

# Evaluation of the Dyeing Process for Paiche (*Arapaima gigas*) Leather Using Colorant Extracted from Walnut Leaves (*Juglans neotropica*) for Scaling-Up

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## ABSTRACT

The technological development of paiche leather tanning is projected as a high-impact economic activity and requires the application of clean technologies, such as replacing highly toxic synthetic chemicals. This study aimed to evaluate the extraction process of naphthoquinone from walnut leaves for dyeing chrome-free tanned paiche leather and to establish the feasibility of scaling it up. The active principle extracted in an aqueous medium was characterized using HPLC chromatography and UV spectrophotometry. A factorial design was implemented to evaluate leather dyeing, and the results were scaled up at the pilot level. The factors studied were dyeing time (60, 90, and 120 minutes) and walnut dye concentration (161.6, 209.8, and 250.0 g/L), with Color Intensity in the dyed leather as the response variable. Juglone (5-hydroxy-1,4, naphthoquinone) was identified as the primary dye-producing compound with maximum absorption at 210 nm in aqueous solution. The results of the experimental design fit a linear model, with a determination coefficient ( $R^2$ ) of 77.36% and an adjusted coefficient of 74.34%. The values obtained for the finished leather at the pilot scale, including tear resistance (88.90 N), tensile strength (8.34 N/mm<sup>2</sup>), elongation percentage (53.76 %), dry (4/5), wet (4), and artificial sweat rub (4) and lightfastness (5), indicate that walnut-dyed leather can be used in the manufacturing of footwear and clothing. The process is technically scalable to an industrial level.

## KEY WORDS

*Arapaima*, chrome-free tanning techniques, leather dyeing, natural colorant, walnut.

## INTRODUCTION

Scaling up innovative and sustainable processes at the industrial level in the tanning sector is a challenge in Peru. This industry has been severely impacted in recent decades by the entry of imported footwear and requires the recovery of its productivity and competitiveness levels in a sustainable manner [1]. To achieve this, research and development of new products and clean technologies are necessary, involving the incorporation of differentiating elements into their processes and products as a strategy to enter new markets and also to comply with environmental regulations. This strategy become even more relevant when implemented within a circular economy context, based on the reuse of waste.

In Peru, the predominant sources for leather production are traditionally limited to cattle and sheep [2]. However, there are alternative productive sectors, such as aquaculture and fishing, that generate byproducts capable of serving as valuable raw material for the leather industry, thereby establishing the initial link in a new value chain [2,3]. The promotion of aquaculture, particularly focused on paiche (*Arapaima gigas*), has gained significant importance in the Amazonian regions of Peru [2,3], and according to the FAO, the decade spanning from 2010 to 2020 witnessed a total reported paiche production of 19 434 tons from fishing, with 66.14 % originating from Brazil and 32.89 % from Peru. As a byproduct of this activity, skins are generated. Once tanned, these skins can be valorized as exotic leather for the manufacturing of various articles.

Inquiring into the paiche skins exports for the region, Brazil emerges as the leading exporter with 51 316 hides during the period from 2012 to 2020. During this period, the main buyers from Brazil were the Mexico, USA, Italy, and Netherlands [4]. Estimates from CITES indicate that Brazilian exports of paiche leather products during the period from 2012 to 2020 mainly consisted of 47 154 large-sized paiche leather products, 34 255 and small-sized paiche leather products. In smaller quantities, there were exports of clothing items (25 971) and raw leather (850). It is observed that in 2020, corresponding to the time of the pandemic, exports decreased significantly [4].

In the tanning process, the color imparted by the dyeing stage, is important for diversifying manufactured items such as footwear, clothing, and leather goods in accordance with fashion trends and is a determining factor in customer preferences [5]. The main colorants used in the dyeing process are synthetic organic compounds, primarily of acidic, anionic, and water-soluble nature, with azo compounds being prominent among them [6,7]. Azo colorants are the most widely produced globally at an industrial level, representing approximately 70 % of the colorants used by the industry [8]. In Peru, in just the year 2022, 559 000 dollars' worth of diazoic, azoic, or azoxy compounds were imported (recovered from Trade Map) [9].

It has been determined that certain azo compounds can be reduced by skin microorganisms, intestinal bacteria, and liver bacteria, potentially leading to carcinogenic aromatic amines. Therefore, the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) regulation has restricted the use of these colorants since 2009 [10–12]. Besides, traces of azo compounds have been found in environmental samples, such as river water, drinking water, wastewater, suspended particles, sediments, soils, and fish [7], and the toxicity of these substances to ecosystems has been demonstrated [13].

The substitution of chemical products with natural alternatives, characterized by lower toxicity and greater degradability, is a global trend in various industries. This shift aims to mitigate the negative impact of synthetic chemicals on human health and ecosystems. In this regard, several research studies demonstrate the technical

feasibility of applying active principles extracted from plants for leather dyeing [14–18]. However, these substitutes must guarantee batch-to-batch reproducibility, compliance with specifications or technical requirements, flexibility for application to various types of leather, and cost-effectiveness in scaling up [6].

The walnut, scientifically known as *Juglans neotropica Diels*, a medium to large-sized tree [19], belonging to the *Juglandaceae* family within the genus *Juglans*, is a specie with noteworthy properties. These properties are due to the presence of active ingredients found in its fruit (tetradentate tannins and 1,4,5-trihydroxynaphthalene-4-beta-D-glucoside), bark (lignin, hemicellulose, and cellulose), branches (lignin, cellulose, hemicellulose, and tannins), and leaves (naphthoquinones, flavonoids, phenolic acids, and oils) [20–22]. It has been demonstrated that these active principles possess medical properties, for instance, previous studies have reported that the alcoholic-aqueous extract of walnut leaves exhibits antioxidant effects [23,24], dental bleaching capabilities [25,26], antibacterial properties [24,27], and dyeing potential [28]. These beneficial effects are attributed to the abundant presence of phenolic compounds, including naphthoquinones and flavonoids [28].

Juglone (5-hydroxy-1,4, naphthoquinone) is a compound found in walnut leaves, stem bark, and husks [28–31]. Figure 1 illustrates the chemical structure of juglone. Predominantly, it has been used to dye textile fibers such as wool, cotton, silk, flax fabric and nylon fabrics, imparting a natural brown color [28,30,32–34].

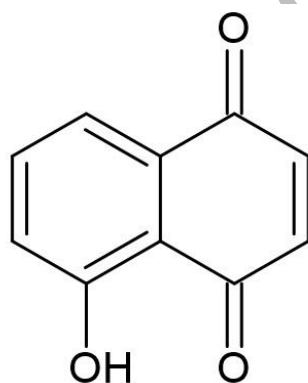


Figure 1. Juglone molecule. The figure was drawn using the software ACD/ChemSketch Freeware Version.

However, natural dyes have the limitation of weak fixation onto fabric, thus requiring the use of mordants to fix the color. Investigated mordants can be grouped into chemical mordants such as aluminum salts, iron, and tannic acid, and biomordants extracted from plant species [30,35,36].

Regarding leather, scientific literature reports that the application of juglone has been conducted on leather pieces, both with and without the application of mordants. Mordants studied in this context include tartaric acid, citric acid, formic acid, and acetic acid [37,38]. In other studies, researchers evaluated the effects of dyeing leather with extracts from green and brown (mature) walnut shell residues.

In Peru, the walnut tree has been identified in various localities, including Amazonas, Cajamarca, Cusco, Huancavelica, Junín, La Libertad, Lambayeque, Lima, and Pasco [39]. It is noteworthy that the utilization of walnut leaves for dye extraction represents an activity that, if expanded, would align with the principles of a circular economy. Between 2010 and 2018, the average production stood at 4,644 cubic meters of roundwood and 2,579 cubic meters for sawn timber. However, there was a noticeable decline to 1,718 and 1,122 cubic meters,

respectively, from 2019 to 2020. This reduction is likely attributed to the impact of the pandemic on productive activities [40]. It is worth highlighting that dyeing with walnut extract is more sustainable when applied to a substrate such as chrome-free tanned leather [41]. This approach aims to produce more biodegradable leather and replace basic chromium salts, the most widely used tanning agent in the industry, thereby avoiding its toxicity to the environment and human health [12,42].

The present study aimed to evaluate the application of aqueous walnut leaf extract in the dyeing process of paiche (*Arapaima gigas*) leather, tanned with phenolic-synton and glutaraldehyde compounds, to determine the technical feasibility of its scaling-up at the pilot level. For this purpose, (i) juglone was characterized as the main tinting active principle obtained through aqueous extraction using diode array chromatography and UV-visible spectrophotometry, and (ii) the application of the dye extract in the dyeing process was evaluated using a 3<sup>2</sup> factorial experimental design, with color intensity determination in the leather as the technical criterion. Additionally, (iii) the dyeing conditions that achieved the highest color intensity were scaled up to the pilot level, and their physical-mechanical properties, including color fastness, were determined. Thus, this research contributes to the technological development of dyeing with natural colorants in the tanning sector.

## EXPERIMENTAL SECTION

### *Raw material*

For the present study, walnut branches were acquired from local suppliers (Mercado La Parada) in the fall season (June 2023), these were leafed out to obtain the walnut leaves, which were used for the extraction of the colorant. The dyeing process was applied on paiche leathers tanned with phenolic-synton and glutaraldehyde compounds, the paiche skins that were transformed into leather in this study are a byproduct from Amazonian aquaculture activity and were acquired in the Pucallpa region (Peru), with certificate of origin No. 167-2021 issued by the Regional Government of Ucayali.

### *Chromatographic characterization of walnut colorant*

Methanol of HPLC grade (Sigma-Aldrich), 2,6-di-tert-butyl-4-methylphenol (butylhydroxytoluene) of HPLC grade (Sigma-Aldrich), phosphoric acid of ACS grade (Analyticals Carlo Erba), acetonitrile (ACN) of HPLC grade (Merck), and water of HPLC grade, microfiltered to 0.22 µm with a conductivity of 1.01 µS/cm, were utilized in this study.

For the chromatographic characterization of the active dyeing principle of the walnut, juglone, walnut leaves were dried in a food dehydrator at 70°C for 120 minutes. Subsequently, they were ground in a grain mill and sieved using a WS TYLER brand ASTM E11 sieve until obtaining 120-mesh particles.

The moisture content of the powdered walnut leaves was determined through gravimetry, using an HV-OVENS brand oven at a temperature of 110 °C, and a Digital Precision brand balance, model ESJ200-4. The ash content was determined using a TOKY brand muffle furnace at a temperature of 520 °C.

The quantification and characterization of the active principle were performed using a high-performance liquid chromatograph (HPLC) with UV and diode array detectors (DAD) from the KONIK 560 brand. The instrument was equipped with a column oven, low-pressure gradient pump, inline degasser, and solvent delivery module,

along with the Konikrom Plus Version 3.0.5.505 Chromatography Station for Windows – 2013 software. For chromatographic separation, a C18 chromatographic column with a particle size of 5 µm and dimensions of 4.60 mm x 150 mm from the RP Thermo Aclaim brand was employed.

The sample was prepared by taking 1000 mg of ground and sieved walnut leaves and 20 ml of 2,6-di-tert-butyl-4-methylphenol- (to avoid oxidation of juglone). This mixture was placed in an ultrasound bath at 25°C for 40 minutes. The extract was then centrifuged at 1200 rpm, and the supernatant was filtered through a polyamide membrane and stored at -20 °C [43].

The mobile phase was prepared using 0.05 % (w/w) phosphoric acid (solution A) in water. The gradient used was as follows: 70 % of solution A and 30% of ACN (5 minutes) y 30 % of solution A and 70 % of ACN (6 minutes). The flow rate of the mobile phase was 1 mL/min, with an injection volume of 5 µL at a constant temperature of 20°C in the oven [43].

The characterization and quantification of juglone were conducted using Juglone–5-hydroxy-1,4-naphthoquinone as the standard, CAS No: 481-39-0, with a molecular weight of 174.15 g/mol and a purity of ≥96.5%, graded as HPLC grade. Propyl gallate (GLP) and quercetin (QCTN) were employed as internal standards. The characterization was carried out at wavelengths of 220, 250, 254, and 260 nm in the ‘Fractal Químicos SRL’ Laboratory.

#### ***Spectrophotometric characterization***

The spectrophotometric characterization was conducted through the analysis of a walnut solution, obtained by boiling 50 g of walnuts in 500 ml of distilled water for 60 minutes. The resulting solution was filtered and diluted to achieve a walnut concentration of 1 g/L. Subsequently, the solution was analyzed using an Agilent spectrophotometer, model Cary 60 UV-VIS, with Cary WinUV software, in the range of 200-600 nm [44]. Also, the characterization was carried out in an alcoholic medium using methanol, HPLC grade.

Furthermore, the absorbance of juglone was assessed concerning the variation in dye concentration in the solution. For this purpose, three aqueous solutions were prepared from dried walnut leaves. The 250 g/L solution was prepared by taking 50 g of walnut leaves in 500 ml of tap water, reducing its volume to 200 ml (Solution B) by boiling. The remaining walnut leaves were boiled again with an additional 500 ml of water and concentrated to 200 ml (Solution C). This process was repeated once more (Solution D) [45]. The experiments were conducted at the R&D Laboratory of CITEccal Lima.

#### ***Experimental design***

An experimental factorial design 3<sup>2</sup> was proposed (Table 1), considering two factors with 3 levels each. The factors analyzed were dyeing time (DT) and dye concentration (DC). The response variable was Color Intensity, expressed as K/S. A replicate was performed for each treatment of the design, obtaining 18 treatments. The dyeing temperature was established at 50 °C.

This design was developed at the pre-pilot level, using pieces of approximately 15 x 25 cm in size, in 6-position stainless steel dyeing test drums of the 7WAY brand, model GB R35-9. This stage was conducted at the Tanning Pilot Plant of CITEccal Lima.

Table 1. Experimental design for the scaling-up at pilot level of the dyeing of paiche leather with walnut leaves colorant.

Factors	Units	Levels			The dye
		Low	Medium	High	
Dyeing time (DT:x1)	minutes	60.0	90.0	120.0	
Dye concentration (DC:x2)	g/L	250.0	209.8	161.6	

extraction was carried out in a reactor with a capacity of up to 25 L. Initially, 1000 g of walnut leaves were taken and mixed with 8 L of tap water. The mixture was heated between 80 °C and 100 °C for one hour, and the volume was reduced to 4 liters, resulting in a concentration of 250 g/L of walnut extract (Solution A). The residual walnut leaves were added to 8 L of water, subjected to the same temperature and time conditions, and reduced to a volume of 4 liters, obtaining (Solution B). This process was repeated for a third time, resulting in (Solution C).

The exact concentration of solutions B and C was determined based on the absorbance of each solution in the range between 204 to 213 nm. The values obtained were 209.8 g/L and 161.6 g/L, respectively. These concentrations were considered as the three levels analyzed for DC in the experimental design. In the dyeing process, 10% sodium chloride (% w/w) was used to increase the exhaustion of natural dye [34,46].

#### **Reflectance and Color Intensity (K/S) measurement**

For the measurement of Color Intensity (K/S) [47,48], a Datacolor spectrophotometer, model SF 550, was utilized. Calibration was performed using a black trap and a white plate, with repeatability diagnosis conducted using a green plate. The calibration conditions included a 9 mm SAV aperture (specular included) and UV filter OFF (UV included). Color Tools vs. 1.1 software was used for analysis. This equipment is located in Laboratory 24 of the Faculty of Chemical and Textile Engineering at the National University of Engineering. The K/S value was calculated by applying the Kubelka-Munk function to the maximum absorption length of juglone, according to equation 1, where R is the measured reflectance.

$$\frac{K}{S} = (1 - R)^2 / 2R \quad (\text{Eq. 1})$$

Additionally, the color measurement value was determined by transforming the reflectance spectra value to the standard Commission International of l'Eclairage (CIELAB) coordinate system. This transformation involves the values of L = luminosity, a\* = red or green, b\* = yellow or blue, c\* = color purity Chroma and h = color hue [15].

#### **Scaling-up of dyeing process at pilot level**

The scaling-up was conducted under optimal dyeing conditions identified in the experimental design at a pH of 5. The process involved four paiche tanned skins in a twin-load stainless steel drum of the 7WAY brand, model GHE II R801-2. This drum is capable of holding up to 15.0 kg of skins or leather, with temperature control up to 80°C (+/- 1 °C) and agitation ranging from 0 to 30 RPM.

In the dyeing process, industrial-grade sodium chloride was utilized as a mordant, along with tap water. The skins underwent fatliquoring with a mixture of natural and synthetic phosphoric esters (FOSPHOLICKER 61.46). In the finishing stage, a combination of a protein binder and polishing wax (LVH67), an aqueous dispersion of

aliphatic polyurethane (TOP L 718), penetrating agent (PE 200), anionic aliphatic polyurethane (TOP L 450), and a water-based lacquer (E.lack 2022) was applied. Both the dyeing and finishing processes were carried out at the Pilot Tannery Plant of CITEccal Lima. The formulation for the dyeing, fatliquoring, and finishing processes is provided in Table 2.

*Table 2. Dyeing, fatliquoring and finishing processes formulation.*

<i>Process</i>	<i>Product</i>	<i>Amount (%)</i>	<i>Duration (min)</i>	<i>Temp. (°C)</i>	<i>Observations</i>
Dyeing	Walnut leaves colorant	x2			Bath pH: 5
	Sodium chloride	10	x1	50	Speed (RPM): 20
	Water	200			Check dye penetration
Fatliquoring	Mixture of natural and synthetic phosphoric esters	2	40	25	Once finished, wash and drain. Dry the leather off at toggling or room temperature.
Protein-based Finishing	Blend of protein binder and polishing wax	40			Apply manually or by spraying.
	Aqueous dispersion of aliphatic polyurethane	3		Room temperature	Dry the leather off.
	Penetrating agent	10			
	Water	47			
Resin-based Finishing	Anionic aliphatic polyurethane	40			Apply manually or by spraying.
	Water based laquer	25		Room temperature	Dry the leather off and polish the grain.
	Penetrating agent	10			
	Water	25			

### *Analysis of the chemical and physical-chemical properties of the leather dyed at pilot level*

After dyeing and finishing the paiche leather at the pilot level, two skins were sampled to characterize their mechanical properties. The evaluated properties included: tear resistance, tensile strength and elongation percentage, seam strength, color fastness to dry rubbing, wet rubbing, and artificial sweat solution, pH and shrinkage. The tests were conducted at the Physical Chemistry Laboratory of CITEccal Lima using the following equipment: electronic dynamometer with a load capacity of 5 kN of the INSTRON brand, model 23-5D; color fastness measurement equipment, MUYER brand, model 5070; leather mill of the FRITSCH brand, model PULVERISETTE 19; potentiometer of the WTW brand, model MULTILINE - P4. Before conducting the tests, the samples were conditioned according to the NTP ISO 2419 standard, at a temperature of  $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and a relative humidity of  $50\% \pm 5\%$ .

### *Statistical analysis*

The data underwent analysis of variance (ANOVA), and the model assumptions, including normal distribution, homoscedasticity, and independence of errors, were tested. Determination coefficients ( $R^2$  and adjusted  $R^2$ ) and

the lack of fit were calculated to assess the model's fit. The statistical analysis was conducted using the "rsm" and "lmtest" packages in R software version 4.2.3 (R Core Team, 2023).

## RESULTS AND DISCUSSION

### *Walnut leaves characterization*

The moisture content of the walnut leaves was determined to be  $4.81\% \pm 0.22$ , and the ash content was found to be  $11.0\% \pm 0.5$ . Figure 2a shows the standard chromatogram for juglone at different concentrations, identifying it at a retention time of 4.5 minutes. In Figure 2b, the chromatogram for the sample is presented, where juglone is also identified at 4.5 minutes.

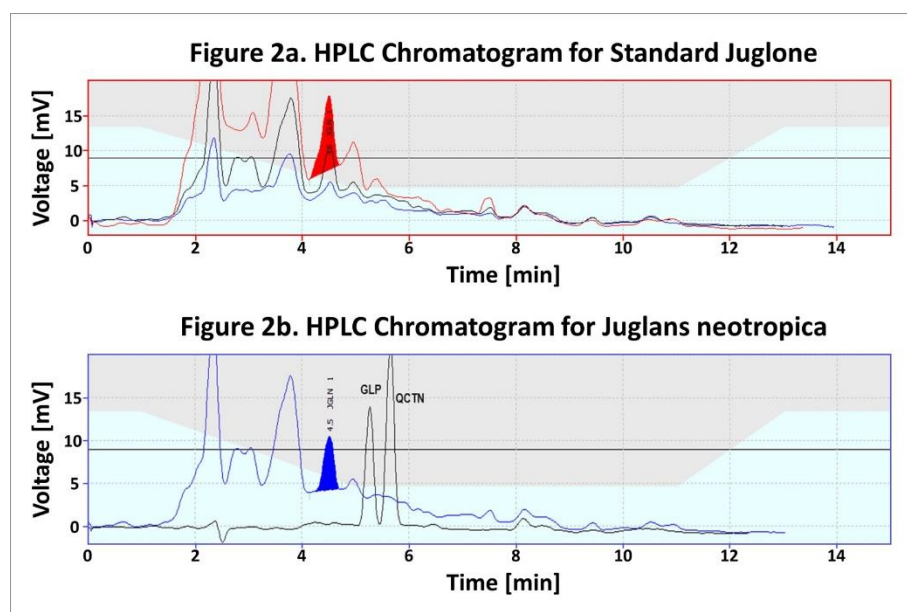


Figure 2. Juglone chromatograms. Figure 2a depicts chromatograms of the juglone standard (5-hydroxy-1,4-naphthoquinone) at concentrations of 6, 12, and 24 ppm. Figure 2b shows the chromatogram of the sample under study (blue line) along with the standard chromatogram at 6 ppm, adding the internal standards propyl gallate (GLP) and quercetin (QCTN) (black line).

The chromatographic characterization results of walnut leaves indicate a juglone content of 423 mg/100 g expressed on a dry weight basis. Medic A. et al. (2022), characterized and quantified the primary phenolic compounds in 6 different walnut species, finding juglone content ranging from 170 to 900 mg/100 g expressed on a dry weight basis. Table 3 shows the values of juglone reported by other authors; the differences between the results could be associated with the walnut leaf species analyzed and their level of maturity. This research used mature walnut leaves [49–54].



**Table 3. Results of juglone in previous studies.**

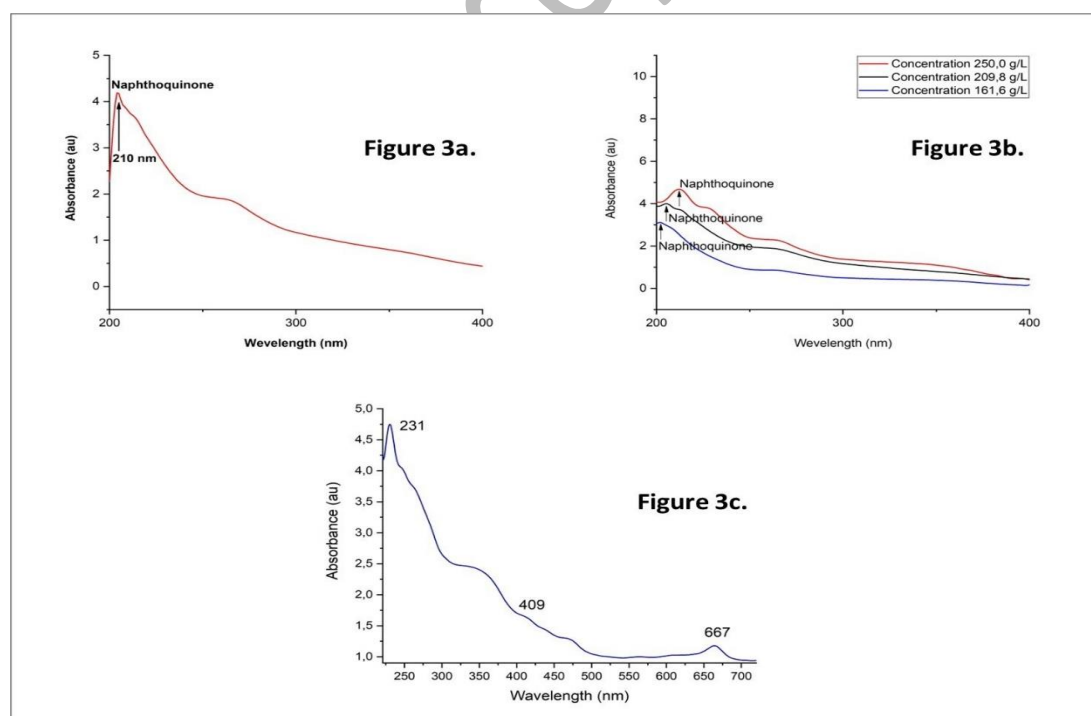
Author	Juglone value in dry weight	Analysis Methods
Girzu M., et al	up to 500 mg/100 g	HPLC - DAD
Medic A., et al	170 -900 mg/100 g	HPLC-MS/MS
Thakur A and Cahallan C.	357 mg/100 g	VERSA max™ tunable micro-plate reader (Molecular Devices Corporation)
Stampar et al.	218 and 1404 mg/100 g	HPLC - PDA*
Cheniany et al.	21.18 and 38.47 mg/100 g	HPLC - PDA
Cosmulescu S. et al.	5.42 to 22.82 mg/100 g	HPLC - RP**

\* High Performance Liquid Chromatography - Photo diode array detector.

\*\* Reverse Phase High Performance Liquid Chromatography.

### Spectrophotometer walnut leaves characterization results

Figure 3a displays the absorption peak of juglone in the aqueous extracted at 210 nm. Likewise, in Figure 3b it is observed that the change in juglone absorbance concerning walnut concentration and the extraction cycle at 80°C, is directly proportional. Figure 3c displays the absorption spectrum of walnut in an alcoholic medium. At 231 nm, 409 nm, and 667 nm, three absorption peaks are observed, corresponding to n- $\pi^*$  transitions of the carbonyl groups in juglone [55].



**Figure 3. Variation in the concentration of naftoquinone per extraction cycle. Figure 3a shows juglone spectra in aqueous solution. Figure 3b shows the spectra of juglone extracts for experimental design. Figure 3c shows the spectra of juglone in alcoholic solution.**

### Results of experimental design

The reflectance and Color Intensity (K/S) results for the experimental design treatments are presented in Table 4. It's worth noting that these values were determined for the leather in crust state, without finishing. It can be observed that the experimental runs 01 and 16, dyed with extreme concentrations of walnut at 161.6 g/L and 250 g/L, respectively, and under the same conditions of temperature (50°C) and time (60 minutes), yielded the lowest and highest K/S values: 0.06 and 0.34, respectively. Thus, it complies with the Kubelka-Munk equation, where the K/S value is proportional to the concentration of the dye in the substrate, in this case, paiche leather, despite the small natural irregularities on its surface.

Table 4. Results of experimental design.

Experimental Run	Paiche weight (g)	leather	Colorant concentration (g/L)	Dyeing time (min)	Reflectance (R)	Color Intensity (K/S)
01	111.56		161.6	60.0	0.85	0.06
02	125.79		161.6	90.0	0.71	0.08
03	141.03		161.6	120.0	0.67	0.08
04	115.86		161.6	60.0	0.67	0.06
05	158.56		161.6	90.0	0.71	0.11
06	134.69		161.6	120.0	0.63	0.08
07	177.87		209.8	60.0	0.67	0.16
08	126.66		209.8	90.0	0.57	0.21
09	151.77		209.8	120.0	0.53	0.17
10	139.35		209.8	60.0	0.56	0.25
11	175.96		209.8	90.0	0.50	0.17
12	169.73		209.8	120.0	0.56	0.19
13	147.40		250.0	60.0	0.54	0.32
14	172.84		250.0	90.0	0.46	0.17
15	150.03		250.0	120.0	0.56	0.20
16	135.95		250.0	60.0	0.54	0.34
17	126.83		250.0	90.0	0.45	0.32
18	153.61		250.0	120.0	0.46	0.36

Table 5 displays the ANOVA analysis of the results from the applied experimental design. The model adequacy was tested through the assumptions of normality (0.6233), homoscedasticity (0.0619), and independence of errors (0.2156), which were no significant, hence the assumptions were accomplished. Additionally, the evaluated lack of fit (0.8540) is not significant [56]. The data obtained adjusted to a linear model, with a determination coefficient ( $R^2$ ) for the model at 77.36 % and an adjusted coefficient equal to 74.34 %. It is shown that DC has a significant effect ( $p < 0.05$ ) on Color Intensity, which is consistent with the dye re-extraction process in the experimental design, where, starting with a concentration of 250 g/L of walnut, depending on the desired color intensities, it is possible to extract the juglone present in walnut leaves for at least 3 extraction cycles, resulting in the color range

shown in Table 4. A darker tone can be achieved with a concentration of 250 g/L after 60 minutes, while a much lighter tone can be obtained in a third extraction cycle at an approximate walnut concentration of 161.6 g/L for 60 minutes. It should be noted that juglone concentrations in the re-extraction processes can be increased by adding more walnut to the reactor to utilize residual juglone and achieve darker shades. On the other hand, it is observed that DT is not a significant factor in the process.

Khalid, B. et al. (2010), determined that in the dyeing of leather pieces with juglone, the most significant variables were dye concentration (10 %) and temperature (70 °C) [37]. The obtained results suggest it would be a chromium tanning process, where the leather exhibits higher temperature resistance compared to tanning with phenolic syntans and glutaraldehyde. In the present research, the dyeing process temperature was set at 50°C.

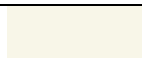



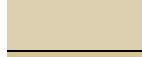














**Table 5. Results of ANOVA for experimental design.**

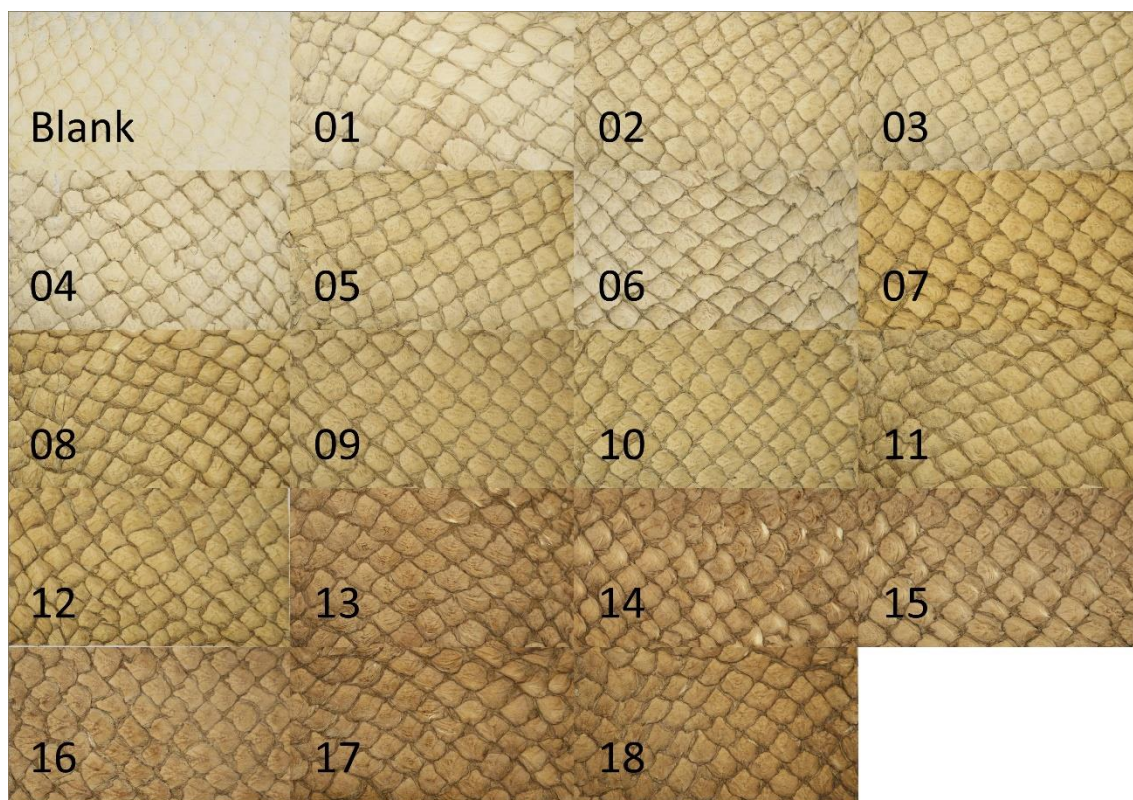
	<i>Estimate</i>	<i>Std. Error</i>	<i>t value</i>	<i>Pr(&gt; t )</i>	
(Intercept)	0.1819	0.0119	15.3391	1.42E-10	***
x1: Dyeing Time (DT) (min)	-0.0092	0.0145	-0.6316	5.37E-01	
x2: Dye Concentration (DC) (g/L)	0.1034	0.0145	7.1314	3.44E-06	***
<b>Response: Nogal Color Strength</b>					
	<i>Df</i>	<i>Sum Sq</i>	<i>Mean Sq</i>	<i>F value</i>	<i>Pr(&gt;F)</i>
FO(x1, x2)	2	0.13	0.06	25.6278	0.0000
Residuals	15	0.04	0.00		
Lack of fit	6	0.01	0.00	0.4114	0.8540
Pure error	9	0.03	0.00		
Multiple R-squared: 77.36 %			Adjusted R-squared: 74.34 %		
<b>Model Assumptions</b>					
Normal Distribution	Shapiro-Wilk normality test			p-value = 0.6233	
Homoscedasticity	studentized Breusch-Pagan test			p-value = 0.0619	
Independence of errors	Breusch-Godfrey test			p-value = 0.2156	

Table 6 shows the results of the optical image calculated from the results and color analysis respecting Color Intensity. Figure 4 presents the results of the dyed leather for each treatment of the experimental design, labeled according to its respective experimental run presented in Table 4 and 6.

The evaluation of the response variable indicates that maximum reflectance is achieved at a concentration of 250 g/L and a time of 60 minutes. The scaling-up was carried out at these optimal conditions, and it included the operations of fatliquoring and finishing according to Table 2.

**Table 6. Results of optical image.**

<i>Experimental Run</i>	<i>L*</i>	<i>a*</i>	<i>b*</i>	<i>C</i>	<i>H</i>	<i>Optical image</i>
Blank	85.19	2.44	27.35	27.46	84.94	
01	75.30	4.11	26.04	26.37	81.03	
02	71.32	5.32	28.70	29.19	79.50	
03	72.13	4.76	28.15	28.56	80.40	
04	75.55	3.86	24.96	25.26	81.20	
05	68.04	6.23	29.89	30.53	78.24	
06	72.27	4.56	25.37	25.78	79.82	
07	63.33	6.97	32.30	33.02	77.82	
08	61.04	6.86	30.31	31.08	77.24	
09	62.67	6.94	30.09	30.89	77.01	
10	60.38	5.89	28.60	29.20	78.37	
11	63.63	6.25	29.48	30.14	78.03	
12	61.86	6.81	30.34	31.10	77.34	
13	53.96	9.65	24.44	26.28	68.46	
14	61.25	8.93	25.94	27.44	71.00	
15	60.13	8.71	25.51	26.96	71.12	
16	53.25	9.71	25.31	27.11	69.03	
17	53.32	9.61	24.37	26.20	68.48	
18	52.81	9.23	26.03	27.62	70.46	



*Figure 4. Results of dyed leather for each treatment and blank. Numbering in the figure is according to the data presented in table 6.*

It is worth noting that the surface of the paiche leather is not uniform due to the inherent characteristics of the skin. It is composed of the imprint left by the extracted scale, forming a thin edge, and the roughness of the rest of the surface. These characteristics could also influence the value of  $R^2$ ; nevertheless, it is observed that the dye penetration into the skin is complete, and the color obtained under optimal conditions is uniform.

#### ***Results of physical-mechanical and chemical properties of dyed leather***

Table 7 presents the results of the evaluation of chemical and physical-mechanical properties of paiche leather samples that were dyed and finished, obtained from the batch in which the process was scaled up from the optimized conditions. This table includes the requirements set by Peruvian technical standards for various types of footwear and garments.

**Table 7. Results of chemical and physical-mechanical properties of paiche leather.**

PARAMETER	Application				Range of values obtained for paiche leather		Test Methods
	Casual Shoes-upper <sup>1</sup>	Men Shoes-upper <sup>2</sup>	Ladies Shoes-upper <sup>3</sup>	Clothing <sup>4</sup>			
Tear Resistance	Unlined: $\geq 100$ N With lining: $\geq 70$ N	$\geq 40$ N	$\geq 40$ N	$\geq 20$ N	88.40 N (571.58 N/cm)	NTP ISO 3377-02:2022. <sup>5</sup>	
Tensile strength	$\geq 10.0$ N/mm <sup>2</sup>	$\geq 15.0$ N/mm <sup>2</sup> in splits	$\geq 15.0$ N/mm <sup>2</sup> in splits	--	8.34 N/mm <sup>2</sup> (834 N/cm <sup>2</sup> )	NTP ISO 3376:2012. <sup>6</sup>	
Elongation at break	$\geq 15\%$ ( $\leftrightarrow$ ) $\geq 7\%$ ( $\uparrow$ )	--	--	--	53.76%	NTP ISO 3376:2012. <sup>6</sup>	
Shrinkage temperature	At least 70°C to 75°C with a shrinkage of no more than 5%.				Above 75°C: shrinkage 4%.	NTP 291.036:2007. <sup>7</sup>	
Seam Strength	--	--	--	--	9.20 N/mm	NTP ISO 17697:2018. <sup>8</sup>	
Dry rubbing color fastness (grain side) 50 cycles 150 cycles	Degradation: $\geq 3$ (150 ciclos) Release: $\geq 3$ (150 ciclos)	Degradation: $\geq 3$ (150 ciclos) Release: $\geq 3$ (150 ciclos)	Degradation: $\geq 3$ (150 ciclos) Release: $\geq 3$ (150 ciclos)	$\geq 3$ (50 cycles)	Degradation: 4/5 (50 cycles) 3/4 (150 cycles) Release: 4/5 (50 cycles) 4 (150 cycles)	NTP ISO 11640:2014. <sup>9</sup>	
Wet rubbing color fastness (grain side) 20 cycles 50 cycles	Degradation: $\geq 3$ (50 ciclos) Release: $\geq 3$ (50 ciclos)	Degradation: $\geq 3$ (50 ciclos) Release: $\geq 3$ (50 ciclos)	Degradation: $\geq 3$ (50 ciclos) Release: $\geq 3$ (50 ciclos)	$\geq 3$ (20 cycles)	Degradation: 4 (20 cycles) 4/5 (50 cycles) Release: 4 (20 cycles) 4/5 (50 cycles)	NTP ISO 11640:2014. <sup>9</sup>	
Artificial sweat solution color fastness (grain side) 20 cycles	--	--	--	$\geq 3$ (20 cycles)	4 (20 cycles)	NTP ISO 11640:2014. <sup>9</sup>	
Color Lightfastness	$\geq 3$ is taken as reference value (72 hours, exposure to yellow light)				5		
Thickness					1.44 mm		
pH	$\geq 3.5$ . If the pH value is below 4, the $\Delta$ must be $\leq 0.7$				7.4	NTP ISO 4045:2008. <sup>10</sup>	

<sup>1</sup> NTP 241.023:2014. SHOES. Casual footwear. Railway applications - Requirements and test methods.

<sup>2</sup> NTP 241.021:2015 FOOTWEAR. Men's footwear. Railway applications - Requirements and test methods.

<sup>3</sup> NTP 241.022:2015 FOOTWEAR. Lady's footwear. Railway applications - Requirements and test methods.

<sup>4</sup> NTP ISO 14931:2016. LEATHER: Guide for the selection of leather for clothing (excluding fur skins).

<sup>5</sup> Tear resistance – NTP ISO 3377-2:2022, 'LEATHER: Physical and mechanical tests - Determination of resistance to tearing - Part 2: Double tear'.

<sup>6</sup> Tensile strength and elongation percentage – NTP ISO 3376:2012, 'LEATHER: Physical and mechanical tests - Determination of tensile strength and elongation percentage'.

<sup>7</sup> Shrinkage temperature - NTP 291.036:2007, revised 2017, 'PELTRY - Physical tests - Determination of shrinkage temperature'.

<sup>8</sup> Seam strength – NTP-ISO 17697:2018, 'Footwear - Test methods for uppers, lining and insoles - Seam strength'.

<sup>9</sup> Color fastness – NTP ISO 11640:2014, 'LEATHER - Colour fastness tests - Colour fastness to and fro rubbing'.

<sup>10</sup> pH – NTP-ISO 4045:2008, revised 2018, 'Leather - Chemical tests - Determination of pH'.

Colorfastness is a crucial attribute that demonstrates the color's durability under different usage conditions. Therefore, dry and wet rubbing colorfastness, as well as exposure to an artificial sweat solution, were evaluated. The results indicate that walnut-dyed leather has a rating of 4/5 and 3/4 for 50 and 150 cycles, respectively, in the

assessment of color fastness to dry rubbing, and 4 for 20 and 50 cycles in the evaluation of color staining. Regarding the leather's fastness under wet conditions, the assessment of degradation and staining is within the range of 4, both at 20 and 50 cycles, and the fastness of the leather exposed to artificial sweat has a rating of 4 for degradation after 20 cycles of rubbing. These results suggest adequate fixation of juglone to the collagen structure of the leather, likely occurring through interactions, primarily between the amino and carboxyl groups of collagen and the hydroxyl groups of juglone.

On the other hand, the high color stability of the dyed material under light exposure, as evidenced by a rating of 5 after being subjected to a yellow light source for 72 hours in this study, could be attributed to the deactivation of the excited electronic states of juglone through the formation of covalent bonds between the dye and the collagen fiber [37].

The analyzed sample, dyed and finished, has a pH of 7.40, a value that meets the established requirements (see Table 7). It is important to note that the pH of the leather before dyeing was 5.0. During dyeing, the -NH<sub>2</sub> groups interact with the -OH groups of the juglone, contributing to the increase in pH. The pH in leather can vary depending on the interaction of collagen with the tanning agent and other chemicals used in the process, including different types of dyes. Table 8 shows the pH values obtained by other researchers in leather tanned with chrome salts and vegetable tannins, and dyed with natural and synthetic colorants [57].

The average thickness of the hides is 1.44 mm, which is suitable for making some clothing and women's footwear. It's worth noting that this attribute can be controlled based on the desired article through greater or lesser thinning in the tanning process. As can be seen, the tear strength average value of 88.40 N, equivalent to 57.16 N/mm (Table 7), allows the leather to be used in any of the articles. Tear strength assesses how prone an opening in the material is to spreading, and in the particular case of paiche, its levels are associated with the parallel arrangement of layers of collagen fibers [57]. Other studies report tensile strength values between 31.14 and 44.99 N/mm, which are comparable to the results obtained in this research.

**Table 8. Comparison of results of physical-mechanical properties with previous studies.**

Research study	Tanning method	Tear resistance <sup>1</sup>			Tensile strength and elongation percentage <sup>2</sup>			pH <sup>3</sup>
		Direction	Thickness (mm)	Tear (N/mm)	Thickness (mm)	Tractive (N/mm <sup>2</sup> )	Stretching (%)	
Marrufo-Saldaña L. et al. (2024)	Tanning with phenolic syntans and glutaraldehyde	Longitudinal	1.58 +/-0.10	55.74 +/- 4.43	1.33 +/-0.01	7.80 +/- 4.26	76.77 +/- 21.61	7.4
		Transverse	1.52 +/-0.32	59.27 +/- 9.94	1.30 +/-0.08	9.98 +/- 3.19	30.74 +/- 1.46	
Cavali J. et al. (2022)	Vegetable tanning	Longitudinal	2.82 +/- 0.63	44.99 +/- 14.36	2.76 +/-0.42	4.48 +/- 0.84	55.00 +/- 24.81	3.43 (0.80)*
		Transverse	2.76 +/- 0.42	31.14 +/- 8.29	2.20 +/- 0.34	4.82 +/- 1.24	59.00 +/- 7.04	
	Diagonal	2.81 +/- 0.37	43.60 +/-10.98	2.36 +/- 0.25	6.65 +/- 2.13	48.57 +/- 6.77		
	Longitudinal	2.15 +/- 0.59	39.41 +/- 8.94	1.77 +/- 0.26	4.71 +/- 1.72	59.57 +/- 18.49		
	Tanning with chromium salts	Transverse	1.85 +/- 0.44	37.69 +/- 9.70	1.82 +/- 0.27	8.49 +/- 2.71	62.57 +/- 15.86	3.38 (0.83)*
		Diagonal	2.16 +/- 0.43	43.40 +/- 9.99	1.94 +/- 0.37	8.61 +/- 1.53	43.29 +/- 10.42	

\* pH differential

<sup>1</sup> ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS (ABNT). NBR/ISO 3376. Couro - Ensaios físicos e mecânicos - Determinação da resistência à tração e percentual de extensão (Leather - Physical and mechanical tests - Determination of tensile strength and percentage extension). Rio de Janeiro: ABNT; 2014.

<sup>2</sup> ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS (ABNT). NBR 11055/ISSO. Couro - Determinação da força de rasgamento progressivo (Leather - Determination of tearing load). Rio de Janeiro: ABNT; 2014.

<sup>3</sup> BNT (Associação Brasileira de Normas Técnicas). (2006). ~NBR 11057: Couro - determinação do pH e da cifra diferencial ~ (pp. 1-3). ABNT

Regarding tensile strength and elongation percentage, this property is important because it indicates the leather's ability to stretch during the shoe upper lasting process. The obtained tensile strength result, equal to 8.34 N/mm<sup>2</sup>, does not reach the values set for casual footwear, which requires a strength greater than or equal to 10 N/mm<sup>2</sup>. For men's and women's footwear, resistances greater than 15 N/mm<sup>2</sup> are established when dealing with suede, a material not compatible with paiche leather. However, the paiche leather can be used as an application in the upper part of the footwear, providing differentiation and sophistication.

Cavali J., et al. (2022), indicate an average tensile strength for paiche leather of around 4.48 and 8.61 N/mm<sup>2</sup> for other types of tanning and thicknesses between 1.85 and 2.82 mm, suggesting that the characteristic tensile strength of this type of tanned leather is lower compared to the standard [57]. The leather applied in the apparel industry requires thicknesses not exceeding 2.4 mm; nevertheless, greater thicknesses would be appropriate for the application of this type of leather, for example, in furniture. The Figure 5 illustrates the validation of this application.

The elongation percentage obtained is 53.76%, which meets the requirements for casual footwear. As for other types of footwear and clothing, no specific value is mentioned for this parameter [36]. However, it is an important attribute for the manufacturing of clothing and leather goods, and it has been successfully validated in the prototyping of such items in this research, as shown in Figure 5.



**Figure 5.** Articles made of paiche leather dyed with walnut leaves colorant. Figure 5a shows a small bookcase with applications of paiche leather dyed with walnut leaves dye. Figure 5b shows off a leather skirt, with applications of paiche leather dyed with walnut leaves dye.



## CONCLUSIONS

This research has demonstrated the feasibility of using walnut as a resource for dyeing chrome-free tanned paiche leather with colorant extracted from walnut leaves and its potential technical scaling-up in a real level environment. The extraction of the active ingredient from walnut leaves in multiple cycles to achieve different color intensities increases its added value. On the other hand, the colorfastness properties it imparts to the leather ensure the quality of the dyeing in terms of durability. Furthermore, it complements the attributes of chrome-free tanning and without metallic mordents, strengthening the image of a more biodegradable and 'clean' product that incorporates elements of biodiversity (paiche and walnut) by applying circular economy principles. It is recommended to conduct an additional study on the potential antifungal properties that walnut-dyed leather may exhibit.

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## REFERENCES

1. Laura-Medina MA. PERU: [Irregular imports and pandemic hit the footwear sector](https://www.serma.net/news/reports/peru/peru-irregular-imports-and-pandemic-hit-the-footwear-sector). Serma [Internet]. 2020; Available from: <https://www.serma.net/news/reports/peru/peru-irregular-imports-and-pandemic-hit-the-footwear-sector>
2. Kristjanson P, Krishna A, Radeny M, Kuan J, Quilca G, Sanchez-Urrelo A, et al. [Poverty dynamics and the role of livestock in the Peruvian Andes](https://linkinghub.elsevier.com/retrieve/pii/S0308521X06001387). Agric Syst [Internet]. 2007;94:294–308. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0308521X06001387>
3. Del Carpio Rodriguez O. [Estudio de prospectiva: la cadena de valor del paiche](https://repositorio.pnipa.gob.pe/handle/20.500.12864/195) [Internet]. Programa Nacional de Innovación en Pesca y Acuicultura; 2020. Available from: <https://repositorio.pnipa.gob.pe/handle/20.500.12864/195>
4. Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). [Base de Datos sobre el Comercio CITES](https://trade.cites.org/es/cites_trade#) [Internet]. 2024 [cited 2024 Jan 22]. Available from: [https://trade.cites.org/es/cites\\_trade#](https://trade.cites.org/es/cites_trade#)
5. Mahdi MM, Zohra FT, Ahmed S. [Dyeing of Shoe Upper Leather with Extracted Dye from Acacia Nilotica Plant Bark-An Eco-Friendly Initiative](https://pccc.icrc.ac.ir/article_81727.html). Prog Color Color Coatings [Internet]. 2020 [cited 2023 Sep 29];14:241–58. Available from: [https://pccc.icrc.ac.ir/article\\_81727.html](https://pccc.icrc.ac.ir/article_81727.html)
6. Page C, Fennen J, Gagliardino D. [Leather Dyes – Properties and Analysis](#). 32nd Congr Int Union Leather Technol Chem Soc. 2009.

7. Tkaczyk A, Mitrowska K, Posyniak A. [Synthetic organic dyes as contaminants of the aquatic environment and their implications for ecosystems: A review](#). *Sci Total Environ* [Internet]. 2020;717:137222. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0048969720307324>
8. Benkhaya S, M'rabet S, El Harfi A. [Classifications, properties, recent synthesis and applications of azo dyes](#). *Heliyon* [Internet]. 2020 [cited 2023 Sep 29];6:e03271. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S240584402030116X>
9. TRADE MAP [Internet]. [List. los mercados proveedores para un Prod. Import. por PerúMetadata - Prod. 2927 Compuestos diazoicos, azoicos o azoxi](#). 2022 [cited 2023 Dec 22]. Available from: [https://www.trademap.org/Country\\_SelProductCountry\\_TS.aspx?nvpm=3%7C604%7C%7C%7C2927%7C%7C%7C4%7C1%7C1%7C1%7C2%7C1%7C2%7C1%7C1%7C1](https://www.trademap.org/Country_SelProductCountry_TS.aspx?nvpm=3%7C604%7C%7C%7C2927%7C%7C%7C4%7C1%7C1%7C1%7C2%7C1%7C2%7C1%7C1%7C1)
10. KAWAKAMI T, ISAMA K, NISHIMURA T. [Survey of Primary Aromatic Amines Originating from Azo Dyes in Commercial Textile Products in Direct Contact with Skin and in Commercial Leather Products in Japan](#). *J Environ Chem* [Internet]. 2012;22:197–204. Available from: [https://www.jstage.jst.go.jp/article/jec/22/4/22\\_197/\\_article](https://www.jstage.jst.go.jp/article/jec/22/4/22_197/_article)
11. REACH. [Azocolourants and Azodyes](#) [Internet]. Reach restricted Subst. List. 2016 [cited 2023 Aug 25]. Available from: [http://www.chemsafetypro.com/Topics/Restriction/REACH\\_annex\\_xiv\\_azocolourants\\_azodyes.html](http://www.chemsafetypro.com/Topics/Restriction/REACH_annex_xiv_azocolourants_azodyes.html)
12. REACH. [Chromium VI compounds](#) [Internet]. Reach restricted Subst. List. 2016 [cited 2023 Aug 25]. Available from: [http://www.chemsafetypro.com/Topics/Restriction/REACH\\_annex\\_xvii\\_Chromium\\_VI\\_compounds.html](http://www.chemsafetypro.com/Topics/Restriction/REACH_annex_xvii_Chromium_VI_compounds.html)
13. Vaiano V, De Marco I. [Removal of Azo Dyes from Wastewater through Heterogeneous Photocatalysis and Supercritical Water Oxidation](#). *Separations* [Internet]. 2023;10:230. Available from: <https://www.mdpi.com/2297-8739/10/4/230>
14. Jing L, Taisheng G, Haiyan X, Yangyi C, Shaoqing H, Huafeng Z. [Study on dyeing of vegetable tanned leather with indigo](#). *J Phys Conf Ser* [Internet]. 2021;1790:012003. Available from: <https://iopscience.iop.org/article/10.1088/1742-6596/1790/1/012003>
15. Kurinjimalar C, Usharani N, Kanimozhi B, Jayakumar GC, Kanth S V. [Extraction and optimization of natural colorant from Garcinia mangostana Linn peels for leather dyeing](#). *Clean Eng Technol*. 2022;7:100423.
16. Mandal S, Venkatramani J. [A review of plant-based natural dyes in leather application with a special focus on color fastness characteristics](#). *Environ Sci Pollut Res* [Internet]. 2023;30:48769–77. Available from: <https://link.springer.com/10.1007/s11356-023-26281-1>
17. Ozdemir MB, Karadag R. [Anatolian Acorn Oak's Economic Potential in the Application to the Textile and Leather Industries](#). *Text Leather Rev* [Internet]. 2023;6:320–32. Available from: <https://www.tlr-journal.com/tlr-2023-044-ozdemir/>
18. Kefale GY, Wodag AF. [Ficus sycomorus as a Sustainable Source of Leather Dyeing Material: An Ecofriendly](#)

[Approach](https://www.hindawi.com/journals/je/2023/7776239/). Sridhar Babu B, editor. J Eng [Internet]. 2023;2023:1–8. Available from: <https://www.hindawi.com/journals/je/2023/7776239/>

19. Reynel C. [Arboles útiles del ande peruano Una guía de identificación, ecología y propagación de las especies de la Sierra y los Bosque montanos en el Perú](#). Tarea Gráfica Educativa, editor. Lima, Peru; 2007.

20. Cano A, Contreras C, Chiralt A, González-Martínez C. [Using tannins as active compounds to develop antioxidant and antimicrobial chitosan and cellulose based films](#). Carbohydr Polym Technol Appl [Internet]. 2021;2:100156. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S2666893921001249>

21. Masek A, Chrzescijanska E, Latos-Brozio M, Zaborski M. [Characteristics of juglone \(5-hydroxy-1,4-naphthoquinone\) using voltammetry and spectrophotometric methods](#). Food Chem [Internet]. 2019;301:125279. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0308814619313895>

22. Hosseinezhad M, Gharanjig K, Iamin H, Rouhani S, Adeel S. [Environmentally Dyeing of Wool Yarns Using of Combination of Myrobalan and Walnut Husk as Bio-mordants](#). Prog Color Color Coatings [Internet]. 2023;16:197–205. Available from: <https://doi.org/10.1001/1.20082134.2023.16.2.7.4>

23. Almeida IF, Fernandes E, Lima JLFC, Costa PC, Fernanda Bahia M. [Walnut \(Juglans regia\) leaf extracts are strong scavengers of pro-oxidant reactive species](#). Food Chem. 2008;106:1014–20.

24. Bhat AA, Shakeel A, Rafiq S, Farooq I, Malik AQ, Alghuthami ME, et al. [Juglans regia Linn.: A Natural Repository of Vital Phytochemical and Pharmacological Compounds](#). Life (Basel, Switzerland) [Internet]. 2023 [cited 2023 Sep 29];13. Available from: <https://pubmed.ncbi.nlm.nih.gov/36836737/>

25. Turki OH, Jafar ZJ. [Antibacterial Activity of Juglans regia L. Dry Husk Extract against Streptococcus mutans and Lactobacillus: An In Vitro Study](#). Dent Hypotheses. 2023;14:29–31.

26. Turki OH, Jafar ZJ. [Whitening Effect of Juglans regia Dry Husk Extract on Primary and Permanent Teeth](#). Agarwal T, editor. Int J Biomater [Internet]. 2023;2023:1–6. Available from: <https://www.hindawi.com/journals/ijbm/2023/1037661/>

27. Kavuncuoglu H, Kavuncuoglu E, Karatas SM, Benli B, Sagdic O, Yalcin H. [Prediction of the antimicrobial activity of walnut \( Juglans regia L. \) kernel aqueous extracts using artificial neural network and multiple linear regression](#). J Microbiol Methods [Internet]. 2018;148:78–86. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0167701218300897>

28. Ojstršek A, Fakin D. [Natural Dyeing of Wool Using Juglans regia \(Common Walnut\) Leaf Extract](#). TEKSTILEC [Internet]. 2019;62:288–98. Available from: <http://www.tekstilec.si/wp-content/uploads/2019/11/10.14502Tekstilec2019.62.288-298.pdf>

29. Bechtold T. [Natural Colorants – Quinoid, Naphthoquinoid and Anthraquinoid Dyes](#). Handb Nat Color [Internet]. Wiley; 2009 [cited 2023 Sep 29]. p. 151–82. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1002/9780470744970.ch10>

30. Arifeen W ul, Rehman FU, Adeel S, Zuber M, Ahmad MN, Ahmad T. [Environmental friendly extraction of walnut bark-based juglone natural colorant for dyeing studies of wool fabric](#). Environ Sci Pollut Res [Internet].

2021 [cited 2023 Sep 29];28:49958–66. Available from: <https://link.springer.com/article/10.1007/s11356-021-14277-8>

31. Gulsoy E, Pehlivan M, Simsek M. [Determination of Fatty Acids,  \$\alpha\$ -Tocopherol,  \$\beta\$ -Caroten, Minerals, and Some Pomological Properties of Walnut Genotypes Selected from Aras Valley \(Eastern Turkey\)](#). Iran J Chem Chem Eng [Internet]. 2019;38:211–21. Available from: <https://doi.org/10.30492/ijcce.2019.31203>

32. Ticha M Ben, Slama N, Dhouibi N, Boudokhane C, Dhaouadi H. [Bark Residues Recovery of Juglans Regia. L For the Dyeing of Wool Fabrics : Development of Microwave-assisted Extraction and Dyeing Processes](#). J Nat Fibers. 2022;19:5282–96.

33. Eser F, Aktas E, Onal A. [Dyeing quality of walnut shells on polyester and polyester/viscose blended fabrics](#). Indian J Fibre Text Res. 2016;41:188–94.

34. Wang L, Li J, Feng H. [Dyeing of flax fabric with natural dye from chestnut shells](#). Pigment Resin Technol. 2009;38:347–52.

35. Habib N, Adeel S, Ali F, Amin N, Khan SR. [Environmental friendly sustainable application of plant-based mordants for cotton dyeing using Arjun bark-based natural colorant](#). Environ Sci Pollut Res [Internet]. 2021;28:54041–7. Available from: <https://link.springer.com/10.1007/s11356-021-14536-8>

36. Salman M, Fazal-ur-Rehman F-R, Adeel S, Habib N, Batool F, Usama M, et al. [Extraction of Anthocyanin from Rose Petals for Coloration of Biomordanted Wool Fabric](#). Coatings [Internet]. 2023;13:623. Available from: <https://www.mdpi.com/2079-6412/13/3/623>

37. Khalid B, Yaqub A, Farooq Arif M, Liaquat L, Iqbal B. [Study of Colour Measurements of Leather Dyed with Walnut Bark Natural Dye](#). Pak J Sci Ind Res. 2010;53:252–7.

38. Doğan-Sağlamtimur N, Turaç E, Arabacıoğlu R, Çivioğlu T. [Production of Dye from Green and Brown Walnut Shells for Leather Coloration](#). Period Eng Nat Sci. 2017;5.

39. Hurtado-Manrique P, Jurado-Teixeira B, Ramos-Llica E, Calixto-Cotos M. [Evaluation of antioxidant activity of the standardized hydroalcoholic extract from juglans Neotropica Diels \(peruvian walnut\) leaves](#). Rev la Soc Química del Perú [Internet]. 2015;81. Available from: [http://www.scielo.org.pe/scielo.php?pid=S1810-634X2015000300010&script=sci\\_arttext&tlng=en](http://www.scielo.org.pe/scielo.php?pid=S1810-634X2015000300010&script=sci_arttext&tlng=en)

40. Servicio Nacional Forestal y de Fauna Silvestre. [Compendio Estadístico Forestal 2010-2020](#). 2021.

41. Hassan MM, Harris J, Busfield JJC, Bilotti E. [A review of the green chemistry approaches to leather tanning in imparting sustainable leather manufacturing](#). Green Chem [Internet]. 2023;25:7441–69. Available from: <http://xlink.rsc.org/?DOI=D3GC02948D>




42. Kirman CR, Suh M, Proctor DM, Hays SM. [Improved physiologically based pharmacokinetic model for oral exposures to chromium in mice, rats, and humans to address temporal variation and sensitive populations](#). Toxicol Appl Pharmacol [Internet]. 2017;325:9–17. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0041008X17301370>

43. Nour V, Trandafir I, Cosmulescu S. [HPLC Determination of Phenolic Acids, Flavonoids and Juglone in Walnut Leaves](#). J Chromatogr Sci [Internet]. 2013;51:883–90. Available from: <https://academic.oup.com/chromsci/article-lookup/doi/10.1093/chromsci/bms180>
44. Zaheer Z. [Eco-friendly walnut shell powder based facile fabrication of biogenic Ag-nanodisks, and their interaction with bovine serum albumin](#). J Photochem Photobiol B Biol [Internet]. 2019;193:8–17. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1011134418313319>
45. Kasmudjiastuti E, Widhiati W. [Pengaruh penggunaan bahan pewarna alam dari ekstrak kayu terhadap sifat fisis kulit ikan kakap merah](#). Maj Kulit, Karet, dan Plast [Internet]. 2002;18:3. Available from: <http://ejournal.kemenperin.go.id/mkkp/article/view/265>
46. Sinnur HD, Verma DK, Kar TR, Samanta AK. [Effect of Dyeing Process Variables on Colour Yield and Colour Fastness Properties for Cotton Khadi Fabric Dyed with De-Oiled Red Sandal Wood Waste](#). J Inst Eng Ser E. 2021;102:273–92.
47. RAO J, PRAKASH A, THANGARAJ E, SREERAM KJ, SARAVANABHAVAN S, NAIR BU. [NATURAL DYEING OF LEATHERS USING NATURAL MATERIALS](#). J Am Leather Chem Assoc [Internet]. 2008 [cited 2023 Aug 25];103:68–75. Available from: <https://journals.uc.edu/index.php/JALCA/article/view/2395>
48. Lykidou S, Pashou M, Vouvoudi E, Nikolaidis N. [Study on the Dyeing Properties of Curcumin on Natural and Synthetic Fibers and Antioxidant and Antibacterial Activities](#). Fibers Polym [Internet]. 2021 [cited 2023 Aug 23];22:3336–42. Available from: <https://link.springer.com/article/10.1007/s12221-021-0412-4>
49. Gîrzu M, Fraisse D, Carnat A-P, Carnat A, Lamaison J-L. [High-performance liquid chromatographic method for the determination of juglone in fresh walnut leaves](#). J Chromatogr A [Internet]. 1998;805:315–8. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0021967397013009>
50. Medic A, Jakopic J, Hudina M, Solar A, Veberic R. [Identification and quantification of major phenolic constituents in Juglans regia L. leaves: healthy vs. infected leaves with Xanthomonas campestris pv. juglandis using HPLC-MS/MS](#). J King Saud Univ - Sci [Internet]. 2022;34:101890. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S1018364722000714>
51. Thakur A, Cahalan C. [Geographical variation of Juglans regia L. in juglone content: Rapid analysis using micro-plate reader](#). Curr Sci. 2011;100:1483–5.
52. Stampar F, Solar A, Hudina M, Veberic R, Colaric M. [Traditional walnut liqueur – cocktail of phenolics](#). Food Chem [Internet]. 2006;95:627–31. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0308814605001317>
53. Cheniany M, Ebrahimzadeh H, Vahdati K, Preece JE, Masoudinejad A, Mirmasoumi M. [Content of different groups of phenolic compounds in microshoots of Juglans regia cultivars and studies on antioxidant activity](#). Acta Physiol Plant [Internet]. 2013;35:443–50. Available from: <http://link.springer.com/10.1007/s11738-012-1087-7>
54. COSMULESCU SN, TRANDAFIR I, ACHIM G, BACIU A. [Juglone Content in Leaf and Green Husk of Five Walnut \(Juglans regia L.\) Cultivars](#). Not Bot Horti Agrobot Cluj-Napoca [Internet]. 2011;39:237. Available

from: <http://www.notulaeobotanicae.ro/index.php/nbha/article/view/5728>

55. Ebrahimi I, Parvinzadeh Gashti M. [Extraction of juglone from Pterocarya fraxinifolia leaves for dyeing, anti-fungal finishing, and solar UV protection of wool](#). Color Technol. 2015;131:451–7.

56. Poyyamozi N, Kumar SS, Kumar RA, Soundararajan G. [An investigation into enhancing energy storage capacity of solar ponds integrated with nanoparticles through PCM coupling and RSM optimization](#). Renew Energy [Internet]. 2024;221:119733. Available from: <https://linkinghub.elsevier.com/retrieve/pii/S0960148123016488>

57. Cavali J, 1  ID, Rodrigues De Souza ML, 2  ID, Silva De Oliveira P, 1  K, et al. [Tanned leather of the paiche Arapaima gigas Schinz, 1822 \(Arapaimidae\) with extracts of vegetable origin to replace chromium salts](#). PLoS One [Internet]. 2022 [cited 2023 Aug 23];17:e0261781. Available from: <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0261781>

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