

CHARACTERIZATION OF A NATURAL DYE FROM AVOCADO (PERSEA AMERICANA) SEED FOR DYEING TEXTILE FIBERS.

CARACTERIZACIÓN DE UN TINTE NATURAL DE LA SEMILLA DE AGUACATE (PERSEA AMERICANA) PARA TEÑIR FIBRAS TEXTILES.

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Summary

The coloration of vegetation is given by the presence of anthocyanins. Anthocyanins are water-soluble pigments capable of providing a wide range of colorations with an extensive market in natural dyes, which have been booming in the last decade due to their biodegradability and low toxicity compared to synthetic dyes. Natural dyes can be extracted from different parts of the plant. In the present research work, the extraction and evaluation of a natural dye extracted from avocado seeds was carried out. The extraction of the dye was carried out by grinding the seed, extraction with sodium hydroxide, then filtering and, finally, centrifugation. For the evaluation of this dye, its yield was calculated, obtaining an average of 90.15%. Subsequently, after measuring the absorbances of the sample with the spectrophotometer, its concentration was determined using the Lambert-Beer Law. Next, it was applied to cotton, polyester and mixed textile fibers, resulting in a reddish-brown coloration, with greater adherence observed in the case of cotton. Finally, a washing fastness test was carried out, obtaining a good fixation in cotton fibers dyed with natural dye.

Key words: anthocyanins, coloration, water-soluble pigments, low toxicity, wash fastness, biodegradability.



Abstract

The vegetation coloration is determined by the presence of anthocyanins. Anthocyanins are water-soluble pigments capable of providing a wide range of colors with a significant market in natural dyes, which have experienced a surge in the last decade due to their biodegradability and low toxicity compared to synthetic dyes. Natural dyes can be extracted from different parts of the plant. In this research study, the extraction and evaluation of a natural dye extracted from avocado seeds were carried out. The dye extraction process involved grinding the seed, followed by extraction with Sodium Hydroxide, filtration, and finally centrifugation. The yield of this dye was calculated for its evaluation, resulting in an average of 90.15%. Subsequently, after measuring the sample's absorbance using a spectrophotometer, its concentration was determined using the Lambert-Beer Law. Then, it was applied to cotton, polyester, and blended textile fibers, resulting in a brownish-red coloration, with higher adhesion observed in the case of cotton. Finally, a wash fastness test was performed, obtaining good fixation on the cotton fibers dyed with the natural dye.

Key words: anthocyanins, coloring, water-soluble pigments, low toxicity, Wash fastness, biodegradability

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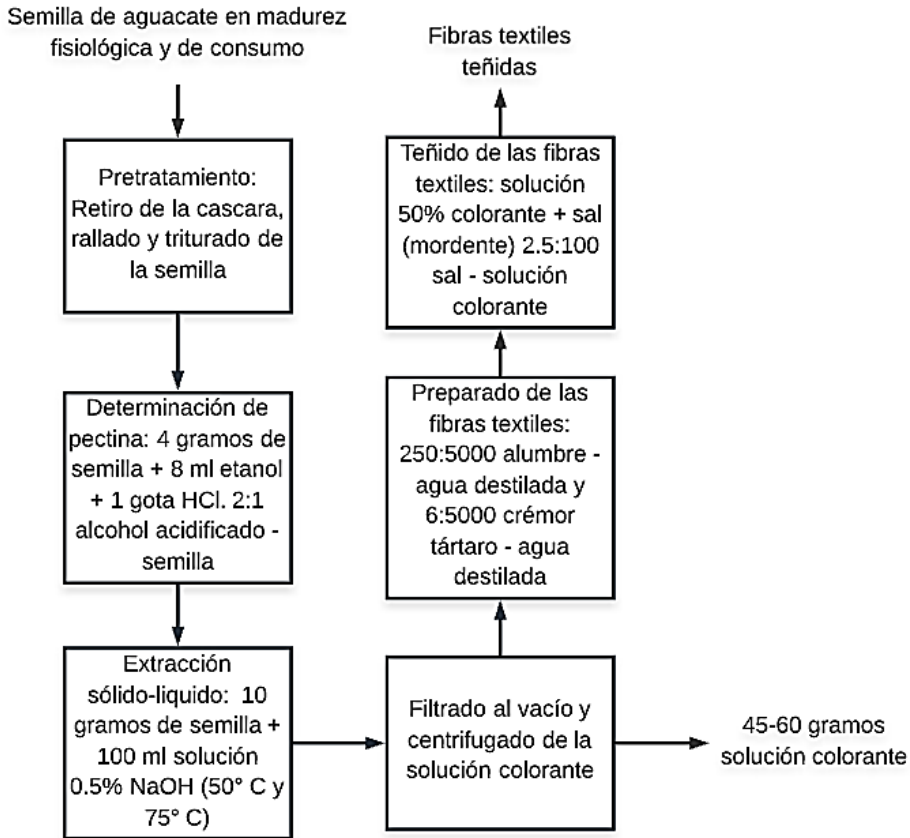
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1. Introduction

The avocado (*Persea Americana*) is an exotic, fleshy and unisemillado fruit from Mexico and Guatemala. This fruit has been present in the American culture for centuries since, besides being rich in natural fats, it has great medicinal properties through the use of its seed, which has been used in different industries such as pharmacological and cosmetological. In addition to these applications, we can highlight the use of this seed to obtain a natural dye with unique properties in the textile industry. Nowadays, the use of dyes has become a very common practice in the industries, with a very varied application. In the case of the textile industry, the use of natural dyes has been taken advantage of since they do not require the use of synthetic products that increase the cost of the final product. Currently, both the environmental impact and the health consequences caused by the use of synthetic components are being questioned. Obtaining colorants from avocado seed is an alternative for the use of waste, since this seed is wasted in daily life. It is important to promote the use of natural dyes, which have been widely accepted due to their biodegradability and low toxicity. In the present research work, the extraction of the natural dye is carried out using sodium hydroxide (NaOH) solution, resulting in a reddish-brown dye, as well as the calculation of the yield and, using a spectrophotometer, the concentration. Finally, it is applied to different textile fibers in order to evaluate the dyeing capacity of the natural dye and its fixation to them.

2. Experimentation

Figure 1. Diagram of the process for obtaining the dye and dyed textile fibers.



Source: Own elaboration.

2.1 Determination of pectin in avocado seed

Before starting the extraction of the colorant, the determination of pectin in the seed to be used is carried out, it is necessary that it is completely free of pectin or with minimum quantities to avoid the thickening of the solution. For this process, the methodology of the Pectin Test carried out by Novozymes Switzerland AG for the determination of pectinases in winemaking was followed. For this procedure, an acidified alcohol solution was prepared by mixing 8 ml of Ethanol and 1 drop of hydrochloric acid in a test tube. Then, 4 grams of previously grated

avocado seed were weighed and placed in the test tube together with the previous solution and allowed to stand for 10 minutes. After 10 minutes, if a white gelatinous precipitate is present, it means that pectin is present in the avocado seed, since pectin precipitates in the presence of acidified alcohol (Novozymes Switzerland AG, 2009).

2.2 Extraction of the dye from avocado seed

Once the lack of pectin in the avocado seed was determined, the extraction of the colorant from the seed was carried out. The following procedure was carried out based on the methodologies of the following authors: Devia & Saldarriaga and Moreno; with certain changes decided at the time of the bibliographical revision and as a solution to certain problems presented when carrying out the experiments.

First, remove the skin from the avocado seed and wash it properly to remove any impurities. Next, the avocado seed is grated to reduce its size. Then, with a mortar and pestle, grind the grated avocado seed so that a paste-like consistency is obtained as a result. Subsequently, 10 grams of the grated and crushed avocado seed are weighed to carry out the extraction in a ratio of 10:100, 10 grams of avocado seed sample per 100 ml of 0.5% sodium hydroxide solution by weight. The above mentioned elements are mixed in a heating plate, adjusting both the temperature to work and the stirring speed in speed 3 (200 rpm) . The temperature is constantly monitored so that it does not exceed or fall below the temperature at which it is being worked. In the same way, the extraction time is taken with the help of a stopwatch. Table 1 shows the times and temperatures of the extractions with the different seeds to be used.

Table 1. Time and temperature of dye extraction from avocado seed

Seed maturity	Extraction #1	Extraction #2
Physiological	Time: 30 minutes Temperature: 50° C	Time: 30 minutes Temperature: 75° C
Consumer	Time: 30 minutes Temperature: 50° C	Time: 30 minutes Temperature: 75° C

Source: Own elaboration.

2.3 Vacuum filtration and centrifugation of the dye solution

Once the coloring solution is obtained from the avocado seed, it is filtered and centrifuged to completely eliminate unwanted residues. First, vacuum filtration is carried out by connecting the vertical Venturi hose to the detachment tube of the filtration flask. Subsequently, sufficient glass wool is placed over the Buchner funnel. The water flow is opened and kept that way while the vacuum filtration is carried out. Finally, the dye solution is poured over the glass wool and a few minutes are waited, dropping the filtered solution into the filtration flask, until it is observed that only residues remain on the glass wool. When filtering the dye solution, the unwanted residues are not completely removed. For this reason, the dye solution obtained after filtration is subjected to a centrifugation, so that these residues are completely removed. For this, the filtered dye solution is poured into test tubes suitable to be placed in the centrifuge. These test tubes are placed inside the centrifuge for thirty (30) minutes and at speed 8 (3200 rpm). After this time, the dye solution is completely free of residues and is transferred from the test tubes to a clean beaker.

2.4 Dye solution tests

2.4.1 Performance calculation

For the calculation of the yield, the dye solution was weighed immediately after finishing the extraction and then weighed again after filtration and centrifugation, recording both values. The yield was calculated using the following equation 1:

$$\text{Rendimiento} = \frac{\text{g sol colorante filtrada y centrifugada}}{\text{g sol colorante antes de filtrar y centrifugar}} \cdot 100\% \quad (1)$$

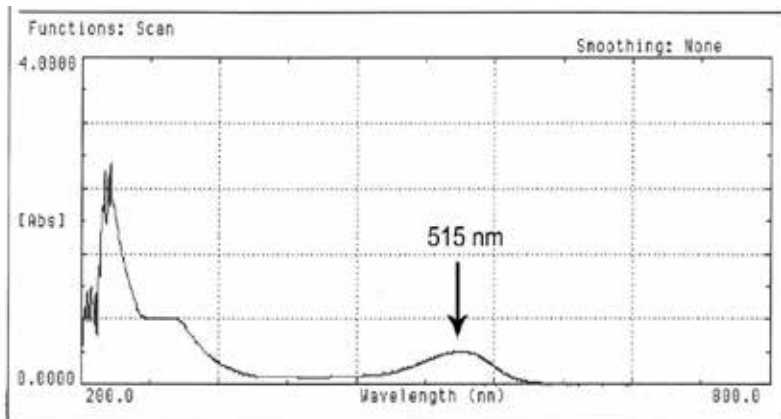
2.4.2 Characterization of natural dye using FTIR

It is necessary to check the presence of anthocyanins in the natural dye, since these are the ones that provide the color to the dye solution. For this purpose, the solution was characterized by Fourier Transform Infrared Spectrometry (FTIR), using a Perkin Elmer Spectrum 100 FTIR spectrometer, in order to analyze its components. First, an investigation was carried out to verify the presence of anthocyanins in different fruits using FTIR. Then, having a reference with which to compare the results, we proceeded to analyze our solution in the FTIR. For this, previously, the "Background" was carried out with the empty cell, in order to verify that the cell is in the necessary conditions to carry out the characterization. Once this was done, the cell was filled with the natural dye and the corresponding infrared spectrum for the natural dye was obtained and then compared with the previously investigated spectrum.

2.5 Calculation of the concentration of the dye solution

Once the natural dye is obtained, the absorbance of the sample is determined using the spectrophotometer, using a UV-visible spectrophotometer, Fisher 4001/4, Thermo Scientific (Genesys 20) and, with this obtained value, the calculation of the concentration is made by means of the Lambert-Beer Law. When the natural colorant is obtained, it is diluted with a dilution factor of forty (40). Through the corresponding bibliographic review (Gross, 1987, among others) it was determined that the optimum wavelength, in the visible region, for anthocyanins is 515 nm as shown in Figure 2. After filling the cuvettes with the different samples to be analyzed and using sodium hydroxide solution as a blank for the spectrophotometer, the absorbance of the samples is measured using the aforementioned wavelength.

Figure 2. Ultraviolet-visible spectrum, characteristic absorption band for anthocyanins.



Source: Martínez-Cruz et al., 2011.

Once the absorbance values have been obtained for each of the dye samples, the calculations corresponding to the concentration are made using an extinction coefficient of $26900 \text{ M}^{-1} \text{ cm}^{-1}$. The concentration value obtained is multiplied by the dilution factor used to obtain the real concentration of the dye. The following equation 2 was used:

$$C = \frac{A \cdot FD}{\xi \cdot L} \quad (2)$$

Where: A = Absorbance

C = Chromophore Concentration

L = Length of the optical path containing the sample

ξ = Extinction Coefficient

FD = Dilution factor

2.6 Dyeing of textile fibers with the dye solution

2.6.1 Preparation of textile fibers for dyeing

It is important to prepare the textile fibers before dyeing, in order to ensure a uniform, intense and bright dyeing. By carrying out this process known as “pre-mordanting”, the molecular chains of the fiber are broken in order to better fix the dye in it (Arroyo, 2010). The following procedure was carried out based on the methodology of the author Leticia Arroyo.

First, the textile fiber was cut into equal sizes measuring approximately 5 cm wide and 5 cm long. Then, the textile fiber was washed with neutral pH soap in order to eliminate any impurity that could affect the dyeing. Next, the potassium aluminum sulfate (alum) ratio 250:5000, 250 grams of alum per 5000 ml of water and potassium bitartrate (cremora tartar) ratio 6:5000, 6 grams of potassium bitartrate per 5000 ml of water were weighed. In a beaker all the elements mentioned above are mixed. This beaker with the solution is placed on a heating plate and the textile fibers are left to circulate in the bath for 40 minutes at boiling point. Finally, they are drained and left to dry.

2.6.2 Application of the dyeing substance to textile fibers

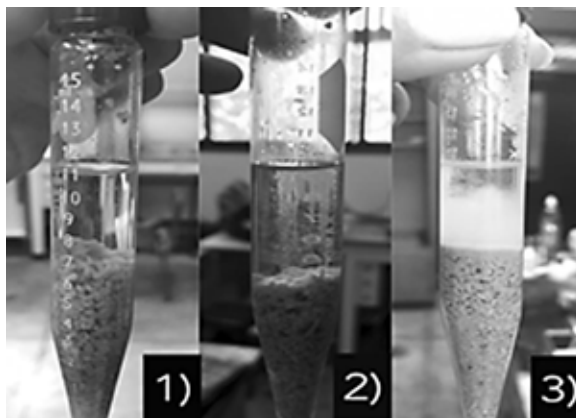
In this process, the application of the coloring substance to the different textile fibers to be used was carried out. The dyeing substance is diluted with equal amounts of water and dye to obtain a 50% dye solution. To this diluted solution, 2.5 grams of mordant (common salt, NaCl) are added for each 100 ml of solution, in this case common salt (NaCl) is used as mordant. The beaker with the solution is placed on the heating plate, the temperature of the plate is adjusted and the textile fibers, after being prepared, are placed in the dyeing bath. The textile fibers are left to circulate in the dyeing bath for 40 minutes at 40° C. Once the time has elapsed, they are removed from the iron and left to cool until the following day, at which time the textile fibers are removed from the dyeing bath and left to dry on a line.

3. Results and discussion

3.1 Extraction of natural dye from avocado seed

Before starting the extraction of the natural colorant from the avocado seed, the presence or lack of pectin in the avocado seed was determined by means of a Pectin Test. It is necessary that the seed be free of pectin in order to use it in the extraction because, being a gelling agent, if the extraction is carried out with an avocado seed containing pectin, the solution obtained will have a thick consistency. As shown in Figure 3, in test tube 1) there is an absence of pectin since the liquid is clear, in test tube 2) there is a slight presence of pectin due to the formation of haze and, finally, in test tube 3) there is a high presence of pectin due to the observed flocculation.

Figure 3. Avocado seed pectin test
(1) absence of pectin, (2) slight presence, (3) high presence of pectin.



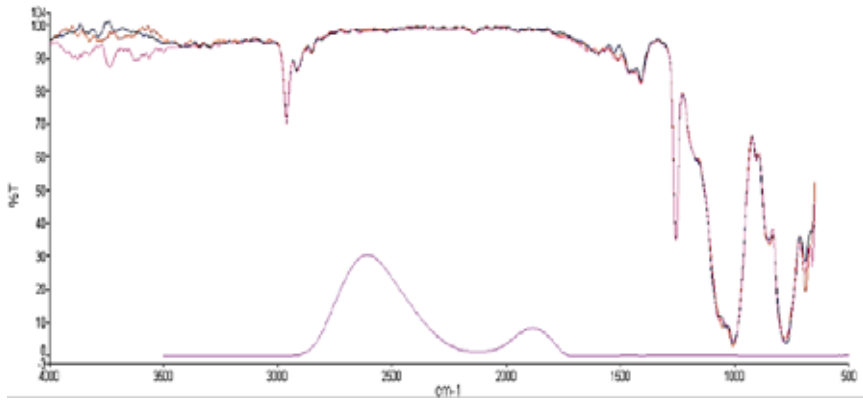
Source: Own elaboration.

The extraction of the natural dye from avocado seed was successfully carried out using Sodium Hydroxide 0.5% by weight as solvent. The decision was made to use sodium hydroxide as solvent since, according to Devia & Saldarriaga “After several solid-liquid extraction trials of the dye (...), it was found that the most suitable solvent is a dilute solution of sodium hydroxide” (2004).

This extraction was carried out for thirty (30) minutes at temperatures of 50°C and 75°C with avocado seeds at physiological maturity and consumption maturity. It is important to mention that in the methodology of the authors mentioned above, extractions are carried out with a duration of one (1) hour, as well as with a temperature of 100°C. Regarding the one (1) hour extraction, according to Rosales “The structural conversions of anthocyanins are endothermic reactions. They resist processes at high temperatures for short periods of time” (2015). As when performing the corresponding literature review, it was determined that anthocyanin degrades when in the presence of temperatures greater than or equal to 100°C (Simpson et al, 1976). For the reasons mentioned above, the decision was made to discard those extractions lasting one (1) hour or at a temperature of 100°C.

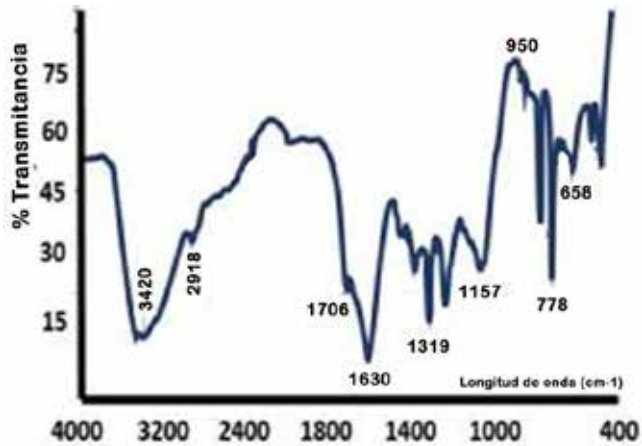
The colorant obtained is an anthocyanin. To verify the presence of anthocyanins in this dye, a Fourier Transform Infrared Spectrometry (FTIR) test was carried out, where the results obtained are shown in Figure 4 and their respective comparison with the anthocyanin reference is shown in Figure 5.

Figure 4. FTIR spectra of anthocyanins present in avocado seed.



Source: Own elaboration using FTIR.

Figure 5. Infrared spectrum of a flavinyl dye fraction.



Source: Barragan et al., 2017

Analyzing the infrared spectrum graph obtained and comparing with the infrared spectrum for a flavinyl dye, it is established that between 2900 and 3000 cm^{-1} C-H stretching occurs, which is due to the fact that, as the atom attached to the carbon increases in mass, the vibrational frequency decreases, i.e., the wave numbers become smaller. These peaks are seen in Figure 5 at 2914.29 and 2971.42 cm^{-1} , peaks which in turn signify the presence of methyl C-H groups. On the other hand, there is the presence of a C=C stretching of the aromatic ring between 1600 and 1475 cm^{-1} , which is specifically observed at 1475.43 cm^{-1} in Figure 6. The

band at 1428.57 belongs to the vibration of the carbon-nitrogen (CN) bond. Approximately at the height of 1050, 1260 and 1515 cm^{-1} the deformation occurs in the aromatic =C-H (benzene) plane and flavonoid group = C-O-C, which is shown in Figure 6 at 1271.43 and at 1071.43 cm^{-1} along with the bending vibration of carbonyl groups (COC) which may indicate the presence of carbohydrates. The bands between 860 and 780 cm^{-1} correspond to aromatic ring vibrations. All these vibrational frequency characteristics suggest a flavonoid-like structure of the anthocyanin type in the FTIR spectra for the dye extracted from avocado seed.

3.2 Determination of the yield of natural colorant

Table 2 shows the results of the yield obtained according to each natural colorant extraction for each avocado seed according to its maturity.

Table 2. Yield of natural dye extractions from avocado seed .

Seed maturity	Temperature (°C)	Yield (%)	Average (%)
Physiological	50	89.39	90.88
		90.86	
		92.39	
	75	88.82	87.61
		87.97	
		86.06	
Consumer	50	95.48	94.04
		92.25	
		94.39	
	75	86.74	88.10
		89.60	
		87.96	

Source: Own elaboration

It should be noted that good performance is related to the nature of the solvent and depends on many factors such as the ionizing power of the solvent, its polarity, binding forces and other physicochemical factors. Therefore, using sodium hydroxide as a solvent results in a high yield.

On the other hand, when comparing the extraction temperatures used with avocado seeds at different stages of maturity, it can be observed that extractions carried out at 50°C have higher yields than those carried out at 75°C. While, when comparing the extractions in terms of the state of maturity of the seed used, it can be established that a higher yield is obtained when using avocado seed at consumer maturity. In this sense, it can be said that by obtaining a higher yield using the avocado seed at consumption maturity, it is a matter of taking advantage of a residue without compromising the fruit pulp, while if it were the avocado seed at physiological maturity, the whole fruit would be wasted. The yield obtained in this research work is considerably higher in comparison with that obtained by the authors Devia & Saldarriaga and Moreno, who are in charge of the investigation of the colorant in solid state (powder). Therefore, it is evident that the dye in liquid state, with an average yield of 90.15%, presents a higher yield than the dye in solid state (powder), with an approximate yield of 45% among the consulted authors.

3.3 Determination of natural dye concentration

Table 3 shows the results corresponding to the concentration of the dyes obtained at the temperatures worked and with the seeds at physiological and consumption maturity.

Table 3. Absorbances and concentrations of the natural dye.

Seed maturity	Temperature (°C)	Absorbance	Concentration (M)	Average concentration (M)
Physiological	50	0.318	9.18	9.28
		0.321	9.27	
		0.325	9.38	
	75	0.469	13.54	13.54
		0.473	13.66	
		0.465	13.43	
Consumer	50	0.547	15.79	15.79
		0.545	15.74	
		0.549	15.85	
	75	0.725	20.93	20.93
		0.723	20.88	
		0.727	20.99	

Source: Own elaboration

Once the absorbance values were obtained, we proceeded to calculate the concentrations using the Lambert-Beer Law. For the use of this law, it was determined that the extinction coefficient for cyanidin is $26900 \text{ M}^{-1} \text{ cm}^{-1}$. It was decided to use the aforementioned extinction coefficient because both the peel and the seed of the avocado have a high content of flavonoids, including procyanidin, flavonols and hydroxycinnamic acids. In this sense, procyanidins are a class of anthocyanin from flavonoids, which upon oxidation depolymerize turning into cyanidins, (Tremocoldi, et al, 2018).

When analyzing the concentration values obtained, it was found that, comparing the natural dyes in terms of the maturity of the avocado seed used, the dye extracted from the avocado seed at consumption maturity presents a higher concentration than the dye extracted from the avocado seed at physiological maturity. This is due to the fact that, as the avocado ripens, modifications occur in both its structure and composition, due to the depolymerization of the fruit cell wall (Ceballos & Montoya, 2013, p.105). Therefore, it was determined that as avocado ripens, the concentration of anthocyanins in the avocado seed increases, which coincides with the statement of Anttonen and Karjalainen (2005) who “found wide variation in anthocyanin content, (...) among cultivars and environments; the degree of fruit ripening also influences the content and type of anthocyanins, as well as other phenolic compounds.”

On the other hand, when comparing the concentrations in terms of the temperature used in the extraction, those carried out at 75°C present a higher concentration than the extractions carried out at 50°C . The reason for this is that, when carrying out the extraction using a water-based solvent and, in turn, working with temperatures close to 100°C , part of the liquid evaporates due to the greater kinetic energy present between the water molecules (Jaramillo, 2007).

3.4 Verification of the dyeing capacity of the natural dyestuff by application to different textile fibers

For the fulfillment of this objective, the application of the natural dye was carried out using textile fibers: 100% cotton, 100% polyester and 50% cotton 50% polyester, with the due previous preparation of the fabric and using common salt (NaCl) as mordant during dyeing for the fixation of the dye in the textile fiber.

Natural dyes are recognized for their coloring power in natural protein fibers such as wool, silk and cotton, (Kumar & Agarwal, 2009). Whereas, they are not effective in synthetic fabrics, (Xicota, 2015). The results obtained, verify the above mentioned. When applying the natural dye in the 100% cotton textile fiber, a dyeing with intense and uniform coloration was obtained, since it is a fabric of natural origin. On the other hand, when carrying out the dyeing in the textile fiber 50% cotton 50% polyester, a less intense coloration was obtained than in the fiber 100% cotton, but with equal uniformity of dyeing, having the presence of polyester generates the less intense dyeing. Finally, evaluating the dyeing of the 100% polyester textile fiber, it is observed

that the coloration is not very intense and, in turn, presents little uniformity with the presence of slight darker stains than the rest of the fabric, since it is a synthetic fabric.

It was verified that the intensity of the coloration depends on the concentration of the dye used. Regarding the dyes extracted from the avocado seed at consumption maturity, it is considered that, when evaluating the dyeing carried out with the dye extracted at 75°C, the most intense coloration was observed, since it is the dye with the highest concentration. On the other hand, when evaluating the dyeing carried out with the dye obtained at 50°C, a less intense coloration was observed than with the dye at 75°C, due to the decrease in concentration. In relation to the dye extracted from the avocado seed at physiological maturity, when analyzing the dyeing carried out with the dye obtained at 75°C, it presented a coloration similar to that obtained with the dye from the avocado seed at consumption maturity at 50°C due to the similar concentration of both dye solutions. The dye extracted at 50°C presented the least intense coloration of all due to its low concentration.

3.5 Evaluation of natural dye fixation by a wash fastness test

The textile fibers, when completely dry, were subjected to a process that simulates a digital color sensor under the CMYK (Cyan, Magenta, Yellow, Key) model.

Comparing the results obtained, it can be determined that the 100% cotton textile fiber presents the highest dye fixation with natural dye from avocado seed. Then, comparing this textile fiber in terms of the dye used, the highest dye fixation was presented with the dye extracted from the avocado seed at consumption maturity, with a similar fixation between the dyeing with the dye extracted at 50°C and 75°C, with a tendency to the loss of black color (Key) when washing the textile fiber.

As for the textile fiber 50% cotton 50% polyester, it presented an intermediate discoloration, with greater discoloration in those dyeings carried out with the natural dye extracted from the avocado seed in physiological maturity, having the presence of polyester, the fixation will be lower due to the low affinity between natural dyes and artificial textile fibers. With respect to the 100% polyester textile fiber, there was a great discoloration of approximately 55% in all colors (Cyan, Magenta, Yellow, Key), since it is an artificial textile fiber and natural dyes are not effective in this type of fabrics.

Both the 50% cotton 50% polyester and 100% polyester textile fibers showed a greater discoloration of the Magenta and Yellow colors in the first wash and, in the second wash, a greater loss of the black color (Key). Meanwhile, all the textile fibers showed a tendency to the loss of black color (Key) throughout the washes.

Finally, the 100% polyester textile fiber showed the lowest fixation because it is a man-made fabric. The average total color loss among all the polyester fabrics dyed was 54.3% Magenta, 49.6% Yellow and 58.6% Key. Therefore, it is evident that this dye is not effective in this type of textile fibers.

4. Conclusions

- Reddish-brown dye was efficiently extracted from avocado seed at physiological and consumption maturity, using 0.5% sodium hydroxide solution by weight, at temperatures of 50°C and 75°C.
- The dye extracted from the avocado seed contains anthocyanins.
- The average yield of the extractions was 90.15%.
- The use of sodium hydroxide as a solvent proved to be efficient for this experiment since a high yield was obtained.
- The concentration of the dye depends on the maturity of the avocado seed used.
- The low color intensity is due to the partial or complete presence of artificial textile fibers.
- The most intense dyes were obtained with the dye extracted from the avocado seed at eating maturity at 75°C.
- The dye obtained in this work is not effective on man-made textile fibers.
- The highest fixation of the natural dye was obtained in the 100% cotton textile fiber, specifically with the dye extracted from the avocado seed at consumption maturity.
- The 50% cotton 50% polyester and 100% polyester fibers showed less fixation when using this dye, since they are artificial textile fibers.
- After washing the textile fiber, there was a tendency for the black color to fade (Key).

Conflict of interest

The authors declare that there is no conflict of interest with respect to the publication of this document.

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