

# Lycopene's Stability in Watermelon Juice (*Citrullus lanatus*) Regarding to Technological Routes

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## Abstract

Highly prized by consumers, watermelon is rich in water, but also in micronutrients including lycopene, pigment responsible for the red color. It is also a powerful antioxidant which has many virtues including the prevention and treatment of certain diseases. The transformation into nectar of watermelons combined with treatment could cause several modifications including the alteration of coloring. It is in this context that this study focuses on the variation of the lycopene content in nectars. Thus, nectars of 12 °Brix and 15 °Brix were prepared from three varieties of watermelon (Sugar Baby, Crimson Sweet and Charleston Gray). To study the stability, two pasteurization scales (85°C/15min and 95°C/15min) and one sterilization scale (105°C/15min) were applied to the different nectars produced. The results obtained showed that the Sugar Baby variety is richer in lycopene (24.39 mg·kg<sup>-1</sup>) with a higher pH (5.80). In addition, the study showed, for the Sugar Baby variety, an increase of lycopene with the addition of sugar and the heat treatment (a maximum of 42.83 mg·kg<sup>-1</sup> for SbF<sub>12</sub>T<sub>105</sub>). On the other hand, for the Crimson Sweet and Charleston varieties, the highest rate of lycopene, except the heat-treated ones, are those formulated at 12 °B (10.46 mg·kg<sup>-1</sup> for CrF<sub>12</sub>T<sub>105</sub> and 18.40 mg·kg<sup>-1</sup> for ChF<sub>12</sub>T<sub>105</sub>). Without any health consequences, the formulation combined with heat treatment would preserve the lycopene content of watermelon nectars.

## Keywords

*Citrullus lanatus*, Nectars, Variety, Thermal Treatment, Micronutrient

## 1. Introduction

Watermelon (*Citrullus lanatus*) is a plant of the Cucurbitaceae family. The fruit is a particular berry, spherical in shape, more or less oblong [1]. Its flesh which can be red, yellow, greenish or white generally weighs between 4 to 16 kg however there is watermelon with bigger weight (120 kg) [1]. Several bioactive compounds have been determined and the beneficial effects demonstrated by in vivo and in vitro studies [2]. Watermelon is also very rich in phenolic compounds, which are mainly derivatives of hydroxycinnamic acid and lycopene which gives watermelon its characteristic flesh red color and powerful antioxidant activity [3] [4]. The main pigment that causes red flesh color in watermelon is lycopene, which is considered one of the most important natural carotenoids in fruits [5]. It has the highest rate of oxygen quenching singlet of all carotenoids tested from biological systems [6]. Lycopene has been a research focus in many areas, including health care products, cosmetics, and nutrition, and has been shown to serve physiological functions in the human body [5] [7] [8]. Watermelon juice is produced commercially with heat treatment which can cause different types of changes such as coloring, flavor or other attributes, but, nevertheless, non-heat treatments are used [9] [10] [11]. Thus, the objective of this work is to study the impact of heat treatment and the addition of sugar during formulation on the lycopene concentration of watermelon nectars.

## 2. Material and Methods

### 2.1. Plant Material

For the study, three varieties of watermelon grown in Senegal have been used. These varieties are Sugar Baby (Figure 1(a)), Crimson Sweet (Figure 1(b)) and Charleston Gray (Figure 1(c)).

### 2.2. Formulation

Separately, the three (3) varieties of watermelon (Sugar Baby (Sb), Crimson Sweet (Cr) and Charleston Gray (Ch)) were cut into pieces and the red pulps collected. To obtain raw nectar (SbF<sub>0</sub>T<sub>0</sub> for Sugar Baby, CrF<sub>0</sub>T<sub>0</sub> for Crimson Sweet and ChF<sub>0</sub>T<sub>0</sub> for Charleston Gray), the pulp was ground using an electric juice blender (LOKI type LBL 201-C). Two formulations, at 12 °Brix (F12) and



**Figure 1.** Variety of watermelon: Sugar Baby (a), Crimson Sweet (b) and Charleston Gray (c).

at 14 °Brix (F14), were then carried out with each variety. The amount of sucrose added depends on the initial and final soluble solids content (**Table 1**) desired. All formulated and raw products were conditioned in glass bottles wrapped in aluminum foil.

### 2.3. Heat Treatment

All formulated and raw products of each variety have been stabilized by heat treatment. For stabilization, two pasteurization scales [(85 ± 0.2)°C/15min and (95 ± 0.2)°C/15min] and one sterilization scale [(105 ± 0.2)°C/15min] were chosen. Pasteurization was carried out using a water bath (Mettler) and sterilization in an autoclave. For pasteurization, a control flask is used to follow the evolution of the core temperature.

### 2.4. Codification of Samples

The coding of the samples was carried out at three levels (Depending on the variety V, the formulation made F and the heat treatment applied T). Thus, the denomination of each sample was presented in the form VFyTz with:

V equals **Sb** for Sugar Baby, **Cr** for Crimson Sweet and **Ch** for Charleston Gray;

Fy equals formulation and brix with **F<sub>0</sub>** for raw products, **F<sub>12</sub>** for samples formulate at 12 °B and **F<sub>14</sub>** for those 14 °B;

Tz equals thermal treatment with **T<sub>0</sub>** for products without heat treatment, **T<sub>85</sub>** and **T<sub>95</sub>** for pasteurized samples at 85°C and 95°C for 15 minutes and **T<sub>105</sub>** for sterilized products at 105°C.

### 2.5. Analytical Methods

For the study, different analyzes were carried out. The soluble solids content (brix), which is expressed in g-100 g<sup>-1</sup> of product, was determined using a digital refractometer ATAGO PAL-α. The pH was determined using an analog pH meter (HANNA HI 223) with an accuracy of 0.05 at 25°C (Standards NF V76-122). To determine the color component (L\* a\* b) of the nectars, a colorimeter (CM-5, Konica Minolta Sensing Americas Inc., US) was used. The different color components allow us to define the product according to the three-dimensional space with L\* indicating the lightness or luminance which varies from black to white; a\* which corresponds to the green-red antagonist couple and b\* corresponding to the blue-yellow antagonist couple. The lycopene content was determined by using the method described by Benakmoum *et al.* [12]. For this, centrifugation of the extract with an Hexane/Acetone/Ethanol (50/50/1) mixture is made at 5000 rpm for 15 minutes. The organic phase obtained was recovered with hexane, before carrying out a measurement of the absorbance at 472 nm using a UV spectrophotometer (Shimadzu UVmini-1240).

### 2.6. Statistical Analyzes

The results of the various analyses carried out were subject to statistical processing.

Statistica (version 7.1) and Minitab (version 17.3.1) softwares were used for the analysis of variance (ANOVA) and correlation analysis (Pearson's correlation coefficients) at a significance level of 5%.

### 3. Results and Discussions

The physicochemical and biochemical properties of the raw nectars are given in **Table 1**. The results of the study of correlations between the various parameters of the raw products are presented in **Table 2** and lycopene content's results by **Figure 2** and **Figure 3**.

#### 3.1. Characterization of Watermelons

The characterization results of the three varieties of watermelon are given in **Table 1**. This table shows overall significant differences between watermelons. In terms of pH, there are no significant differences between the varieties Crimson Sweet (5.20) and Charleston Gray (5.21). These values are higher than the pH of watermelon with seeds, from the eastern USA, obtained by Perkins [13]. But differences are noted with the Sugar Baby variety (5.80) which is less acidic and quite similar to ripe watermelon without seeds observed by Perkins [13]. However, significant differences are noted, for the soluble solids content (Brix), between watermelons (9.17 g·100g<sup>-1</sup> for Sugar Baby, 6.57 g·100g<sup>-1</sup> for Crimson Sweet and 8.07 g·100g<sup>-1</sup> for Charleston Gray). The high solids content of the Sugar Baby variety can also be explained by the high pH. According to lycopene, values differ significantly for all varieties (24.39 mg·kg<sup>-1</sup> for Sugar Baby, 6.23 mg·kg<sup>-1</sup> for Crimson Sweet and 13.61 mg·kg<sup>-1</sup> for Charleston Grey). The Sugar Baby variety has the highest lycopene content. The lowest lycopene content is the Crimson Sweet variety (quarter of the Sugar Baby). This significant difference could be explained by the growing conditions and the varieties. The concentrations of all watermelon varieties are, much lower than those obtained by Adetutu [14] (45.38 mg·kg<sup>-1</sup>) and Perkins [13] (43.4 mg·kg<sup>-1</sup> for watermelons with seeds and 71.2 mg·kg<sup>-1</sup> for those without seeds). These low concentrations can be explained by the degree of ripeness or the growing conditions.

As lycopene, significant differences are noted between the varieties, for a\*-values (red color). Sugar Baby variety has the highest intensity with  $10.76 \pm 0.46$ . Crimson Sweet variety, is with a lower intensity of red coloration ( $4.50 \pm 0.74$ ), with the highest color difference with Sugar Baby ( $7.48 \pm 1.30$ ). In addition, all varieties show the same for color component of luminosity (L\*-values). On the other hand, the brown index (BI) of the different varieties, which characterizes the ratio between yellow coloring and red one, shows significant differences ( $24.67 \pm 1.23$  for the Sugar Baby variety,  $5.23 \pm 1.94$  for Crimson Sweet and  $16.08 \pm 1.80$  for Charleston Gray). Low index of Crimson Sweet could be explained by the intensity of b\*-values ( $-0.93 \pm 0.36$ ) which is characterized by the low presence of yellow-colored compounds such as xanthophylls and  $\beta$ -carotene [15].

### 3.2. Statistical Analyzes

Comparison between the physicochemical and biochemical parameters (**Table 1**) indicates significant differences for the variation of the red coloration ( $a^*$ -values) for all the watermelons (10.76 for Sugar Baby, 4.50 for Crimson Sweet and 7.09 for Charleston Gray). These large variations of  $a^*$ -values are confirmed by a strong correlation (**Table 2**) with the lycopene content (0.98). All  $a^*$ -values, obtained for watermelons, are much lower than those observed by Perkins [16], on varieties grown in the state of Oklahoma (USA) including, among others, the variety Crimson Sweet ( $a^*$ -values = 20.0). These differences are also observed between Sugar Baby and other watermelons (7.48 with Crimson Sweet and 3.91 with Charleston Gray). It exceeds the minimum threshold of perceptibility of the color by an observer of 2.00 defined by Mokrzycki [17]. The correlations (**Table 2**) between other analytical parameters shows overall

**Table 1.** Physicochemical and biochemical characteristics of raw watermelon nectars

Parameters	Sugar Baby	Crimson Sweet	Charleston Grey
pH	5.80 ± 0.02 <sup>a</sup>	5.20 ± 0.03 <sup>b</sup>	5.21 ± 0.01 <sup>b</sup>
Brix (g·100g <sup>-1</sup> )	9.17 ± 0.15 <sup>a</sup>	6.57 ± 0.06 <sup>b</sup>	8.07 ± 0.06 <sup>c</sup>
Lycopene content (mg·kg <sup>-1</sup> )	24.39 ± 0.15 <sup>a</sup>	6.23 ± 0.05 <sup>b</sup>	13.61 ± 0.56 <sup>c</sup>
L*	43.04 ± 0.30 <sup>a</sup>	42.88 ± 0.22 <sup>a</sup>	43.17 ± 0.17 <sup>a</sup>
a*	10.76 ± 0.46 <sup>a</sup>	4.50 ± 0.74 <sup>b</sup>	7.09 ± 0.30 <sup>c</sup>
b*	3.15 ± 0.22 <sup>a</sup>	-0.93 ± 0.36 <sup>b</sup>	2.00 ± 0.71 <sup>c</sup>
Brown Index (BI)	24.67 ± 1.23 <sup>a</sup>	5.23 ± 1.94 <sup>b</sup>	16.08 ± 1.80 <sup>c</sup>
ΔE	0.00 ± 0.00 <sup>a</sup>	7.48 ± 1.30 <sup>b</sup>	3.91 ± 0.62 <sup>c</sup>

**Table 2.** Correlations between the biochemical parameters of raw watermelon nectars

	pH	Brix g·100g <sup>-1</sup>	Lycopene e g·kg <sup>-1</sup>	L*	a*	b*	IB	ΔE
pH								
Brix g·100 g <sup>-1</sup>	0.77 p 0.03							
Lycopene mg·kg <sup>-1</sup>	0.89 p 0.00	0.97 p 0.00						
L*	-0.30 p 0.47	0.16 p 0.71	0.02 p 0.97					
a*	0.88 p 0.00	0.96 p 0.00	0.98 p 0.00	0.11 p 0.79				
b*	0.65 p 0.08	0.96 p 0.00	0.91 p 0.00	0.41 p 0.32	0.92 p 0.00			
Brown Index (BI)	0.79 p 0.02	0.98 p 0.00	0.97 p 0.00	0.26 p 0.54	0.98 p 0.00	0.98 p 0.00		
ΔE	-0.83 p 0.01	-0.96 p 0.00	-0.97 p 0.00	-0.23 p 0.59	0.99 p 0.00	-0.95 p 0.00	-0.99 p 0.00	

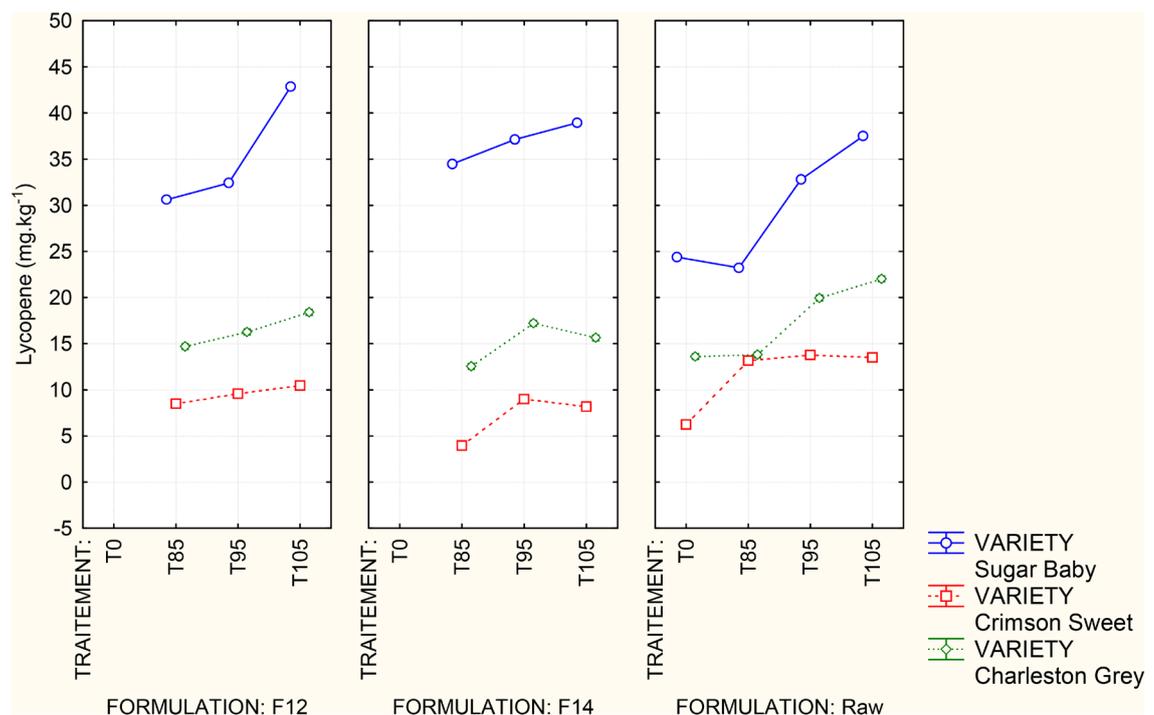
that they are significant, except for the luminance ( $L^*$ -values) but also between  $b^*$ -values and the pH. In the other hand, lycopene concentration increases sharply relative to pH (0.89), brix (0.97) and color component (0.98 for  $a^*$ -values, 0.91 for  $b^*$ -values and 0.97 for Brown Index). These strong correlations could be explained by the biochemical properties of lycopene which are carotenoids (acidic soluble pigments). The correlation table also shows that an increase in the lycopene content of the Crimson Sweet and Charleston Gray varieties would reduce the color differences with Sugar Baby ( $-0.97$ ).

### 3.3. Study of the Evolution of Lycopene Concentration

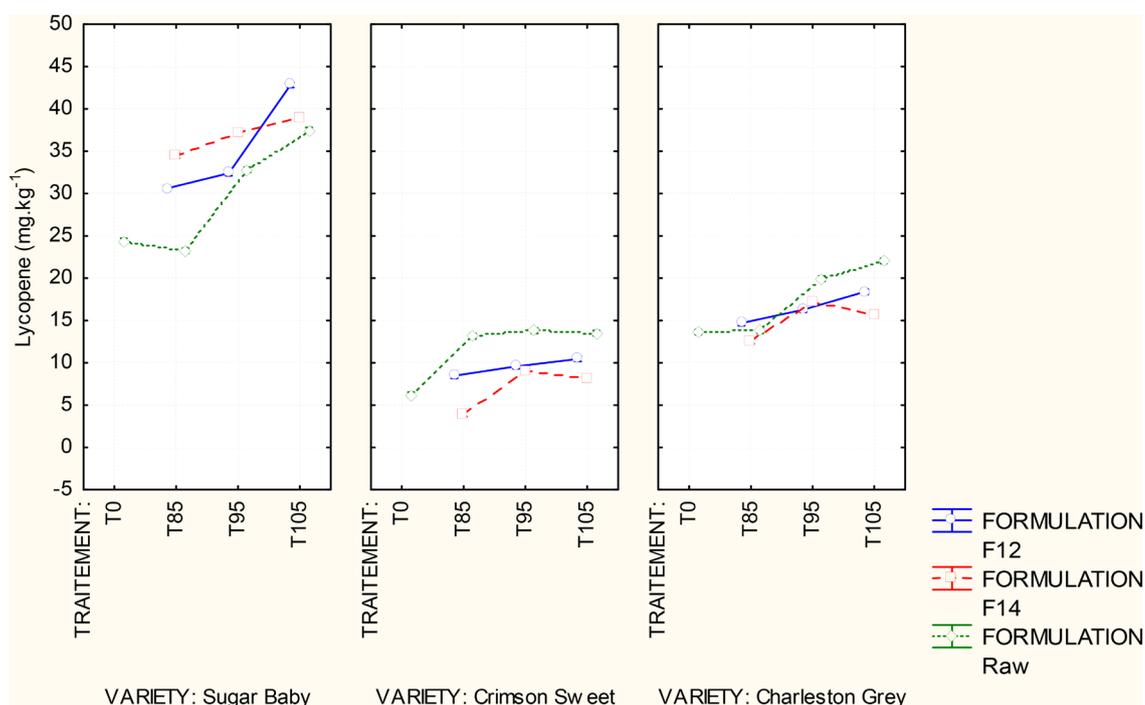
The lycopene content in raw products and those formulated and having undergone a heat treatment, is determined and the various results are represented by **Figure 2** and **Figure 3**. These figures represent the evolution of lycopene concentration based on sample characteristics (variety, formulation and heat treatment).

**Figure 3** represents the change in the lycopene content of the various nectars formulated as a function of the heat treatment for each variety.

Analysis of **Figure 2** shows that, regardless of the formulation performed, there is an increase of lycopene in Sugar Baby due to the thermal treatment ( $24.39 \text{ mg}\cdot\text{kg}^{-1}$  to  $\text{SbF}_0\text{T}_0$  to  $42.83 \text{ mg}\cdot\text{kg}^{-1}$  for  $\text{SbF}_{12}\text{T}_{105}$ ). In addition, the highest increase is obtained for the formulation at  $12^\circ\text{B}$  ( $\text{F}_{12}$ ) with  $\text{SbF}_{12}\text{T}_{85}$  ( $30.61 \text{ mg}\cdot\text{kg}^{-1}$ ) and  $\text{SbF}_{12}\text{T}_{105}$ . This sharp increase can be explained by the low addition of sugar which would have allowed, with the effect of treatment heat, the release of lycopene contained in watermelon chromoplasts [18] [19]. These high lycopene



**Figure 2.** Evolution of lycopene content by heat treatment for each formulation (brix of raw material are  $9.17 \pm 0.15$  for Sugar Baby,  $6.57 \pm 0.06$  for Crimson Sweet and  $8.07 \pm 0.06$  for Charleston Grey).



**Figure 3.** Evolution of lycopene content by variety for each formulation.

concentrations obtained are much lower than those observed by Choudhary [20] on different watermelon genotypes in India including Sugar Baby ( $53.6 \text{ mg}\cdot\text{kg}^{-1}$ ). Indeed, studies carried out on tomatoes have shown that lycopene is stored in globular or tubular form, in the form of crystalloids whether or not surrounded by membranes, or dissolved in plastoglobules, lipid droplets enclosing carotenoids and tocopherols [21] [22]. A decrease of the initial lycopene concentration is also noted for pasteurized raw watermelon at  $85^\circ\text{C}/15 \text{ min}$  ( $23.23 \text{ mg}\cdot\text{kg}^{-1}$  for  $\text{SbF}_{12}\text{T}_{85}$ ). This could be explained, by the rigidity of the chromoplast envelope which resists the heating applied or by the degradation of lycopene in solution. Concerning the Charleston Gray variety (Ch), an increase according to the heat treatment is noted only for the raw products ( $F_0$ ) and those at  $12^\circ\text{B}$  ( $F_{12}$ ). The concentration increase is lower than those obtained with Sugar Baby. This low increase could be explained by the initial low lycopene concentration in the raw nectar. The maximum lycopene concentration obtained after heat treatment ( $22.03 \text{ mg}\cdot\text{kg}^{-1}$ ) is lower than that of raw Sugar Baby nectar ( $24.39 \text{ mg}\cdot\text{kg}^{-1}$  for  $\text{SbF}_0\text{T}_0$ ). For products formulated at  $12^\circ\text{B}$  of Crimson Sweet variety, a small increase in lycopene was noted. For the crude, having undergone a heat treatment, a drop in the lycopene concentration was observed above  $85^\circ\text{C}$ . For the Crimson Sweet and Charleston Gray varieties, formulated at  $14^\circ\text{B}$ , the highest concentrations were obtained with heat treatment at  $95^\circ\text{C}$  ( $9.00 \text{ mg}\cdot\text{kg}^{-1}$  for  $\text{CrF}_{14}\text{T}_{95}$  and  $17.21 \text{ mg}\cdot\text{kg}^{-1}$  for  $\text{ChF}_{14}\text{T}_{95}$ ). These results could be due to the low lycopene concentration of the two varieties of watermelon. The results obtained by Tlili [23] on watermelons studied in Tunisia ( $53.5 \text{ mg}\cdot\text{kg}^{-1}$  for the Crimson Sweet) are superior to those obtained in this study.

Thus, for the Sugar Baby variety, an overall increase was observed for heat treatments at 85°C and 95°C. For the treatment at 105°C, a degradation of the lycopene is observed above 12 °B. This can be explained by the amount of sugar added, but also by the relatively high heat treatment, which can cause lycopene degradation. For the Crimson Sweet variety, there is an increase in lycopene for all raw products (from 6.23 mg·kg<sup>-1</sup> for CrF<sub>0</sub>T<sub>0</sub> to 13.14 mg·kg<sup>-1</sup> for CrF<sub>0</sub>T<sub>85</sub>, 13.78 mg·kg<sup>-1</sup> for CrF<sub>0</sub>T<sub>95</sub> and 13.50 mg·kg<sup>-1</sup> for CrF<sub>0</sub>T<sub>105</sub>).

A significant decrease in the lycopene concentration is observed for all the formulated products (3.94 mg·kg<sup>-1</sup> for CrF<sub>14</sub>T<sub>85</sub>, 9.00 mg·kg<sup>-1</sup> for CrF<sub>14</sub>T<sub>95</sub> and 8.18 mg·kg<sup>-1</sup> for CrF<sub>14</sub>T<sub>105</sub>). This decrease can be explained by the low lycopene concentration of the Crimson Sweet variety. For the Charleston Gray variety, there is no overall trend that emerges depending on the heat treatment. For the heat treatment at 85°C, the maximum increase is obtained with products formulated at 12 °B.

For the treatments at 95°C and 105°C, the maximum concentrations are obtained with the crude products (19.95 mg·kg<sup>-1</sup> for ChF<sub>0</sub>T<sub>95</sub> and 22.03 mg·kg<sup>-1</sup> for ChF<sub>0</sub>T<sub>105</sub>). These results can be explained, as for the Crimson Sweet variety, by the low concentration of lycopene in the fruit.

**Figure 3** shows the impact of the formulation on the heat treatment for each variety. For the Sugar Baby variety, there is an increase in lycopene concentration depending on the formulation for the 85°C and 95°C treatments (from 24.39 mg·kg<sup>-1</sup> for SbF<sub>0</sub>T<sub>0</sub> to 34.48 mg·kg<sup>-1</sup> for SbF<sub>14</sub>T<sub>85</sub> and 37.14 mg·kg<sup>-1</sup> for SbF<sub>14</sub>T<sub>95</sub>).

For the 105°C treatment, the increase is no longer observed above 12 °B. This can be explained by the fact that the added sugar could act as a brake on the release of lycopene contained in the chloroplasts. For the Crimson Sweet variety, there is a significant drop in lycopene, regardless of the treatment applied, for products formulated at 12 °B and 15 °B. The same observation was noted for the Charleston Gray variety for the 95°C and 105°C treatments. These results show that the amount of added sugar can have a negative impact on the increase in lycopene. On the other hand, for the Charleston Gray nectars treated at 85°C (13.80 mg·kg<sup>-1</sup> for ChF<sub>0</sub>T<sub>85</sub>, 14.69 mg·kg<sup>-1</sup> for ChF<sub>12</sub>T<sub>85</sub> and 12.54 mg·kg<sup>-1</sup> for ChF<sub>14</sub>T<sub>85</sub>), there are no significant differences compared to the crude product (13.61 mg·kg<sup>-1</sup> for ChF<sub>0</sub>T<sub>0</sub>), for all formulations. This can be explained by the fact that the treatment used is weak to allow the rupture of the envelopes of the chromoplasts and by the degradation of the lycopene present in solution.

In short, it appears that, for the Sugar Baby variety, the addition of sugar but also the heat treatment temperature promotes the increase in the lycopene content. Conversely, for the Crimson Sweet and Charleston Gray varieties, the addition of sugar combined with the heat treatment reduces the lycopene concentration in the nectars. Apart from the raw nectars of Crimson Sweet and Charleston Gray, those formulated at 12 °B show the best increases in lycopene concentration.

## 4. Conclusion

This study allows us to observe that the Sugar Baby variety is much richer in lycopene than the Crimson Sweet and Charleston Gray varieties. On the other hand, there is not a general tendency which emerges on the impact of the heat treatment and the formulation on the nectars of all the varieties studied. Nevertheless, it emerges that for all the watermelons, a formulation at 12 °B combined with high heat treatment (high pasteurization or sterilization) would allow to have a significant increase in lycopene.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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