen at -18 °C, for use in each experiment repetition. The use of coconuts from the same field and harvested at the same day was to avoid differences in the composition of the coconut water related to the maturation degree. Statistical analysis was conducted by evaluating the mean and standard deviation among the times and treatments.

2.3 Chemical, physicochemical, enzymatic and color analyses

The color evaluation was performed using the CIE LAB system with a CR-400 colorimeter (Konica Minolta Sensing, Inc.) in reflectance mode (L*, a*, b*) according to the methodology described by AOAC (2005). Turbidity was read in a Tecnopon TB-1000 turbidimeter. Quantitative analysis of residual sulphite was performed using the optimized M-W method, as indicated by the AOAC 990.28 method (2005). Enzymatic activity of the polyphenol oxidase (PPO) and peroxidase (POD) were performed according to the methods developed by Ponting and Joslyn (1948) and adapted by Campos et al. (1996). All analyses were performed in triplicate.

3. Results and Discussion

3.1 Experiment 1: Evaluation of temperature effect on enzymatic activity and color change in processed coconut water

The results obtained from the color and enzymatic activity analyses of the fresh coconut water samples before UHT processing and after processing are presented in Table 2. It is observed that the fresh coconut water, without the sterilization process, presented higher values for polyphenol oxidase (PPO) and peroxidase (POD) enzymatic activities, being 9.76 and 13.21 UAE mL^{-1}, respectively. PPO had a higher value than POD, with the standard deviation being higher because the coconut water came from different harvest periods in each replicate. It can be observed that the heat treatment causes a decrease in the enzymatic activity, with values close to the method detection limits, being 0.07 and 0.12 for POD and PPO, respectively.

Table 2 : Results of enzymatic activities and color of fresh and UHT sterilized coconut water.

Treatments	Color			Enzymatic activity[1]	
	L*	a*	b*	POD	PPO
In natura	$28,45^{a} \pm 1,10$	$-0,09^{b} \pm 0,12$	$-1,19^{a} \pm 0,08$	$9,76^{a} \pm 12,70$	$13,21^{a} \pm 21,56$
110 °C/4,1s	$27,87^{a} \pm 2,65$	$0,29^{ab} \pm 0,31$	$-0,15^{a} \pm 0,20$	$1,41^{b} \pm 0,28$	$0,56^{b} \pm 0,97$
120 °C/4,1s	$28,87^{a} \pm 2,52$	$0,53^{a} \pm 0,32$	$-0,16^{a} \pm 0,90$	$0,28^{b} \pm 0,41$	$0,40^{b} \pm 0,69$
138 °C/5,7s	$28,90^{a} \pm 1,57$	$0,12^{ab} \pm 0,19$	$-0,52^{a} \pm 0,36$	$0,03^{b} \pm 0,05$	$0,29^{b} \pm 0,23$
142 °C/4,1s	$29,37^{a} \pm 1,99$	$0,15^{ab} \pm 0,25$	$0,07^{a} \pm 1,04$	$0,36^{b} \pm 0,62$	$0,10^{b} \pm 0,08$

[1] Enzymatic activity units ($UAE.mL^{-1}$). Results as mean of three repetitions ± standard deviation. Different letters following the mean, in the columns, indicate significant difference in Tukey´s test ($p < 0.05$).

Results from the PPO and POD thermal inactivation are in agreement with those reported by Fontan et al. (2012), who verified that peroxidase is heat resistant, but the temperature of 95 °C / 3 min drastically reduced the enzyme activity from 30.23 to 0.53 $UAE.mL^{-1}$. Tan et al. (2014) observed that the heat treatment for peroxidase inactivation in green coconut water was efficient at temperatures above 90 °C, corroborating the findings of Campos et al. (1996).

Results of the color evaluation, performed 24 hours after processing, did not indicate a significant difference ($p > 0.05$) for the parameters L* (luminosity) and b* (blue to yellow). The results of the color parameter a* (green to red) presented a significant difference between the treatment at 120ºC and the *in natura* coconut water ($p < 0.05$). Samples treated at 110 °C, and 120 °C showed higher a* values (0.29 and 0.53), with a tendency towards red. This result was also confirmed by visual observation, as shown in Figure 1, with the formation of a pink color in coconut water samples during storage.

In the visual color evaluation, as indicated in Figure 1, it was observed that the thermal treatments equivalent to sub-processing (110 °C and 120 °C) presented a pink color, whereas the samples with treatment equivalent to the commercial sterilization did not present this color. This indicates that heat treatment temperatures of 110 to 120 °C contribute to the appearance of this color, pointing to a probable chemical reaction which forms the pink pigment. However, it was not possible to associate the color change to the PPO or POD enzymes activity. The pink color appearance in this experiment was not caused by the enzymatic activity, since the treatments that presented this coloration (110 ºC and 120 ºC) exhibited similar enzymatic activity when compared to the non-pink samples (Table 2). It is interesting to note that the color change is time dependent, as shown in Figure 1, occurring 24 to 48 h after thermal processing, and remaining for one to two weeks and then dissipating, or with the colored particles precipitating on the bottom of the package.

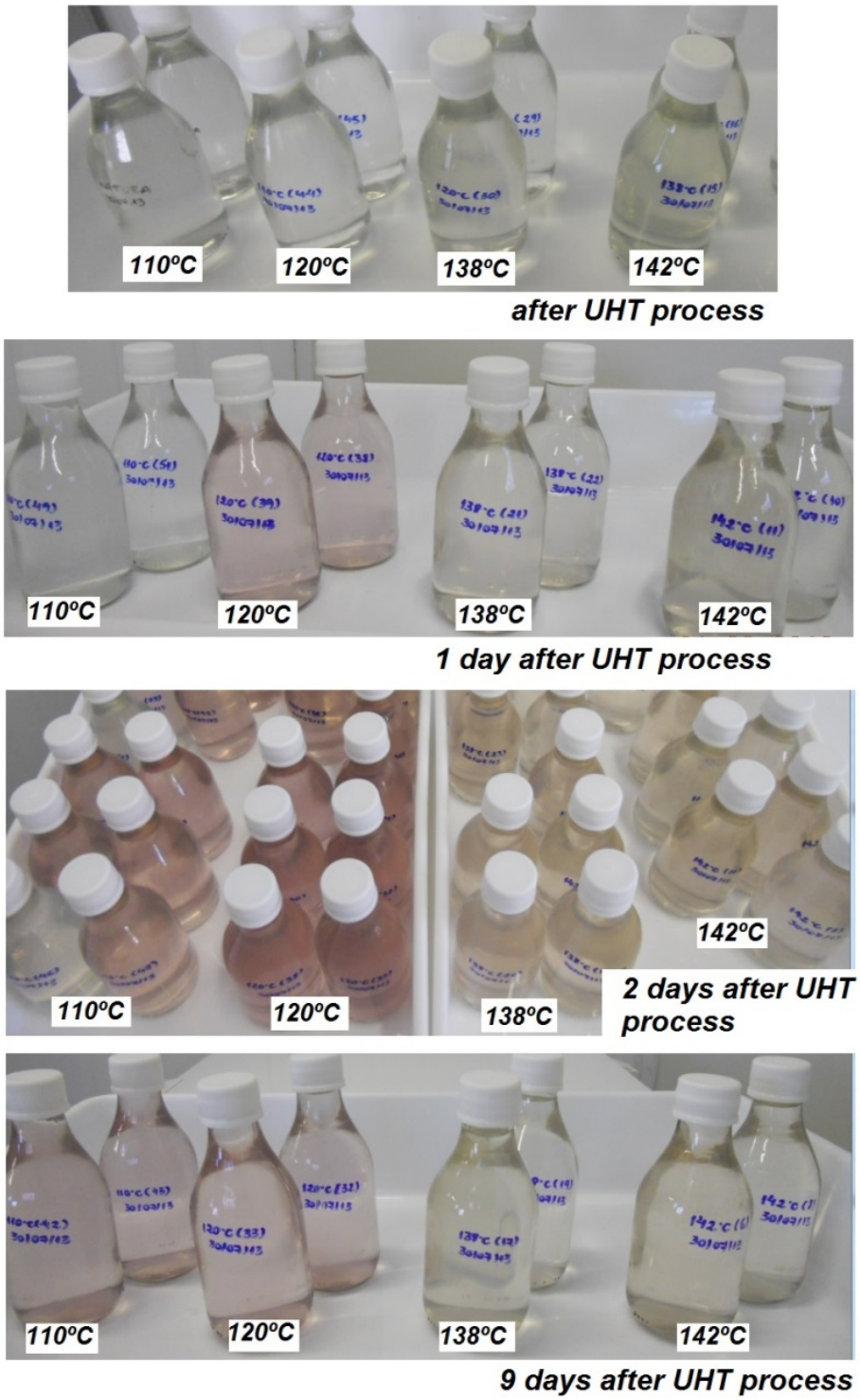

Figure 1 : Coloring of coconut water after different UHT sterilization processing severities and storing at room temperature

3.2 Experiment 2: Effect of thermal processing and sulphite use to inactivate enzymes and control the pink color during processed coconut water storage

The objective of this experiment was to evaluate the possible activation (or not) of PPO and POD enzymatic, as well as to verify sulphite performance in controlling the pink coloration in UHT sub-processing (treatment at 110 °C / 8s without sulphite addition). It was observed that the coconut water presented few variations during

the 28 days of storage, and only C treatment (110 ºC / 8 s) presented a pinkish color change. Only the a* color parameter (0,53) presented variations among treatments and also as a function of storage time (Figure 2), when C treatment differs from A, B and D treatments. The L* values ranged from 45.4 to 47.1, and b* ranged from 1,7 a 3,4, with relative stability during storage, and even when turning pink in treatment C, this was not related to a significant variation in the values of L* or b* parameter.

The average values for the a* parameter shown in Figure 2, treatment C, related to the sub-processing at 110 ºC without sulphite addition, showed higher values (0.26), and with a pink coloration. A decay in the value of this coordinate from 14 to 28 days of storage was also observed. Treatment C showed higher values of a*, differing statistically ($p < 0.05$) from treatments containing sulphite (B and D), and not differing from sample A (136 °C / 8 s, without sulphite addition). Although no statistical difference was observed between treatments A and C, the color change of sample C (processed at 110° C / 8 s without sulphite) was visually verified for the pink color. Delfiya and Thangavel (2016) also observed a pink color one day after ohmic heating process using 100 ºC / 3 min and stored at 25 ºC.

Damar (2006) studied the formation of pink color in stored coconut water and found that aeration and heating favor the emergence whereas ascorbic acid and sulphite stabilize the color variation, preventing the pink color appearance. The author also stated that this color in coconut water due to microbial or enzymatic action is unlikely since boiling the coconut water did not prevent the pink color formation.

The pH results presented values close to 5.0 and soluble solids (ºBrix) ranging from 5.6 to 5.7, with similar behavior and showing no changes during the 28 days of storage at room temperature.

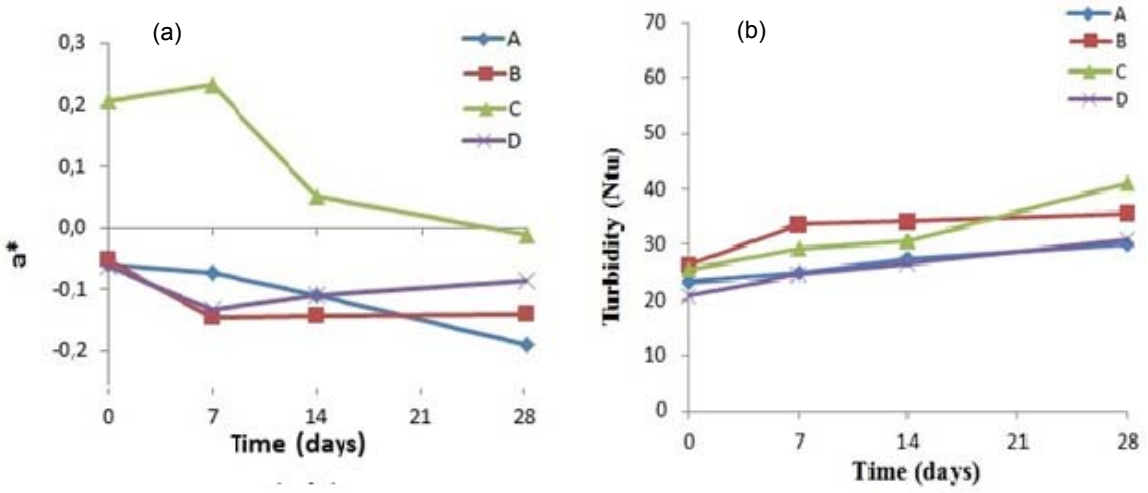

Figure 2 – Evaluation of color parameter a (2a) and turbidity(2b) during storage.*

Regarding the turbidity analysis of coconut water, there was no significant statistical difference between the average values of the treatments over time, but there was an increase in turbidity for all treatments analysed, from an initial value of 25 Ntu after process, to 40 Ntu at the end of the 28 days storage period. Leber (2001) evaluated the turbidity changes of chilled and frozen coconut water throughout storage. Over time, the turbidity of the product increased. Coconut water became more turbid due to the physical-chemical transformations, such as protein aggregation and enzymes performance in phenolic compounds or microbiological contamination.

Enzymatic activities results for PPO and POD enzymatic activities remained low throughout the storage, with values lower than 0.5 UAE.mL^{-1}, close to the methods detection limit, with no statistical difference among treatments over time, suggesting that the heat treatment was effective in inactivating the activity of these enzymes. Thermal processing is one of the most widely used conservation methods in the food industry since high temperature can lead to microbial inactivation and enzymatic activity (AGUIAR, YAMASHITA; GUT, 2012).

For residual sulphite, the analysis was performed for treatments B and D, which were added with sulphite. There was a decrease during storage in which the two treatments studied decreased to 30% of their initial values (data not reported). Treatment D (110 °C / 8s) exhibited a greater reduction in the residual sulphite content until the 14th day of storage and remained almost constant until the 28th day at the end of storage at room temperature 28 ± 2 °C. This result is similar to that reported by Sucupira *et al*. (2015), noting that the residual sulphite content was approximately 25% relative to the sulphite content added before the UHT process.

4 Conclusion

The pink color formed during heat processed coconut water storage cannot be related to the enzymatic activity of polyphenol oxidase and peroxidase, because the sample with pink color showing low activity for PPO and POD (0.28 and 0.40 UAE.mL^{-1}, respectively. In conclusion, the use of ultra-high temperature (UHT) processing associated with the use of sulphite inhibited polyphenol oxidase and peroxidase enzymes activity in coconut water. Addition of sulphite was effective in avoiding the pink coloration during storage of coconut water and after sterilization process. Sub-processing temperatures (110 °C / 4.1s and 120 °C / 4.1s) favored the appearance of a pinkish coloration.

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