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Color Analysis of the Parameters through Time for the Juice of *Rubus Glaucus* by Addiction of the Insecticide Chlorpyrifos by Colorimetry

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Abstract:

The juice is one of the ways more popular of preparing the Andean blackberry, because it has their nutritional value, pleasant aroma and flavor. In this harvested fruit, the presence of the insecticide Chlorpyrifos was found. Due to the poor application of pesticides on the fruit and inadequate process of post - harvest, it is common find in juices residues of these pesticides. Due to the intrinsic toxicity of the compound, it can produce serious damage to some organs of the body human. Reason why is required to analyze the metabolism of this compound, when is present in the juice. In order to make that, it was performed the characterization of the pesticide used and the blackberry juice used. 100 mL of 0.024 M of Chlorpyrifos was added to 100 mL of blackberry juice, in the experiment, also was evaluated decreasing of the concentration of the insecticide from time 0 to 107 min. A base color of -7.13 degrees (Hue angle) was obtained, and a Cab chroma of 6. In the addition of the pesticide increased the values in 18 degrees and 13 for the Cab respectively. For the CIE Lab color space, the relationships were obtained as a function of time, with a higher coefficient of determination of 0.92 for "L" and "b", and 0.97 for the parameter "a".

Keywords: Blackberry juice, CIE xyz, Pesticide, Hue Angle, Cab chroma.

INTRODUCTION

In colombia, one of the fruits of greater commercialization is the Andean Blackberry *(sp. Rubus Glaucus),* because his nutritional properties, flavor and aroma [1], [2]. This variety is grown in mountainous areas with a tropical climate. Due to achieve an efficient harvest, it requires an average temperature of 15 °C with high relative humidity, in order to achieve a proper fruit development [3], [4]. However, these conditions attract different pests that attack crops, such as fruit fly, some ants, fungi in the roots, among others. To reduce the loss in crops, chemical pesticides are used that prevent the occurrence of these pests [5].

Currently, chemical pesticides used in agriculture produce side effects when are ingested by humans. The main reason for this phenomenon is the intrinsic toxicity that require this compounds in order to act as pest controllers [6]–[8]. Given this, the use thereof is governed by different national and foreign government agencies such as the Environmental Protection Agency of the United States, the Colombian Agricultural Institute (ICA), among others [5], [8]. One of the most widely used pesticides in crops of blackberry is the insecticide Chlorpyrifos [6], [9]. Which it is a synthetic compound, in other words, produced by man, reason why is not normally found this compound in the nature.

In agriculture there is a technological gap, which also affects the application of pesticides on crops, reason why there is an increase in the number of intoxicated people in rural areas. The O, O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate, known in its commercial form as Chlorpyrifos, his principal function is the organophosphate site in the molecule [10], [11]. When is ingested, attacks the central nervous system, by blocking the acetylcholinesterase (AChE). Generating problems in the communication between the brain and muscles, which can lead to tachycardia, excessive salivation, cramps and even death [7].

Due to an increased improper pesticide application and an inopportune treatment of post-harvest fruits, the poisoning seen with this pesticide in humans have been rising. In order to avoid this, some tests are performed to determine the amount of pesticide containing fruits, however, this technique is destructive, whereby, the fruit is no longer available for consumption. The main technique to achieved this is the chromatography, in which the sample is subjected to various chemical processes in order to isolate the molecule of interest and compare it with a pattern in order to determine his concentration [12]–[14].

One of the most desirable forms for consumption the Andean blackberry is the juice, because, it is a refreshing drink that is capable of providing all the benefits of the fruit, however, for a mishandling, the pesticide present in the fruit can reach the juice, causing the same intoxication [15]. One of the methodologies non-destructive for the analysis of these substances is the colorimetry. Which it is based on color analysis (the amount of light reflected on a surface) [16], [17].

To bring this about, they have different parameters such as Hue angle, which indicates the base color reflected and chroma Cab, which indicates the color saturation in the sample. In addition, there are different color spaces for measuring the same as the CIE Lab and CIE XYZ. These seek to unify the color in order to obtain reproducibility of the same [18], [19].

Based on the above, in this paper is presented the analysis of the blackberry juice *(sp. Rubus Glaucus)* in function of the time when was applied the insecticide Chlorpyrifos on it, through colorimetry. Obtaining the color parameters for the juice, the commercial pesticide and his change over time.

MATERIALS AND METHODS

The experimental part was made on the campus of Military University Nueva Granada in Cajicá, Colombia. Due to this area is producer of the Andean Blackberry, therefore, the environmental conditions replicate those used during harvest. Within these, the temperature was controlled, keeping it at 19°C, not direct sunlight and wind exposure. In order to perform the tests, commercial Chlorpyrifos was used and the recommended dose for application to crops was prepared.

Molar concentration was determined using a commercial solution with the equation 1, where MW was the molecular weight and ρ was density. The initial concentration of the commercial version of the pesticide was 480 g / L.

$$C_1\left[\frac{mol}{L}\right] = \frac{C_1[\frac{g}{L}]}{MW[\frac{g}{mol}]} = \frac{480\frac{g}{L}}{359.59\frac{g}{mol}} = 1.335\frac{mol}{L} \quad \text{Eq. 1}$$

Distilled water was used to prepare the solution used to contaminate the samples. The concentration of the solution used was given by equation 2, where C was the concentration and V was the volume.

$$C_1 * V_1 = C_2 * V_2$$

$$\frac{C_1 * V_1}{V_2} = C_2 = \frac{1.335\ M * 5\ mL}{275\ mL} = 0.024\ M \qquad \text{Eq. 2}$$

This was achieved, because, for a solute in a solution, the mass before and after was the same. This dose was recommended by the data sheet of the pesticide, in order to treat minor infestations on crops.

Andean blackberry (*sp. Rubus Glaucus*)

In order to obtain the blackberry necessary for the experimentation, a sampling for 3 different markets in Bogota was made, collecting at each station 500 g of good quality fruit, for which it was observed that the fruit was not in decomposition process by the action of pests.

In the laboratory, 500 g of the collected blackberry was selected (Figure 1). The fruit was washed and dried to remove the excess of impurities on the shell. First, the sepal was removed (the remnant of plant leaves that still remaining on the mora), then placed in a blender with 100 mL of distilled water for 1 min at 2000 rpm.

Figure 1: Preparation of blackberry juice.

For the prepared juice, 100 mL of juice were selected and 100 mL of the pesticide solution was added. Adding 0.0024 moles in total (0863 grams) of the pesticide.

COLORIMETRY

The samples of colorimetry for the blackberry were taken with a non-contact spectrophotometer Flame-S of Ocean Optics (Figure 2, No. 2), which has a DET2B detector with a detection range from 200 to 1100 nm, with a slit INTSMA-025 and cells of quartz of 1 cm step. The light source used was a DH-2000-BAL with deuterium and tungsten lamp from Ocean Optics (Figure 2, No. 1). A fiber of reflectance of premium grade of 400 um with solarization-resistant was used, which had a range of wavelength of 200 to 1100 nm. In order to made the equipment calibration, a standard diffuse of reflectance from Ocean Optics was used, made of polytetrafluoroethylene (PTFE), with anodized aluminum cover, hydrophobic, chemically inert and stable, which increased the reflectivity to 98%.

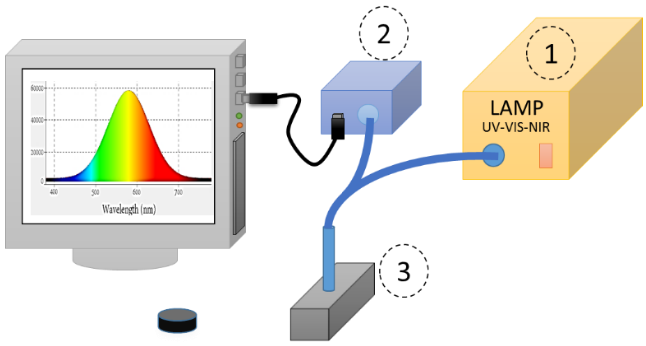

Figure 2: Mounting used in the colorimetry experiment.

All measurements of colorimetry were taken by triplicate, in order to ensure the accuracy in each simple.

In the measurement of color, the Hue angle and the Cab chroma were parameters used to identify the characteristic color of a sample. These indicate the base color of the sample and the color saturation respectively. These were related through the reflection spectrum. In which the wavelength with a greater light reflectance was the Hue angle, while the Cab chroma was the peak intensity.

In order to standardize the measures, the color spaces CIE Lab and CIE XYZ were used. Where for the space CIE Lab, the "L" indicates the brightness, "a *" was a scale of values between green and magenta colors, and "b *" was the scale between blue and yellow. While the CIE XYZ color space was used to define the colors, in a scale perceived by the human eye. In this model, the parameter "Y" means brightness; "Z" was approximately equal to the blue stimulus (S cones), and X was a mixture aimed at the sensitivity curve from red to green (cones L and M).

The values for the color spaces were taken with the Ocean View™ software, which collects the data used by the spectrophotometer. Then, they were represented in Matlab R2016a®, in order to determine the average and the standard deviation of each sample taken by triplicate. Color space CIE XYZ was normalized according to equation 3, in order to determine the variation of the parameters.

$$x = \frac{X}{X+Y+Z} \quad y = \frac{Y}{X+Y+Z} \quad z = \frac{Z}{X+Y+Z} \quad \text{Eq. 3}$$

RESULTS AND DISCUSSION

To each sample was assigned an alphanumeric code, in order to identify them during the process, this code was shown in Table 1, in addition, the time at which the measurements were made were indicated after applying the pesticide on Blackberry juice.

Table 1: Codes used for simple identification.

Code	Value	Time
Pure	Chlorpyrifos comercial	-----
JM	Blackberry juice	0 min
JM0	Blackberry juice	0 min
JM1	Blackberry juice	28 min
JM2	Blackberry juice	62 min
JM3	Blackberry juice	107 min

In total, 0.863 grams of the insecticide Chlorpyrifos were added on the 100 mL of juice prepared at time 0. With the initial solution, it was proceeded to perform the measurement by colorimetry and track them through the time. One of the parameters obtained was the Cab chroma, which as noted previously, it makes relative to the color saturation. In Figure 3 the different values obtained for each one of the samples were observed, also the black lines indicate the standard deviation of the average of each sample.

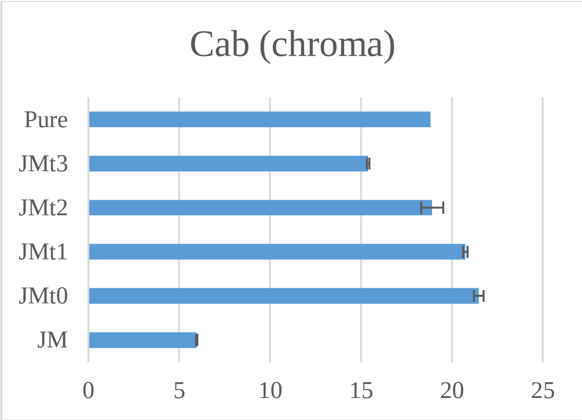

Figure 3: Cab chroma for the analyzed samples.

The standard deviation of each samples was low, whereby an adequate characterization of blackberry juice and the pure compound were achieved, which was denoted a high difference between these samples. The contaminated samples had intermediate values, which decrease with time. This parameter was related to the Hue angle and the color space CIE xyz, which were shown in Table 2 with their respective deviation.

Table 2: Hue angle and standard space color for the samples of blackberry juice.

Values	Hue angle	x	y	z
JM	-7,128+-0,664	0,4650+-0,0002	0,3921+-0,0002	0,1429+-0,0003
JMt0	11,131+-0,132	0,5071+-0,0015	0,3738+-0,0010	0,1192+-0,0006
JMt1	11,706+-0,135	0,5101+-0,0003	0,3725+-0,0000	0,1174+-0,0002
JMt2	12,395+-0,812	0,5013+-0,0031	0,3780+-0,0012	0,1206+-0,0019
JMt3	10,432+-0,081	0,4948+-0,004	0,3804+-0,0003	0,1248+-0,0001
Pure	171,327+-1,474	0,4179+-0,0010	0,4349+-0,0010	0,1472+-0,0010

As for the Hue angle, the same relationship was observed, where the pure samples had external values and the contaminated juice had intermediate values. A variation of approximately 18 degrees by adding the insecticide on blackberry juice was observed, due to the wide difference in the base color of each sample. However, thorough time, there was not significant variation in this parameter. In the CIE xyz color space, it was found that the parameter "x" was the strongest component, with which the relationship between red and green, as expected by the base color of blackberry juice. These parameters did not show a significant variation, able to distinguish between each of the samples. Reason why, was necessary use the CIE Lab color space (Figure 4).

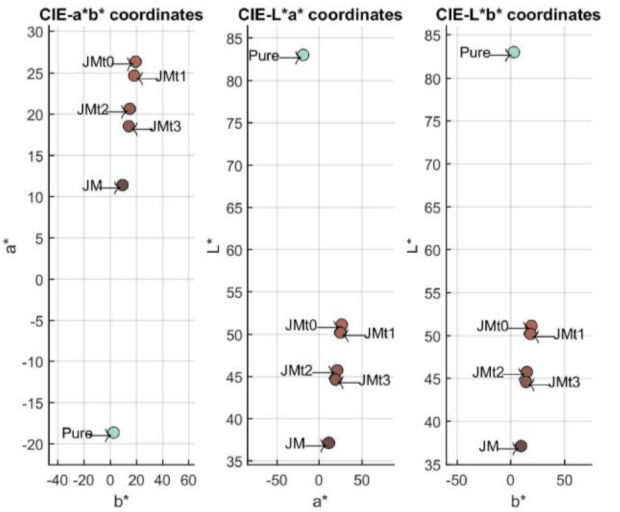

Figure 4: Two-dimensional graphics representing the CIE Lab space color with true colors.

With the two-dimensional graph, the behavior of each substance in relation to the parameters of the CIE color space Lab was observed. The value "b", which represents the variation between the blue and yellow scale, shows no big changes for changes in blackberry juice over time, if not for the parameters "a" and "L", which represent the color scale between green and magenta and brightness respectively.

In the case of variable "a", by adding the insecticide on blackberry juice, on average, they doubled the values, indicating a redder hue, so when applying the insecticide this color will be highlighted. For the variable "L" it has an average variation of 10.7, using as reference the juice without pesticides.

In general, the addition of pesticide on blackberry juice, contributes to an enhancement of the red color hue, a saturation increasing, without distort the component of the color blue or yellow. In order to determine the relationship function of time for the parameters of the CIE Lab space color, the trend lines were shown in Figure 5 and their respective equation of adjustment in Table 3.

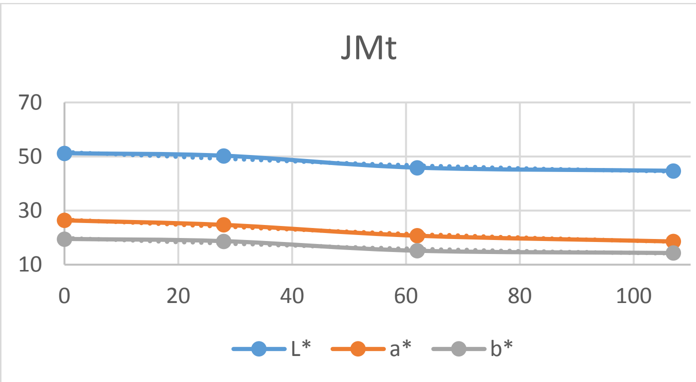

Figure 5: Relationships for the CIE Lab color space.

In Figure 5, the standard deviation for each value was shown, however, the value was not numerically relevant for the data, indicating an adequate precision. Correlations were obtained with a higher coefficient of determination of 0.93 for all cases. Also, it should be noted that the curve generated follows the expected behavior for the blackberry juice with pesticide through the time.

Table 3: Characteristics equations for CIE Lab color space.

	Equation	R^2
L	$y = 0,0003x^2 - 0,0976x + 51,622$	0,9281
a	$y = 0,0002x^2 - 0,1013x + 26,638$	0,9763
b	$y = 0,0002x^2 - 0,0797x + 19,788$	0,9279

The decrease shown in the variables, tend to return to the values of the blackberry juice without pesticide. One of the main reasons for this behavior, was the metabolizing of the pesticide to other substances in the juice, in addition, the oxidation of the juice due to his exposure to the environment. In order to observe the behavior of the pesticide in the juice, the reflection spectra were taken and shown in Figure 6. In this figure is shown the behavior through time, the pure insecticide and blackberry juice.

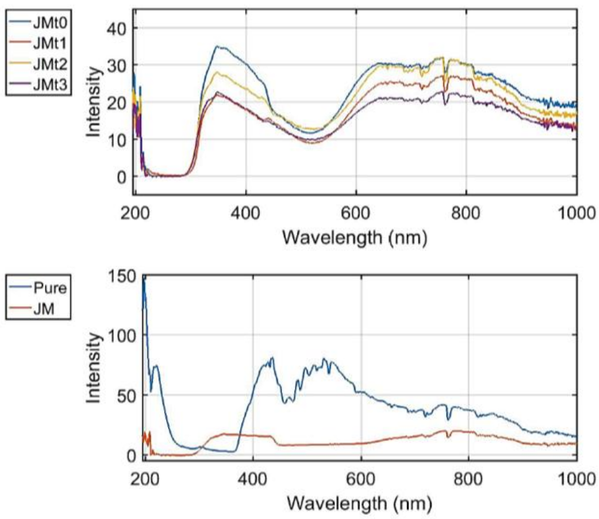

Figure 6: Spectra obtained for the samples of blackberry juice.

The reflection spectrum of the insecticide Chlorpyrifos was very different to the spectrum obtained by the blackberry juice without pesticides. Due to this, was possible perform the analysis when the blackberry juice had presence of the pesticide. Through time there was a decrease in the signal intensity, obtaining the same pattern as for the color space CIE Lab. However, several of the characteristic peaks of sample of commercial Chlorpyrifos not had an adequate definition in the blackberry juice.

CONCLUSIONS

The insecticide Chlorpyrifos causes damage to the neurological system, when is ingested by humans, this was due to the blockage of the acetylcholinesterase. Reason why, the analysis of it was made when the pesticide comes to the humans through the preparation of the blackberry juice, which is one of the main fruits that use this pesticide for pest control. The Andean blackberry due to the extensive production of this fruit in the savannah of Bogota, where the experiment was conducted.

The characterization to the blackberry juice and the insecticide in his commercial form were made, for which the Cab chroma (with values of 18.8 and 6) and the Hue angle (with values of 171.33 and -7.13 respectively) were obtained. The values found for the blackberry juice polluted with pesticide had intermediate values. Showing a trend towards the value of the blackberry juice without pesticide through time. One of the reasons for this behavior was the possible compound metabolizing and the oxidation of the juice due to his exposure to the environment.

The relations for the blackberry juice contaminated through the time with the CIE Lab space color were determined. For which the characteristic equation describes the behavior, with a higher coefficient of determination to 0.92 for the parameters "L" and " b ", while for the parameter" a " the coefficient increased to 0.97. In the reflection spectra of the pattern found was the decreases of the intensity versus the time of contamination of the juice.

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