

A Novel Eco-friendly Route of Sustainable and Natural Indigo Dyeing and Coating for Denim Textiles

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January 30, 2023

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Abstract

Denim dyeing is an essential industrial process that produces daily clothing for billions of people worldwide. However, the current process relies heavily on toxic chemicals that are detrimental to the environment. Therefore, we propose a new environmentally friendly alternative. This pioneering project uses proteins extracted from imperfect coffee beans as an adhesive for coloring denim textiles and natural indigo extracted from Assam indigo leaves.

The acid precipitation method was used to extract denatured plant proteins as dye adhesive. Coffee-denatured protein precipitated most when it was treated with hydrochloric acid at pH 4.3. Soybean juice treated with food-grade acetic acid yielded the maximum precipitant at pH 5.1 at 24°C. Additionally, we constructed a washing fastness and a rubbing fastness machine using discarded appliance materials to test the color grading of natural indigo-dyed textiles. The natural indigo-dyed textiles achieved grade 4-5 results after both tests. The result was comparable to commercially available textile samples.

Excluding the cost of the laboratory-grade hydrochloric acid used to denature coffee bean proteins, the cost of other materials used in our novel dyeing method is also comparable to the current synthetic indigo dyeing process, even at a small laboratory scale. This suggests the cost-saving potential of our proof-of-principle approach when it is industrialized in the future.

Overall, this project has demonstrated the feasibility of replacing synthetic dyes and adhesives currently used in the denim dyeing industry with low-cost, natural, and biodegradable plant-based protein adhesives and dyes to achieve a commercial-grade result in denim textiles.

Keywords: denim; indigo; dyeing; protein; adhesive

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Introduction

Motivation

Denim is an indispensable textile in people's wardrobes. In 2022, the global denim textiles market was valued at approximately 27.1 billion U.S. dollars, and it is projected to increase to 71.8 billion U.S. dollars by 2027.¹ However, these popular blue denim textiles are dyed with toxic synthetically produced indigo. According to the report, the denim industry alone uses over 45,000 tons of synthetic indigo annually;² it has raised environmental contamination due to the use of pollutants and hazardous chemicals. The synthetic dyes toxic effluents inhibit plant growth, kill fish, and potentially cause various bodily cancers. Moreover, Canadian scientists have found microfibers released from washing jeans in Arctic regions.³ This is one of the significant indicators of anthropogenic pollution footprint. Therefore, we conceived the idea of producing a natural indigo dye and adhesive to replace synthetic chemical dye.

Until roughly 1940, casein glue was used in aircraft manufacturing.⁴ Since casein protein derived from cow milk is used as an adhesive for wood aircraft, we hypothesize that protein from plant seeds maintains strong adhesive properties that can be used to replace chemical resin in dyeing. A cup of aroma coffee is brewed from carefully selected beans, and the remaining imperfect beans are disposed of as waste. Defective coffee beans constitute approximately 20% of total coffee production mass by weight.⁵ Instead of discarding defective beans, we extract the protein from them as a bio-based adhesive for natural indigo dye. This process will solve the pollution problem caused by petroleum-based resin.

Research Objectives

In this experiment, the natural indigo dye was extracted from Assam indigo leaves. Grounded raw coffee beans were dissolved by adding alkali and denatured by adding acid for precipitation reaction. The soybean juice was heated and precipitated with acid. Both precipitates were used separately as adhesives in denim dyeing. We used self-made washing and rubbing fastness machines to check the fastness of dyeing with reference to the dyeing fastness measurement standard. This experiment was divided as follows:

- Extraction of Natural Indigo Dye from Assam Indigo Leaves
- Quantification of Protein Precipitation Amount of Coffee Beans After Acid Denaturation
- Quantification of Protein Precipitation Amount of Soybeans After Acid Denaturation
- Washing and Rubbing Fastness of Natural Indigo Dyeing and Coating for Denim Textiles
- Feasibility of Replacing Synthetic Dyes with Natural Indigo Dyes

Materials

Raw Materials

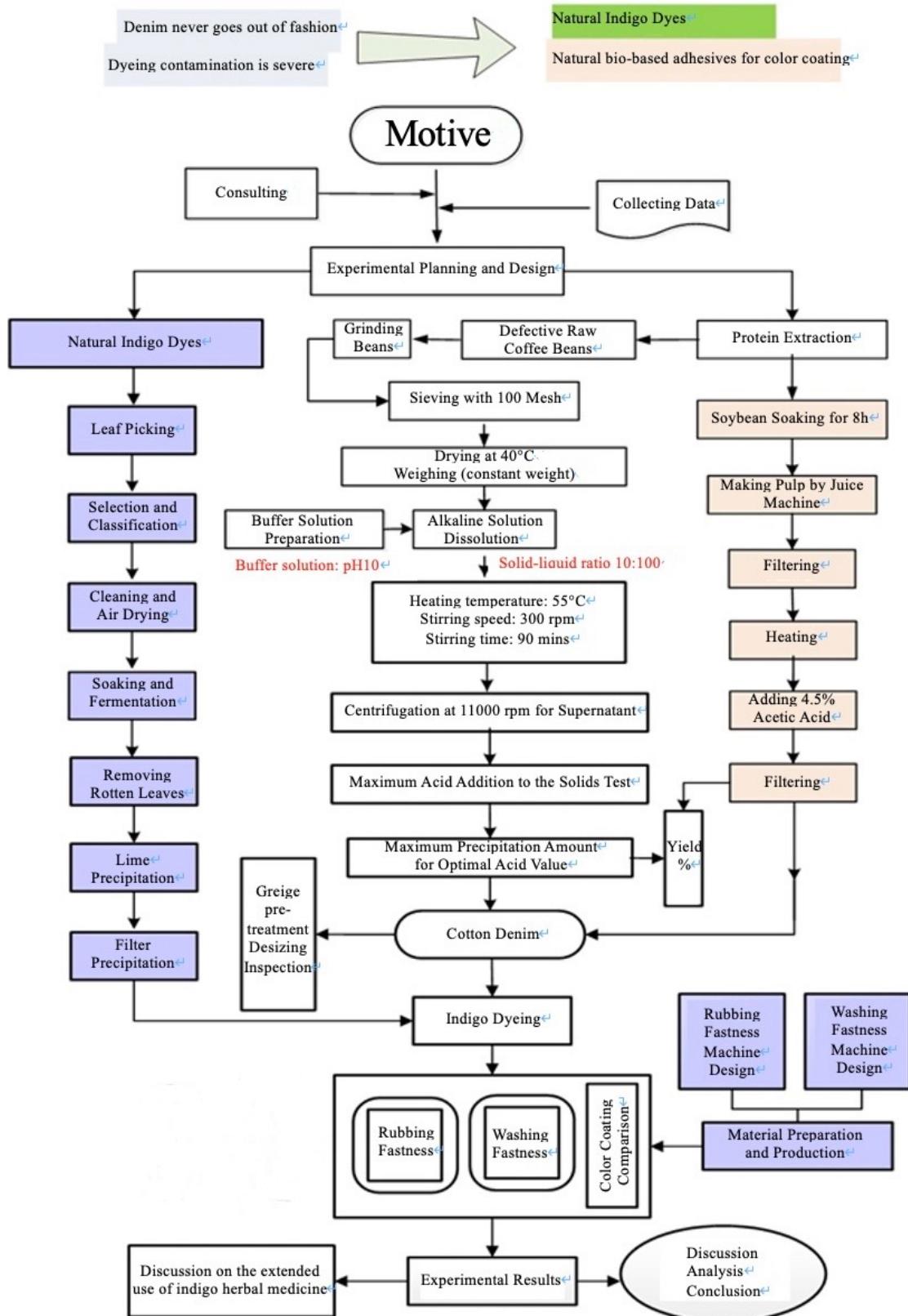
Cotton fabric, sundried coffee beans, soybeans, Assam indigo leaves, surfactant, baking soda, 4.5% acetic acid, salad oil, sodium hydroxide, sodium carbonate, printing resin (made in Japan), pH-test paper, hydrated lime, agar.

Equipment and Devices

Stainless steel pot, induction stove, grinder, sieve, weighing scale, beakers, dropper, magnet heating stirrer, micro dropper, oven, stirring stick, pH meter, filter gauze, juice machine, thermometer, filter paper, funnel, high-pressure sterilizer, iron, lab goggles, spectrophotometer, motor, DC power supply, I inch brush, measuring cup, marbles, gar, timer, safety gloves, scissors, measuring cylinder.

Methods

Experiment Process



Denim Textiles Manufacturing

1. Manufacturing Process of Denim Textiles

This experiment was conducted with reference to the following books for a comprehensive discourse.^{6,7,8} The denim fabric used in this experiment was purchased from Yongle Market, Dihua Street, Taipei, and it was 10×7/80×30 singed cotton greige that had not been pre-treated. This experiment started with the desizing process of pre-treatment. Before weaving, the warp was sized to avoid static electricity, increase strength and reduce frictional resistance to make the weaving machine run smoothly. The sizing material usually contains starch or polyvinyl alcohol. Before dyeing work, the sizing materials must be removed to avoid dyeing failure. The pre-treatment work, which includes desizing, refining and bleaching, is combined in the current process. For this experiment: hot water was used to remove sizing materials, a surfactant was added in the refining process to remove impurities, and hydrogen peroxide was used to bleach the fabric without harming it. Existing literature documents that this needs to be done in an alkaline environment. Therefore, sodium carbonate was added to this experiment to obtain better pre-treatment results.⁹

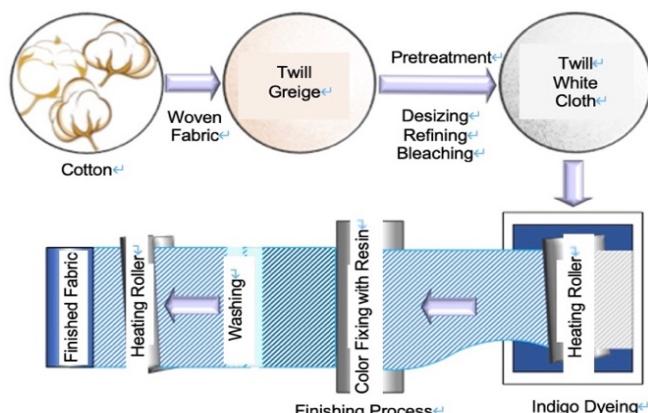


Figure 1. Denim Textiles Manufacturing Process

2. Dye

Many natural dyes are extracted from minerals and plants. Because denim textile is in blue series, we use Assam indigo leaves to make the blue puree, which is the "indigo" substance in Assam indigo leaves. Traditional blue dyeing is done in alkaline with complex work, and the color is applied to the cotton fabric after oxidation, but the biggest difference of this experimental coloring is that the color is applied by adhesive. In fact, the earliest indigo dye used on denim textiles was obtained from natural plants. Later, due to mass production, chemically synthesized non-natural dyes were used.

Natural Indigo Dye

The indigo dye initially used was natural and derived from a plant source; due to mass production, the trend shifted to synthetic indigo dye.¹⁰

1. Assam Leaves

There are many plants that can be used to make natural indigo dyes. In Taiwan, Assam indigo is an indigenous plant. Also known as Dajing, it is a perennial subshrub in the Acanthus family, with cross-opposite leaves and lavender flowers. The plant can be located in the low and mid-altitude mountain areas of northern Taiwan.¹¹ The collected leaves undergo a process of oxidation and extraction to obtain blue puree, which is then sun-dried to become the commercially available indigo dye.

2. Production Method

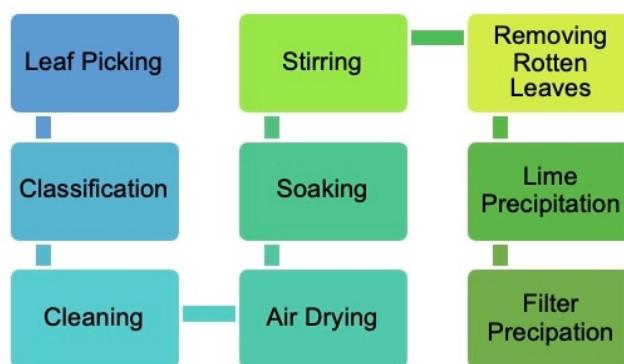


Figure 2. Natural Indigo (from Assam Leaves) Making Process

- 2.1 Fresh Assam indigo leaves are collected.
 - ◆ Wash and remove the sludge.
 - ◆ Pick out the yellow leaves, branches, old leaves, and stems.
 - ◆ Drain and weigh the leaves.
 - ◆ Add 10.0 L of water to each 1000.0 g portion to cover the leaves.
 - ◆ Place a heavy object to press the leaves to prevent them from floating out of the water and wilting.
 - ◆ Place them in a well-ventilated place at room temperature below 30°C.
- 2.2 Turn the leaves up and down daily to increase the oxygen in the water and facilitate the growth of bacteria to dissolve the dyes.
- 2.3 The leaves turn yellow-green as the water surface appears dark blue with an oily film and a fermented odor. The indigo dye has been dissolved.
- 2.4 Remove the rotten leaves and add 10.0 g, 20.0 g, 30.0 g, 40.0 g, and 50.0 g of hydrated lime, respectively, to precipitate indigo dye.

- 2.5 Add 50.0 g of saturated lime milk per 1000.0 g of leaves to produce 200.0 g of aqueous indigo puree. Do not use lime milk for the construction industry; the particles are too large and the blue puree will be agglomerated and insoluble.
- 2.6 Stir slowly and then gradually accelerate. The blue liquid turns to yellow-green. After 3 to 5 minutes, a large amount of blue foam will appear on the surface of the liquid. The foam will increase, and the color will be thicker when the stirring continues. After 30 minutes, the foam will gradually decrease, the color will change from dark blue to light blue, and the blue liquid will be stagnant. Then add 10.0 mL of cooking oil to isolate the blue liquid from the air.
- 2.7 After a day of resting, the blue puree is precipitated. Pour off the top layer of water and drain the blue puree in a filtering bag. The blue puree becomes a paste. Compare the weight of blue puree obtained from hydrated lime of different concentrations.

Bio-based Adhesive

Our dyeing process is combined with natural dye and bio-based adhesive. The conventional printing method uses heated rollers to print continuous printed fabrics. Glue must be added for dyeing, and the chemical glue currently used is mostly water-based and must be heated. There are many kinds of resins, and their pH values are not neutral. To avoid damaging the fabric and causing color differences in dyeing or equipment corrosion failure, neutralization treatment is necessary. The resin wastewater causes water pollution with additional costs for wastewater treatment.

1. *The protein precipitation amount of raw coffee beans after acid denaturation.*
 - 1.1 Raw Coffee Bean Powder Preparation
 - ◆ Grind 1000.0 g of raw coffee beans into powder and then sift with a 100-mesh sieve. After sieving the powder, put it in the oven and dry it at 40°C until it reaches a constant weight.
 - 1.2 Prepare 500.0 mL of borax-sodium hydroxide 0.2 M buffer solution, pH 10.0.
 - 1.3 Add pH 10 buffer solution with 1N aqueous hydrochloric acid solution.
 - ◆ Prepare 10% coffee powder buffer solution: Take 20.0 g of raw coffee bean powder and add 200.0 mL of pH 10 buffer solution.
 - ◆ Magnet stirrer setting: Temperature 55°C, speed 300 rpm, 90 minutes.¹²
 - ◆ Take out 35.0 mL of the homogenized solution and place it in 50.0 mL medium centrifuge tubes. Centrifuged it at 11000 rpm for 15 minutes.

- ◆ Filter the coarse fiber and impurities from the centrifuged solution with #2 filter paper. Measure the pH value.
 - ◆ Take the filtrate and place it in five 50.0 mL centrifuge tubes. Add 1~5 mL of 1N hydrochloric acid solution to the filtrate and shake it at room temperature for 5 minutes and measure the pH value.
 - ◆ Take 20.0 mL for each and place in a centrifuge machine at 11,000 rpm for 5 minutes, pour out the supernatant, and weigh 1-5 precipitated solids.
 - ◆ Remove the bottom solids and place them in sample bottles with marks. Place them in a vacuum dryer overnight. Remove and weigh them the next day.
 - ◆ Test the protein with Biuret reagent.
2. *The protein precipitation amount after acid denaturation by rapid extraction of soybean.*
- ◆ Pulping of soybeans at different temperatures
 - ◆ Put 100.0 g of the soaked soybeans and 1000.0 mL of pure water into the juice machine. Filter the dregs through a filter cloth to leave the soybean juice.
 - ◆ Take 500.0 mL of filtrate and mix it with a magnet heated stirrer at room temperature, 40°C, 60°C, 80°C and 100°C for reaction. Stir continuously at 200 rpm while adding 4.5% acetic acid slowly to observe the production of coagulation. This enables the denaturation reaction to be carried out completely.
 - ◆ When the coagulation gradually increases, the magnet stirring often stops, and the pH value can be measured after the liquid is cooled down by POWER OFF.
 - ◆ Continue the same experiment twice with 100.0 g of soaked soybeans each.
 - ◆ Neutralization: After centrifugation, stir the precipitate with a small amount of baking soda each time and confirm that it becomes neutral with pH test paper.
 - ◆ Protein confirmation: Biuret experimental reagent.

Washing Fastness and Rubbing Fastness

1. Color Fastness Inspection

Denim textiles are made with indigo-dyed warp yarn and undyed weft yarn in traditional dyeing. Interwoven greige cotton and polyester fabric can be dyed via one direct dye or reactive dye to achieve custom-made denim. Polyester is not dyed to remain white. The same will have the effect of denim of colorful warp and white weft. We adopted the printing dyeing method, which can reduce the amount of dyeing agents and pollution.^{13,14} Furthermore, this method cannot be achieved by the traditional immersion dyeing method and DIY alkaline bath reduction method.

Fastness is the ability of a dye to maintain its color without fading or washing away. Color fastness is a resisting property of textiles materials, which includes the material surface's ability to retain color during different mechanical, physical, and chemical treatments.¹⁵ Washing fastness, rubbing fastness, light fastness, and perspiration fastness are the main forms of color fastness that are standardized. In this experiment, we study the washing and rubbing fastness of dyed textiles.

1.1 Washing Fastness (refer to AATCC61)

Washing fastness is the property to stain the color on the surface of textiles materials during washing.¹³ This includes the discoloration of dyed textiles after soaping, the degree to which the original textiles fades, and the color difference appearing after the white cloth is contaminated. After the specimen is tested, rinsed and air dried, it is graded by the "Gray Card" according to the AATCC standard. (Figure 3) This method evaluates the discoloration and staining in the color fastness test. The washing fastness of textiles is categorized from one to five, with a higher number indicating better washing fastness. The color of the contaminated white cloth is also categorized from one to five, with a lower number indicating more contamination. Test fabric sample: 50 mm x 100 mm.

1.2 Rubbing Fastness (in conformity to ISO 105-A03)

Rubbing fastness refers to the resistance of the color of textiles to abrasion or staining. Rubbing can occur under dry and wet conditions. The dry rubbing fastness test refers to the situation of fading and staining when rubbed with a standard white cloth while the wet rubbing fastness test relates to a standard white cloth with water content 95% to 100% when rubbed. The dye concentration will affect the rubbing fastness at dyeing, and the rubbing fastness will be low if the dye concentration is high, and it is easy to cause surface dyeing.

The rubbing fastness of textiles is categorized from one to five by the "Gray Sample Card". A higher grade indicates better rubbing fastness. In the self-made swatch book, RGB indicates the combination of lights of the red, green, and blue colors. Lights of the three primary colors are combined in 0-255 intensity, ranging from all black to all white, with lights of various colors.

- ◆ Dry rubbing fastness of this group:

- ❖ 10 times rubbing back and forth in a straight line on the specimen (104 ± 3.0 mm)
 - ❖ Speed: 1 time/second back and forth; Vertical pressure of 9 ± 0.2 N

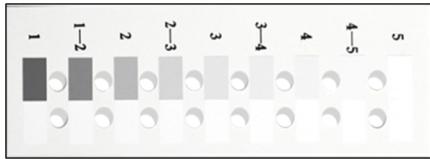


Figure 3. AATCC61 Gray Scale¹⁶

Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
RGB=143	RGB=171	RGB=199	RGB=227	RGB=255

Table 1. Gray Sample Card

2. Self-made washing fastness machine and rubbing fastness machine

All materials of the self-made washing and rubbing fastness machines are from warehouse waste.

2.1 Washing Fastness Machine

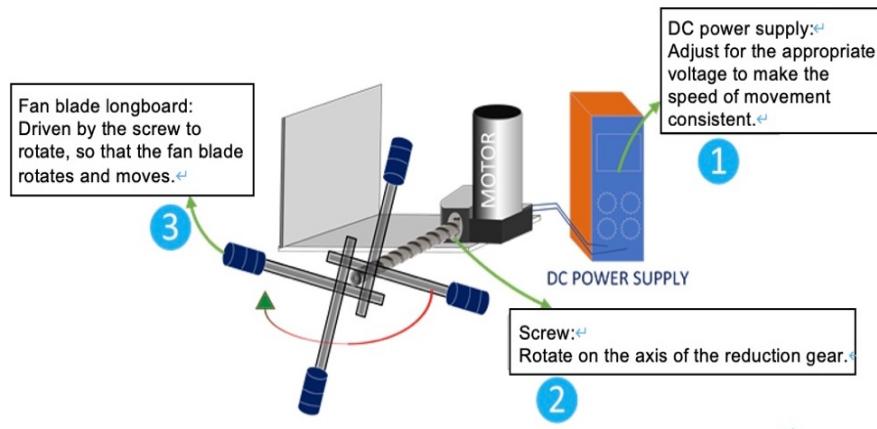


Figure 4. Washing fastness machine concept diagram.

- (1) Remove the motor of a scrap massage chair and tape the front end with an experimental universal angle base iron clip.
- (2) Connect the DC power supply, test and adjust the appropriate rotation speed to 18.0V/0.71A at 40 rpm.

2.2 Rubbing Fastness Machine

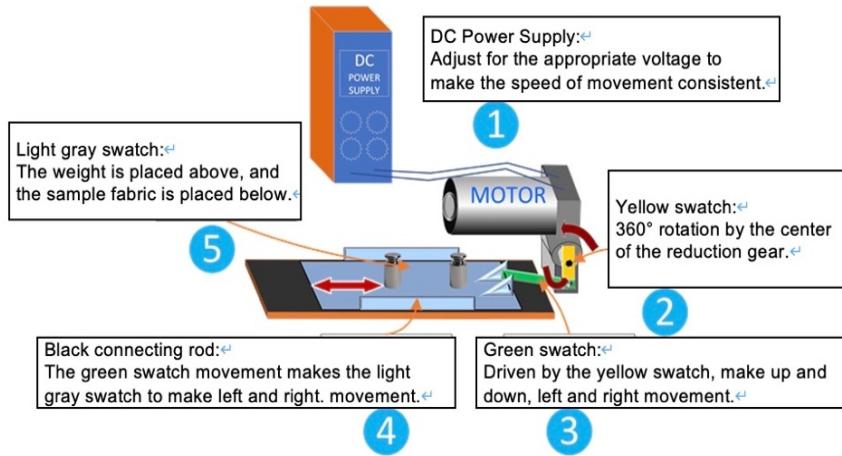


Figure 5. The concept diagram of rubbing fastness machine.

- (1) Simple transmission method: motor → worm gear rotation → worm wheel rotation → connecting rod forward and backward → drive the sliding block to move from side to side. Adjust movement speed of 1 second per round trip with 24.0V/0.21A.
- (2) For the staining Gray Card grading of dry rubbing fastness (in conformity to ISO 105-A03) test, a dry cotton standard rubbing cloth (50 mm x 50 mm ± 2 mm square) is placed on the end of a rubbing finger (16 ± 0.1 mm diameter cylinder) on the test instrument and rubbed back and forth along the straight line of the track on the dry rubbing specimen 10 times (104 ± 3 mm straight back and forth distance) with a vertical pressure of 9 ± 0.2 N.

3. *Washing and Rubbing Fastness Test*

3.1 Pre-treatment of Greige

- ◆ Cut the fabric into 10 cm × 20 cm pieces with a total weight of 514.0 g. The ratio of fabric to water is 1:10, so 5140.0 mL of water was used for the reaction.
- ❖ Bleach with 5.0% concentration of hydrogen peroxide; $5140 \times 5.0\% = 257.0$ g
- ❖ Sodium hydroxide concentration: 5.0 g/L, 25.7 g is required
- ❖ Surfactant concentration: 5.0 g/L, 25.7 g is required
- ❖ Divide the above materials into two parts of 250.0 g. Mix them in a 5-liter iron pot and stir uniformly
- ❖ Add the fabric and set the induction furnace at 80°C for 30 minutes while stirring continuously.
- ❖ Remove and wash with clean water, dry in the oven at 80°C for 8 hours.
- ❖ Iron the uneven fabric to avoid curling when the fabric is colored.

3.2 Desizing Confirmation

Put 1~2 drops of iodine solution on the surface of the fabric and observe the discoloration condition. If the test solution part on the fabric appears blue-purple, it means there is residual starch or PVA (polyvinyl alcohol) on the fabric. The desizing is not complete. If the test solution part on the cotton fabric appears brown, which is the color of iodine liquid, it means the fabric has completed desizing.

3.3 Color Coating

- ◆ Take 10.0 g of neutral protein solids of coffee protein and soybean protein, add 10.0 g of indigo puree, and stir uniformly.
- ◆ Lay the white fabric flat on the table and use a one-inch paint brush to dip a small amount of indigo dye. Coat it directly onto the white fabric in the same direction without dripping.
- ◆ Leave it to air dry, then fully wash the indigo dye on the surface with 2.0% of surfactant and dry. Use 10.0 g of chemical resin and mix thoroughly to make a control group.

3.4 Washing Fastness Test

- ◆ Take the following fabrics which are finished with color coating and washing, and cut into 5.0 cm × 5.0 cm size.
- ◆ Cut cotton white fabrics into 60 pieces of the same size and staple the dyed side to the white fabric.

Experimental Group		Control Group 1	Control Group 2
Coffee bean Adhesive Fabric	Soybean Adhesive Fabric	Printing Resin (Made in Japan)	Commercially Available Jeans

Table 2. Experimental Group and Control Groups in Washing and Rubbing Fastness Test

- ◆ Fill a 350 mL plastic bottle with 1.0% detergent of 150.0 mL.
- ◆ Put 5 marbles with a weight of 26.1 g.
- ◆ Set the DC power supply: 15V/0.3A, measurement speed: 42 rpm
- ◆ Fix it on the iron clip of the motor, and time five minutes to stop.
- ◆ Take out the fabric and wash off the detergent slightly, then blow dry the fabric with a hair dryer and test the color with a colorimeter.

3.5 Dry Rubbing Fastness Test

- ◆ Cut the test fabric into 5.0 cm × 5.0 cm size. (Experimental group and control groups refer to Table 2.)
- ◆ Apply double-sided tape to the 5.0 cm × 10.0 cm side of the test white cotton cloth and attach the same-sized test cloth to the slot surface of the test machine.
- ◆ Glue the white cotton cloth on the upper part of the rubbing mechanism, so that the white cloth is in complete contact with the fabric to be tested.
- ◆ In the plate of the rubbing mechanism, load 100.0 g and maintain a certain pressure.
- ◆ Adjust the rubbing frequency by variable resistance: 40 round trips per minute, timed at 60 seconds.

4. Color Measurement

4.1 Principle

A colorimeter, whose main purpose is to distinguish the color, emits a standard light source along the surface of the object to be measured. The sensor head detects the color of light received and presents it in a digital manner. This enables a variety of colors to have a standard. Currently, CIELAB is the most common way to measure the color of objects. This was developed by the International Commission on Illumination (CIE) in 1976. The L-axis is the luminance axis, 0 is black, and 100 is white; the positive value of the a-axis is red, the negative value is green, and the positive value of the b-axis is yellow, and the negative value is blue. It is a spatial presentation, as shown below, because each color has a position on the spatial coordinates (L_1, a_1, b_1). If two colors have two points, the distance between the points is the color difference value, which is

$$\text{calculated as: } \Delta E_{ab}^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

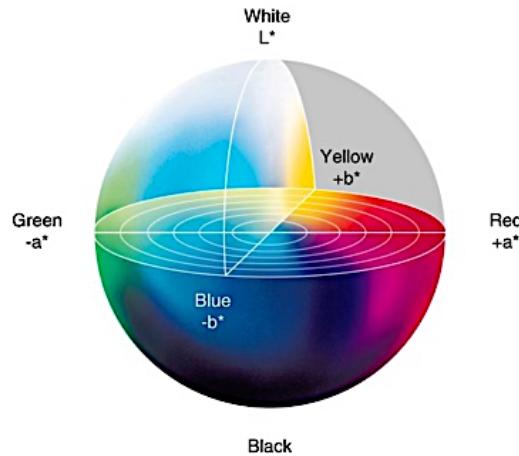


Figure 6. Color 3D Space¹⁷

4.2 Color measurement: Measure the five random positions on the cloth respectively.

- ◆ The use of a colorimeter (color i5 spectrophotometer)

- ❖ Calibration

Warm up the instrument before use for about 15 minutes, and then use black and white swathes, calibrate according to the operating manual, and use the instrument only after completion.

- ❖ Measurement

Align the cloth with the instrument's detection hole position, press the measurement button, and Lab value will appear on the display.

Results and Discussion

➤ Experiment 1

Extraction of Natural Indigo Dye from Assam Indigo Leaves

1.1 Discoloration of soaked leaves

Day	Day 0	Day 1	Day 3	Day 5
Color				

Table 3. Discoloration of Soaked Assam Leaves

1.2 Hydrated lime added to obtain blue puree weight

Hydrated Lime	10.0 g	20.0 g	30.0 g	40.0 g	50.0 g
pH Value	10.5	12.5	12.7	12.8	12.7
Blue Puree Obtained (g)	37.27	42.19	71.69	74.11	74.73 g
Appearance					

Table 4. Weight of blue puree obtained after the addition of hydrated lime.

The pH value of the leaves and branches before adding hydrated lime is 5.9 to 6.2

Results: We obtained the blue puree by adding different amounts of hydrated lime. The highest yield of blue puree was obtained by adding 50.0 g of hydrated lime, and the color was close to blue-green. The rest of the groups were dark green, and the more hydrated lime was added, the lighter the color was.

1.3 Discussion

- ◆ Blue grasses, such as *Indigofera tinctoria*, *Isatis indigotica Fortune ex Linndl.*, *Baphicacanthus cusia Bremek.*, *Polygonum tinctorium Ait.*, have been used as a dye for thousands of years. However, the creation of chemical pigments, and the

development of other plants, such as tea, led to the decline of the blue dyeing industry. It is only in recent years that this traditional craft has been taken seriously again due to the efforts of environmentalists to revive it. We obtained fresh Assam indigo leaves from the mountains and tried to make our own blue puree dyeing. The indigo plant is composed of Indican ($C_{14}H_{17}NO_6$), which contains glucose in its structure, and is fermented in an alkaline solution to produce indoxyl molecules, which are finally oxidized to form indigo. The indigo powder in the market is the dried blue puree obtained from this step.

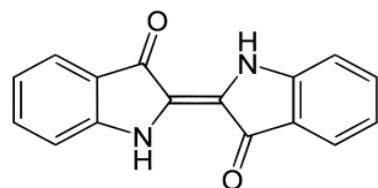


Figure 7. The chemical formula of Indigo (from Wikipedia)

- ◆ Since indigo does not adhere easily to textiles, natural coffee, and soybean protein are extracted as adhesives for coloring via a reduction reaction by alkaline solution. In the indigo reaction, different amounts of hydrated lime were added to the fermented solution of Assam indigo leaves at 10.0 liters per 1000.0 g leaves, and the amount of blue puree and the actual color of dyeing were observed.
- ◆ The solubility of hydrated lime was 0.15 g/100.0 g at 30°C, which decreased with an increase in temperature, so the color was darkest when 10.0 g of hydrated lime was added, and the color was lighter when 50.0 g of hydrated lime was attached to the surface with the least amount of dye. There are many determining factors in the production of indigo, such as picking time, soaking time, soaking temperature, water quality, limewater concentration, pH value of the soaking solution, etc. When 50.0 g of lime was used, the actual dyeing showed blue-green color. This showed that the higher the concentration of hydrated lime, the lighter the color would be after dyeing.

➤ *Experiment 2*

Quantification of Protein Precipitation Amount of Raw Coffee Beans After Acid Denaturation

- 2.1 The pH 10 buffer solution was divided into 5 groups of filtrates, which were combined with 1~5 mL of 1N hydrochloric acid, respectively, and the filtrates produced precipitates.
- 2.2 After mixing, centrifuge and pour out the supernatant, measure the pH value, and dry

and weigh 1~5 precipitated solids.

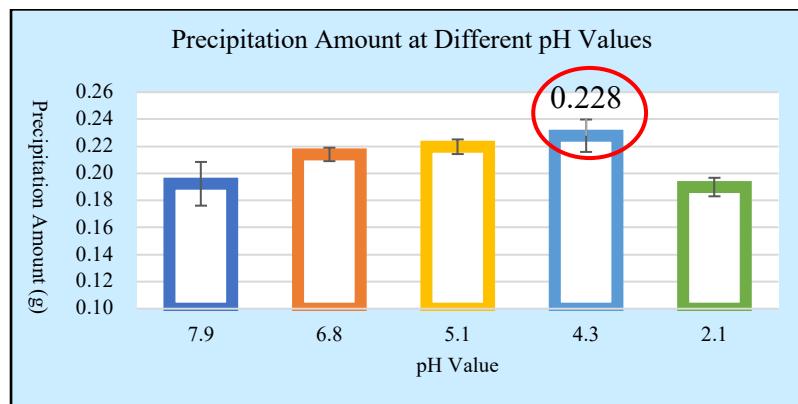


Figure 8. Precipitation Amount of Coffee-denatured Protein at Different pH Values (N=3)

Results: The maximum precipitation amount was at pH 4.3.

2.3 Discussion

- ◆ The borax-sodium hydroxide 0.2 M buffer solution with pH 10.0 was used in this experiment with reference to the experimental results of Coffee Bean Protein Extraction Conditions and Constituents Optimization¹², because the protein content of coffee beans was about 11-13%, and an attempt was made to use the fast extraction method of experiment 3 with soybean juice. However, the solids obtained had too many impurities, so there was no effective viscosity to fix the dye.
- ◆ In the experiment, 1N of hydrochloric acid was added quantitatively, and denatured protein precipitation could be produced at normal temperature. We first soaked the beans in water for a week before grinding them into bean juice. Our first attempt to isolate the coffee bean protein had limited success due to the limited shelf-life of raw coffee beans. In order to refine the extraction process, freshly collected defective coffee beans were ground in a dry grinder and sieved with a 100-meshed sieve. Coffee pigment remained in the isolated coffee protein. The color of coffee bean protein is yellowish. After mixing with indigo puree, the color becomes blue-green, which can be used as the discoloration for denim textiles due to the different shades of vegetable protein sources.

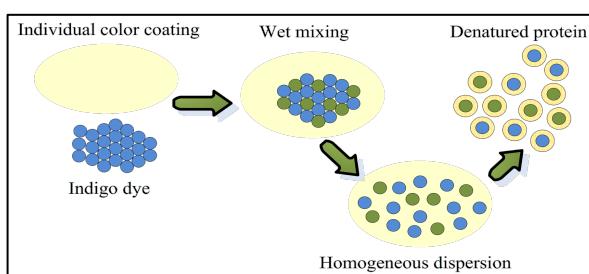


Figure 9. The Indigo Dye and Coffee Bean Protein Mixing Process

➤ Experiment 3

Quantification of Protein Precipitation Amount of Soybeans After Acid Denaturation

3.1 After taking 12 mL for reaction and mixing uniformly, make three portions of each, centrifuge at 4000 rpm for 10 minutes, pour out the supernatant, measure the pH value, and weigh the solids.

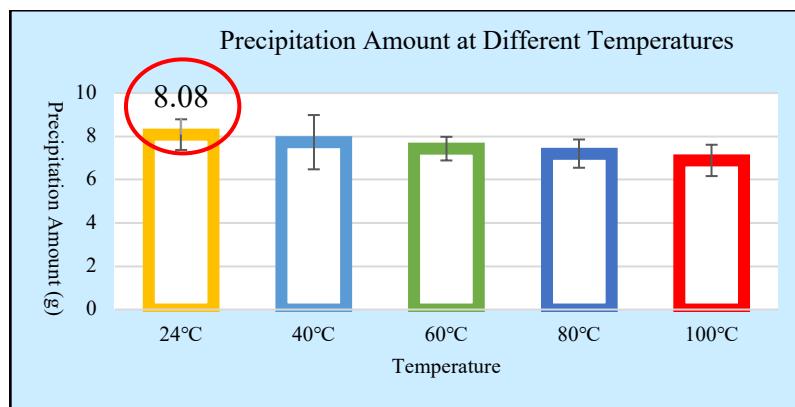


Figure 10. Precipitation Amounts of Soybean-denatured Protein at Different Temperatures (N=3)

Results: The largest number of denatured solids occurred at 24°C. The amount of precipitation decreased gradually with higher heating temperature.

3.2 Discussion

- ◆ The experiment of denaturing soybean juice was conducted by using food-grade 4.5% acetic acid, which was added 1-2 dropwise. The solution was stirred to observe the protein precipitation until no more observable gain in the precipitant amount. The soybean protein precipitation amount varies at different acidity levels.²¹ To obtain the maximum precipitation, the group that added the juice from pH 6.8 to pH 5.0 had the highest solid protein yield. This method is similar to the acid addition to milk, which precipitates casein to make adhesives.

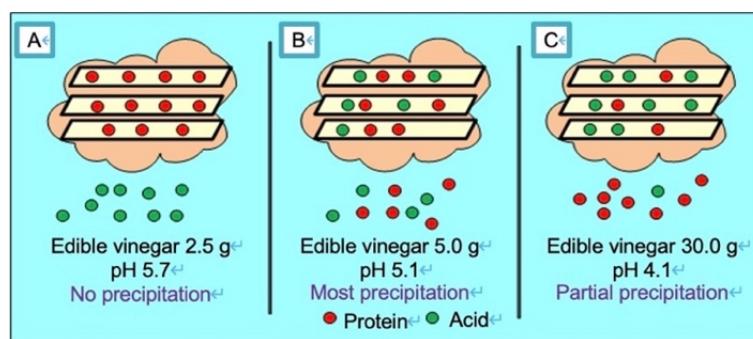


Figure 11. The precipitated particles of protein and acid when the pH of buffer solutions. (A) above isoelectric point (B) reaches isoelectric point (C) below isoelectric point

- ◆ The experimental results showed normal temperature had the highest solid protein yield. We questioned the reason for the inversely proportional relationship between

temperature and solid protein yield. It is believed that the higher temperature may have denatured protein. It is also possible the plant protein may be unstable for prolonged exposure to environmental heat, resulting in a lowered yield.

➤ *Experiment 4*

Washing and Rubbing Fastness of Natural Indigo Dyeing and Coating for Denim

Textiles

4.1 Washing fastness after dyeing with the adhesives made from raw coffee beans and soybeans. (Gray Sample Card refer to Table 1.)

Test	Experimental Group		Control Group 1	Control Group 2
	Coffee Bean Adhesive	Soybean Adhesive	Printing Resin (Made In Japan)	Commercially Available Jeans
1				
AVG L/a/b	30.747/-1.244/-1.057	32.821/-0.957/-2.132	31.247/-0.834/-3.187	28.151/-0.804/-5.132
Grade	4~5	4~5	4	5
2				
AVG L/a/b	30.527/-1.314/-1.178	33.127/-1.025/-2.357	30.928/-0.758/-3.345	27.367/-0.782/-5.470
Grade	4	4~5	4	5
3				
AVG L/a/b	31.315/-2.030/-1.002	32.748/-1.728/-2.433	29.854/-0.571/-3.832	25.749/-0.649/-5.934
Grade	4~5	4	4	5

Table 5. Washing Fastness Grade Test Results

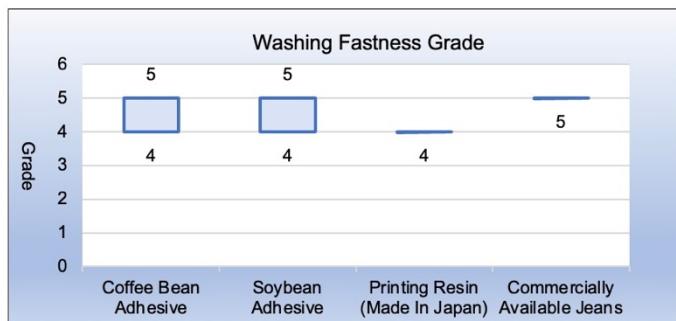


Figure 12. Washing Fastness Test Grade (N=3)

Results: The grade of adhesives made from natural protein can reach 4-5 grades.

4.2 Rubbing fastness after dyeing with the adhesives made from raw coffee beans and soybeans.

Test	Results		AVG L/a/b	Grade
Experimental Groups		Coffee Bean Adhesive	30.931/-2.045/-1.024	5
			30.556/-1.287/-1.214	4~5
			30.763/-1.281/-1.035	4~5
		Soybean Adhesive	32.574/-1.698/-2.312	4~5
			32.985/-1.039/-2.315	5
			32.903/-0.981/-2.214	4~5
Control Group 1		Printing Resin (Made in Japan)	29.137/-0.614/-3.927	4
			31.248/-0.767/-3.288	4
			31.571/-0.865/-3.241	4
Control Group 2		Commercially Available Jeans	25.778/-0.634/-5.874	5
			27.389/-0.794/-5.525	5
			29.054/-0.837/-5.236	4~5

Table 6. Rubbing Fastness Grade Test Results

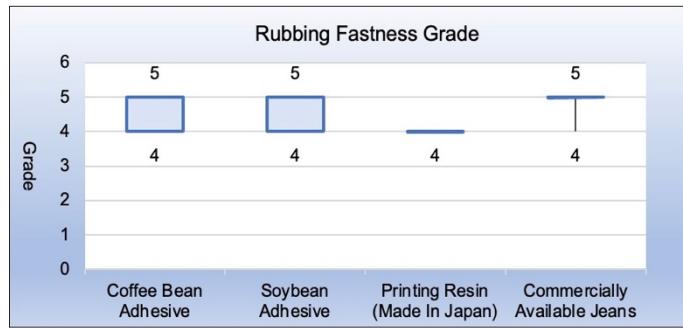


Figure 13. Rubbing Fastness Test Grade (N=3)

Results: The grade of adhesives made from natural protein can reach 4~5 grades.

4.3 Discussion

- ◆ Besides observing the effect of washing after dyeing, the purpose of measuring the washing fastness is to avoid contaminating other fabrics. This experiment used 1.0% detergent at room temperature for cleaning and it showed that the washing and rubbing fastness were above grades 4~5. The color would also have the vintage effect due to the number of times of washes.
- ◆ The rubbing fastness was measured by a self-made machine. The experiment results showed that the effect of our self-made natural dye was superior to the commercial indigo powder, while the amount of hydrated lime added also made a difference. We used the resin provided by the vendor to compare it with the self-made modified protein adhesive. The groups using 10.0 g and 20.0 g hydrated lime addition produced similar results to those using the resin. One major disadvantage of using resin is that it is a non-natural material that needs to be processed with heat, and the wastewater after dyeing needs to be treated for proper disposal. When denim is processed in the pad-dyeing plant, the dark blue pigment combined with the viscous nature of the resin also results in higher cleaning costs for the machine rollers. In contrast, we used denatured vegetable protein as an adhesive, which produced a fabric with a softer texture. Our product also has a slightly lighter color. These superior qualities allow it to be promoted over products made with the current commercial process.
- ◆ This experiment used self-made indigo dye with natural protein for washing fastness and rubbing fastness experiments. Since these materials were harnessed from nature, they align with the ultimate goal of reducing pollution. The color emission of organic botanic pigments differs from that of inorganic mineral pigments. The color of inorganic minerals mainly comes from the arrangement of the metals contained in the crystals, and the refractive index is different, which affects the reflected color of

the color light. After inorganic mineral pigments are grounded, their particle sizes are in proportion to their color intensity. The finer the particles, the lighter the color; the darker the color, the coarser they are. However, the larger color particles easily fall off due to external force, so the larger the particles, the worse the fastness after color coating. The fading in color is especially evident in the rubbing and washing fastness. The organic botanic pigment can be sourced from a wide variety of anthocyanins containing vegetation. For example, pigment can be isolated from beet, gardenia, onion skin, and yam. The current industrial dyeing method adds heavy metal as a dyeing aid. Although the additives can enhance the color fastness and intensify the brightness, the remnants of the process heavily pollute the environment. Our methods of using soybean protein adhesives have shown the potential to remediate the negative environmental impact caused by these chemical additives.

Feasibility of Replacing Synthetic Dyes with Natural Indigo Dyes

- ◆ According to a published report, the cost of the synthetic indigo dyeing process is approximately \$27.24 USD per kilogram.²³ The table below (Table 7) outlines the itemized cost breakdown of our natural material-based indigo dyeing process. The most significant cost incurred in hydrochloric acid. Since it was a laboratory-graded hydrochloric acid, the price per liter is significantly higher than industrial-graded acid. Excluding the cost of the hydrochloric acid, the cost of other materials used in our novel dyeing method is comparable to the current synthetic indigo dyeing process even at a small laboratory scale. This suggests the cost-saving potential of our proof-of-principle approach when it is industrialized in the future.

Required Materials	Price/Unit (USD)	Required Amounts	Cost (USD)
a. Assam Leaves	free	1230.77 g ~ 3981.64 g (Depending on the color shades)	free
b. Lime	2.67/kg	25.0 g	0.07
c. Coffee Beans	free	87.719 kg	free
d. 1N Hydrochloric acid	8.67/L	17.543 L	152.10
e. Borax	7.33/kg	1673.68 g	12.27
f. Sodium Hydroxide	10.0/kg	701.75 g	7.02
g. Soybeans	1.83/kg	3355.0 g	6.14
h. Acetic acid (4.5%)	0.43/kg	1677.0 g	0.72

Table 7. The cost of natural dyes per kilogram.

- (1) Natural indigo + bio-based adhesive (coffee-denatured protein) (a+b+c+d+e+f) = 171.46 (USD)
(2) Natural indigo + bio-based adhesive (soybean-denatured protein) (a+b+g+h) = 6.93 (USD)

Conclusion

- ***Extraction of Natural Indigo Dye from Assam Indigo Leaves***
 - 10.0 g, 20.0 g, 30.0 g, 40.0 g, and 50.0 g of hydrated lime were added to precipitate indigo dyes. 10.0 g of hydrated lime extracted the darkest color and 50.0 g of hydrated lime extracted the lightest color.
 - 50.0 g of saturated lime milk was added to 1000.0 g of leaves to produce about 200.0 g of aqueous indigo puree.
- ***Quantification of Protein Precipitation Amount of Raw Coffee Beans After Acid Denaturation***

The raw coffee beans were ground and sieved with 100 mesh, the proteins were dissolved in a pH 10 buffer solution and denatured with 1N hydrochloric acid. 0.228 g of protein was precipitated at pH 4.3, which was the maximum.
- ***Quantification of Protein Precipitation Amount of Soybeans After Acid Denaturation***

Soybean juice was heated at different temperatures, and the weight of the solid was compared after acid addition and centrifuged. The maximum amount of protein was extracted at 24°C, The solid protein yield decreased with increasing temperature in the order of 40°C, 60°C, 80°C, and 100°C.
- ***Washing and Rubbing Fastness of Natural Indigo Dye and Coating for Denim Textiles***

The fastness was tested with self-made washing and rubbing machines. The washing, fastness and rubbing fastness were above grades 4~5. The fastness was better than printing resin (made in Japan). Although it is a half grade lower than commercially available ones, it is within the acceptable range.
- ***Feasibility of Replacing Synthetic Dyes with Natural Indigo Dyes***
 - After several washes, the color remains stable, and the rubbing fastness test can reach 4-5 grades. The results show that color coating using gelatin after denaturation of natural protein not only gives the denim a faded and vintage effect, but also fulfills the need for environmental protection. Therefore, it is highly feasible to replace petroleum-based resin with biodegradable adhesives from natural origins.
 - Our experiment shows the potential reduced environmental impact and costs in dyeing denim textiles by using natural indigo dyes.

Future Prospective

This project extracted indigo dye from Assam indigo leaves. To our knowledge, this is the first attempt to replace chemical resin with coffee beans protein by acid denaturation as the adhesive for denim coloring. Our future goals are to find better natural coloring materials and production conditions to improve the luster of the fabric and to explore alternative proteins that can also be used as adhesives. For example, we will investigate the feasibility of extracting yeast protein from barrel remnants of the grape wine fermentation process and using the extracted protein as an adhesive in the dying process. The advantage of using yeast is its ultra-low cost and rapid production cycle. It is also ready to upscale to an industrial level. Furthermore, we need to standardize the natural indigo dye extracted from the Assam indigo leaves to avoid inconsistent color depths for mass production.

Overall, indigo dye from Assam indigo leaves has the potential for mass production in Taiwan because it is an indigenous plant species and grows year-long in the wild. Taiwan also has an experienced complete industrial chain to adopt large-scale production. Yet, this will not occur without challenges. For instance, indigo leaf harvesting can be a labor-intensive process; plants tend to grow in rural areas, which limits agricultural machinery access. Although the proposed plant-based adhesive extraction method generates significantly less wastewater than using resin adhesive, the strong acid used in the extraction would still require proper neutralization before disposal. These two major challenges ought to be overcome before full-scale industrialization. As a member who is conscious of our environment, it is our ultimate goal to continuously explore new avenues to refine current industrial processes while maintaining environmental sustainability.

Acknowledgement

I would like to thank my mentor, Dr. Chin-Wen Chen, for suggesting the research topic, and providing invaluable guidance and resources throughout this research process. Without the support from Dr. Chen, my research could not have been accomplished during pandemic.

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