Quality Blues: Indigenous Knowledge of Natural Indigo Identification in Southern China

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Research

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Abstract

Background: As one of the oldest traditional dyes, natural indigo is commonly used for centuries by the people worldwide. In the process of indigo production, indigenous people have formed unique knowledge of indigo identification because the indigo identification is crucial for indigo quality control and the dyeing effects. However, such indigenous knowledge is rarely documented and explained. Therefore, the aims of this study were to i) document and assess the indigenous knowledge of local people identifying the natural indigo paste and ii) to explore the characteristics and material basis of such indigenous knowledge.

Method: Three ethnobotanical studies were conducted in 2019 and 2020. A total of 283 traditional indigo-paste artisans were interviewed in Guizhou, Yunnan, and Fujian Provinces. Frequency of citation, Mention index, and Fidelity level of each indigo-paste quality criterion were calculated to determine the most commonly used, most recognized, and most important quality criterion. To explore the characteristics and material basis of such traditional knowledge, we analyzed 21 samples we collected by using HPLC, pH and particle size analysis methods.

Results: Local people possess unique knowledge to identify natural indigo. After thousands of years, they finally chose four criteria (color, taste, touch, and dyeing ability) and based on this, the nature indigo was divided into five quality grades. The best folk criterion was as following: dark blue with purple-red luster, smooth and difficult to wipe off; it should have a "sweet" or "spicy" taste, and dye cloth easily. It found that the higher the indigo and indirubin contents, especially indirubin content, the better the quality of the indigo paste. Within the 9-12 range, pH of high quality indigo-paste was lower. However, there was no significance difference between particle size and quality.

Conclusion: The ancient methods used by the local people for identifying natural indigo are comprehensive and unique. This study revealed the importance of indirubin and pH for assessing the quality of indigo paste by documenting the various folk quality criteria and quantitative experiments. These findings differ from existing synthetic indigo-quality standards. Amid rapid modernization, traditional knowledge remains invaluable in world heritage of humanity that warrants preservation.

Background

Natural indigo is considered one of the oldest dyestuffs known to humanity, and one of the most commonly used natural dyestuffs worldwide, particularly, in Egypt, India, China, and Africa [1, 2]. It is extracted from indigo-yielding plant species (e.g., *Indigofera tinctoria* and *I. suffruticosa*) and processed by a variety of methods developed in different regions, such as *sukumo* (*Persicaria tinctoria*) in Japan, and woad (*Isatis tinctoria*) balls in Europe. Indigo paste is the traditional product used in China. Over thousands of years, people have cherished natural indigo. Further, indigo color has been endowed with a unique significance and is commonly used a symbol of independence and individualism, whereby it has been called “the king of colors” and “the color of kings” [3]. However, with the rise of industrialization, synthetic indigo is now used in almost the entire dyeing industry, due advantages such as high purity, low price, and better reproducibility [4–7]. Therefore, the production and use of natural indigo has declined and is currently threatened with gradual disappearance, existing only in a few remote areas in China, India, and a few other countries [8, 9]. However, the disadvantages of using synthetic dyes include the high costs of the raw materials, their toxicity, and environmental pollution [4, 5, 10–14]. Therefore, in recent years, consumer interest in natural indigo has been on the rise, which has attracted the attention of dyeing enterprises.

In contrast with synthetic indigo, natural indigo is originally a natural green dye that bears a harmonious and sustainable relationship with the environment, as it is biodegradable [8, 12, 15]. In terms of dyeing, natural indigo has many advantages over synthetic indigo. Thus, whereas synthetic indigo contains residual chemical impurities and only the indigo component, natural indigo is a mixture containing 7–45% indigo, as well as indirubin, dark brown indigo, and yellow indigo [1, 16]. These components give fabrics dyed with natural indigo a richer color and better colorfastness compared to fabrics dyed with synthetic indigo [1, 17, 18]. In addition to its pleasant natural fragrance, fabrics dyed with natural indigo have certain health benefits, as they show insect-repellent and disinfectant actions [15, 19].

Unfortunately, the quality of natural indigo currently found in the market vary greatly and, to date, there is no reliable method for the evaluation of its quality. However, the indigo identification significantly influenced the indigo quality control and the dyeing effects [20–23]. While a large number of studies related to indigo-yielding plant species, traditional extraction methods or dyeing processes, very little is known of folk criteria of indigo identification.

Previously, we found that people still cultivate, use, and trade with natural indigo (indigo paste) on a large scale, in parts of Guizhou, Yunnan, and Fujian Provinces in southern China. Further, we have learned that the traditional knowledge of indigo paste is passed on from generation to generation. Moreover, local people separate indigo paste into different quality grades by folk judging criteria. However, such indigenous knowledge is rarely documented and explained. Based on this, the aims of this study were: i) document and assess the indigenous knowledge of local people identifying the natural indigo paste and ii) to explore the characteristics and material basis of such indigenous knowledge.

Materials And Methods

Study site

Information provided by the local governments and a preliminary survey played a decisive role in the selection of the research site. We selected 15 villages and three markets in Guizhou, Yunnan, and Fujian Provinces for survey sites, as people frequently used indigo paste and had a good heritage of traditional knowledge in these areas. Guizhou and Yunnan Provinces are located in Southwest China, while Fujian Province is located in Southeast China (Fig. 1).

Congjiang County (25°16'–26°05' N; 108°05'–109°12' E) and Zhenfeng County (25°07'–25°44' N; 105°25'–105°56' E) [24] belong to the Qiandongnan Miao and Dong Autonomous Prefecture and Qianxinan Buyi and Miao Autonomous Prefecture, respectively. Congjiang County is located in the middle reaches of the Duliu River. The climate is humid monsoon of the mid-subtropical zone. Annual average temperature and precipitation are 18.5 °C and 1185.9 mm, respectively. Ninety-five percent of the population in Congjiang County is comprised of ethnic minorities, such as the Miao and Dong peoples [25], providing an ideal environment for this study because of their strong cultural inheritance practices. The six villages surveyed in Congjiang County were located in mountainous areas on both sides of the Duliu River, and the residents were all Miao people. Currently, the local area retains a strong indigo-paste culture, and the daily dress of Miao women is still the traditional national costume. Every family in the local area, cultivates indigo-yielding plants (*Strobilanthes cusia*) every year to make indigo paste for dyeing cloth. The climate of Zhenfeng County is subtropical humid monsoon climate. Annual average temperature and precipitation are 16.6 °C and 1276.9 mm, respectively [24]. In Zhenfeng County, indigo paste is produced and traded as a commodity and thus it is produced on a large scale. The main local trade time is market day and the location is the farmers market in each town. Sellers are mostly Han residents from nearby villages and buyers are local ethnic minorities and merchants. In Zhenfeng County, we conducted surveys in two main farmers markets and three villages that produce indigo paste.

Yuanyang County (22°49'–23°19' N; 102°27'–103°13' E) and Jinping County are located in Honghe Hani and Yi Autonomous Prefecture of Yunnan Province. Yuanyang County has a subtropical mountain monsoon climate. Annual average temperature and rainfall are 24.4 °C and 899.5 mm, respectively. Yuanyang County is inhabited by seven ethnic groups including the Hani, Yi, and Han peoples, who have lived in this area for generations. Ethnic minorities account for 89.44% of the total population [26]. Jinping County has a tropical monsoon climate. Annual average temperature and rainfall are 18 °C and 2358.6 mm, respectively. Nine ethnic groups including the Miao and Yao groups have lived in the area for generations, and ethnic minorities account for 87.6% of the total population [27]. In the four villages surveyed in the Yunnan Province (one Yao village and three Hani villages), the elderly still retain the traditional natural-indigo culture and artisanship.

Xianyou County (25°11'–25°43' N; 118°27'–118°56' E) is located approximately at the center of the coastline of the Fujian Province, across from the island of Taiwan. The climate at this site is south subtropical maritime monsoon climate, with an annual average temperature of 20.6 °C and total rainfall ranging from 300 to 2300 mm. In this county, the town of Shufeng is reputedly “the Hometown of Indigo Naturalis," because of its long history of making high-quality Indigo Naturalis [28], which is the powder processed by indigo paste. Its active ingredients are indigo and indirubin, and it is used to treat oral ulcers [29], ulcerative colitis [30], and psoriasis [31–33].

Field survey and data collection

The first field survey was conducted in August and September 2019, in Congjiang and Zhenfeng Counties of Guizhou Province, for approximately 10 days in each case. The second field survey was conducted for 7 days in October 2019 in Xianyou County of Fujian Province. The third field survey was conducted for 6 days in January 2020 in Yuanyang and Jinping Counties of Yunnan Province. During the field investigation, we invited local people who could speak the local language and Mandarin to serve as interpreters. Methods used for data collection included, purposive sampling [34], snowball sampling [35], participatory observation, and questionnaire survey [36]. The interview questionnaire is shown in Table 1. All interviewees possessed traditional knowledge related to indigo paste. Informed consent from all interviewees was obtained orally before conducting the interviews. Once permission was granted, we took photographs [37], audio, video, and other forms of material to assist our research.

As shown in Table 2, a total of 283 informants were interviewed, including 171 from Guizhou Province (139 from Congjiang County and 32 from Zhenfeng County), 42 from Fujian Province, and 70 from Yunnan Province. The age of the interviewees ranged between 31 and 81 years, with 88.0% ranging between 30 and 69 years. The number of female interviewees (n = 219) was almost 3.5 times that of male interviewees (n = 64).

Table 1
Questionnaire for the interviewees.

1 How many types of Indigo-yielding plants in local use?
2 What are the local names of these Indigo-yielding plants?
3 What do these local names mean?
4 How to make Indigo paste after harvesting Indigo-yielding plants?
5 How many ways can you judge the quality of Indigo paste? 6 How to judge specifically?
7 Which of these methods do you like best?

Table 2
Sex and age of the interviewees.

	Number	Percentage
All		
Sex		
Male	64	22.6%
Female	219	77.4%
Age		
30–49	101	35.7%
50–69	148	52.3%
≥ 70	34	12.0%
Guizhou		
Sex		
Male	26 (C:0, Z:26)	15.2% (C:0%, Z:81.3%)
Female	145 (C:139, Z:6)	84.8% (C:100%, Z:18.7%)
Age		
30–49	79 (C:70, Z:9)	46.2% (C:50.4%, Z:28.1%)
50–69	78 (C:56, Z:22)	45.6% (C:40.3%, Z:68.8%)
≥ 70	14 (C:13, Z:1)	8.2% (C:9.3%, Z:3.1%)
Yunnan		
Sex		
Male	8	11.4%
Female	62	88.6%
Age		
30–49	18	25.7%
50–69	38	54.3%
≥ 70	14	20.0%
Fujian		
Sex		
Male	30	71.4%
Female	12	28.6%
Age		
30–49	4	9.5%
50–69	32	76.2%
≥ 70	6	14.3%

Note: C Congjiang county, Z Zhenfeng county.

Quantitative analysis of ethnobotany

In order to screen out the most commonly used, the most recognized, and the most important quality criteria, we used questions 5, 6, and 7 (Table 2) to calculate the Frequency of Citation (FC), Mention Index (QI) [38], and Fidelity Level (FL) [39] of each quality criterion, respectively. The number of interviewees using each quality criterion was counted as the citation frequency for the criterion (FC). QI, used to test homogeneity of the knowledge, was calculated using the following formula: QI = number of mentions/number of interviewees. In turn, FL, used to evaluate the importance of the different quality criteria, was calculated by the following formula: FL = (total number of interviewees providing one quality citation / total number of interviewees providing all quality criteria) × 100%.

Chemical analysis

In order to avoid sample interference, we obtained 21 indigo-paste samples from Guizhou Province, which had the largest number of indigo-paste users among all study sites. These 21 indigo-paste samples were identified and categorized by three key informants using five quality levels, i.e., the best (three samples), good (three samples), general (seven samples), poor (five samples), and worst (three samples). We used the values 1, 2, 3, 4, and 5 to represent the five quality grades of indigo paste, respectively.

An Agilent 1260 series equipment (Agilent Technologies, USA) was used to quantitatively analyze the active ingredients, i.e., indigo and indirubin present in the indigo-paste samples [40]. Ground indigo powder samples of 0.5 g (measured to 0.0004 g accuracy) were ultrasonicated for 30 min to completely disperse the dye in 50 ml of distilled water at pH 7 [41]. A PHS-3C acidity meter and E-201-C composite electrode (Shanghai INESA Scientific Instrument Co. Ltd., Shanghai, China) were used to measure the pH of the 21 indigo-paste samples. An 0.1 g/L indigo-paste suspension was prepared with distilled water and the upper part of the suspension was withdrawn for particle size analysis after ultrasonic dispersion for 10 min. A Malvern Zetasizer ZEN 3600 zeta potential analyzer (Malvern Instruments Ltd., Malvern, U.K.) was used for particle size testing [42].

Statistical analysis

Analysis of variance (ANOVA) was conducted to determine any significant effect of ingredient content and pH ($p \leq 0.05$) on quality grade of the indigo-paste samples. Differences in particle size of indigo paste of different quality grade were analyzed using the Origin Pro learning edition data-analysis software to produce a line graph of the particle size distribution of the 21 indigo-paste samples for comparison.

Results

Indigenous knowledge of indigo identification

As shown in Table 3, five quality grades and four quality criteria for indigo paste were documented. The five quality levels were: Best, good, general, poor, and worst. In turn, the four quality criteria included color, taste, touch, and dyeing ability (Fig. 2). All four criteria were used in Yunnan and Guizhou Provinces, but only two criteria, namely, color and touch, were used in Fujian Province.

Due to differences in the use and storage of indigo paste across the different regions under study, the detailed operation methods were also slightly different. Thus, in Congjiang County of Guizhou Province and Yunnan Province, where people use color to judge quality, they only judged indigo paste in its wet state. However, in the Zhenfeng County of Guizhou Province, people also applied the wet indigo paste to a small area on the back of their hands or arms (Fig. 2a, b) and waited for it to dry naturally. Thus, the colors of the indigo paste in its wet and dry state are combined to assess quality. They believe that high-quality indigo paste should appear dark-blue and purple-red in both its wet and dry states, respectively. In Zhenfeng County, these are known as “water color” and “dry color,” respectively. Because of the inconvenience of transporting wet indigo, people would dry indigo in the sun in the Xianyou County of Fujian Province. They assessed the quality by observing the color of the dried indigo blocks.

People in all regions under study use the touch criterion to judge the quality of the indigo, in which a small amount of moist indigo paste is rubbed between the index finger and thumb. Indigo paste that is smooth and hard to wipe off is considered better quality.

Obvious graininess indicates slightly lesser quality.

In Guizhou and Yunnan Provinces, people think that high-quality indigo paste has a “sweet” or “spicy” taste and dyes fabrics easily. However, it should be noted that there were differences in the description people gave for the taste criterion; furthermore, this phenomenon was more obvious in Guizhou Province. Although more than one fourth of the informants in Guizhou use this criterion, different informants offered contrasting descriptions for taste. Thus, some informants declared that indigo paste is of good quality when it has a “sweet” taste, whereas a “spicy” or “bitter” taste indicates poor quality. Interestingly, other informants held the opposite view.

Table 3
Quality criteria used by folk to assess indigo paste.

criteria	Folk quality levels					Guizhou	Yunnan	Fujian
	1	2	3	4	5	FC/QI/FL	FC/QI/FL	FC/QI/FL
Color	Dark blue, deep purple-red	Dark blue, reddish	Blue	Blue-black, black	Light blue, bluish grey, turquoise	171/1.00/100%	70/1.00/100%	42/1.00/100%
Touch	Exquisite and smooth	Exquisite	Slight granular sensation	Granular sensation	Obvious granular sensation	20/0.12/12%	5/0.07/7%	9/0.21/21%
Taste	-	-	-	-	-	47/0.27/27%	2/0.03/3%	0/0.00/0%
Dyeing ability	Easy	Easy	General	Difficult	Hard	11/0.06/6%	3/0.04/4%	0/0.00/0%
Note: - Meaning that the folk description of this criterion in disagreement.								

Quantitative evaluation of the quality criteria

The color criterion obtained the highest FC value, QI value (QI = 1), and FL value (100%) in all study areas. In contrast, the color criterion was the most commonly used and recognized criterion among people (Table 3). On the other hand, although the touch criterion was used in all study areas, its frequency and importance differed across regions, the highest QI and FL values being obtained in Fujian Province (QI = 0.21/FL = 21%), followed by Guizhou Province (QI = 0.12/FL = 12%), while Yunnan Province showed the lowest values (QI = 0.07/FL = 7%). Taste criterion and dyeing ability criterion were only used in Guizhou and Yunnan Provinces. Similarly, there were differences in the frequency and importance of the two quality criteria in the two regions. Specifically, in Guizhou Province, the taste criterion (FC = 47) was more frequently used than the dyeing ability criterion (FC = 11), whereas people use the dyeing ability criterion (FC = 3) more than the taste criterion (FC = 2) in Yunnan Province, despite the very small difference in the FC value of the two criteria. The QI values of the taste criterion and dyeing ability criterion in Guizhou Province were 0.27 and 0.06, respectively, and the FL values were 27% and 6%, respectively. However, in Yunnan Province, the QI values of the two criteria were 0.03 and 0.04, respectively, and the FL values were 3% and 4%, respectively. This showed that the taste criterion and dyeing ability criterion were more important in Guizhou Province than in Yunnan Province, and were frequently used by the people, especially the taste criterion.

Overall, the most important and recognized evaluation criterion among informants was color, while the other three criteria (taste, touch, and dyeing ability) were only accessory criteria.

Quantitative evaluation of the indigenous knowledge

The main active ingredients in the collected indigo-paste samples were indigo and indirubin, while some samples additionally contained minute amounts of indican or indole (Table 4). As the indigo paste contained water when it was sampled, we also considered water as a factor. Figure 3 shows the average content of the active ingredients in each quality grade of indigo paste. The content of indigo and indirubin decreased with decreasing quality grade. Furthermore, there was a positive correlation between the content of active ingredients and the quality grade of the indigo paste. One-way ANOVA showed that, whether wet or dry, there were significant differences in the content of the active ingredients in the different quality grades.

Table 4

Active ingredients, pH, and particle size of indigo paste samples from Guizhou Province. Significance is indicated by * P < 0.05, ** P < 0.001.

Sample number	Quality grade	Effective components content(ug/g)		Percentage of effective ingredients (%)				PH**	Particle size(d = nm)
		Indigo	Indirubin	Indigo(W) *	Indirubin(W) **	Indigo(D) **	Indirubin(D) **		
1–1	1	11268.55	10221.73	0.37	0.33	1.13	1.02	9.10	531.2–825 (100%)
1–2	1	14486.60	5441.57	0.41	0.15	1.45	0.54	9.47	396.1–825 (100%)
1–3	1	13218.67	4096.02	0.57	0.18	1.32	0.41	9.13	91.28–1281 (87.4%) / 4145–5560 (12.6%)
2 – 1	2	10466.89	3174.17	0.21	0.06	1.05	0.32	10.03	342–825 (90.9%) / 4801–5560 (9.1%)
2–2	2	10583.21	4189.60	0.41	0.16	1.06	0.42	9.18	91.28–164.2 (12.7%) / 396.1–1106 (87.3%)
2–3	2	10590.91	4366.99	0.28	0.12	1.06	0.44	10.92	122.4–255 (17.2%) / 615.1–2669 (82.8%)
3 – 1	3	10164.60	2903.67	0.23	0.07	1.02	0.29	10.98	141.8–255(19%) / 531.2–1718 (81%)
3 – 2	3	8840.08	2357.28	0.20	0.05	0.88	0.24	10.83	220.2-712.4 (100%)
3–3	3	10672.38	2022.68	0.11	0.02	1.07	0.20	10.66	105.7-190.1 (23.6%) / 531.2–1106 (76.4%)
3–4	3	10430.08	1921.20	0.32	0.06	1.04	0.19	10.21	141.8–255 (29.7%) / 825–2305 (70.3%)
3–5	3	10832.98	1193.15	0.46	0.05	1.08	0.12	11.55	220.2-955.4 (96%) / 4145–5560 (4%)
3–6	3	10457.34	2065.58	0.26	0.05	1.05	0.21	10.85	91.28–164.2 (17.4%) / 342–825 (82.6%)
3–7	3	10097.03	1470.28	0.25	0.04	1.01	0.15	11.29	78.82–141.8 (13.2%) / 220.2-955.4 (86.8%)

Note: W Wet weight, D Dry weight.

Sample number	Quality grade	Effective components content(ug/g)		Percentage of effective ingredients (%)				PH**	Particle size(d = nm)
		Indigo	Indirubin	Indigo(W) *	Indirubin(W) **	Indigo(D) **	Indirubin(D) **		
4 – 1	4	7976.97	1818.84	0.16	0.04	0.80	0.18	11.06	91.28–141.8 (8.5%) / 458.7–1106 (88.9%) / 5560 (2.7%)
4 – 2	4	9235.30	1134.66	0.37	0.05	0.92	0.11	10.39	122.4-220.2 (21.5%) / 531.2–1718 (76.6%) / 5560 (1.9%)
4 – 3	4	10334.90	1386.50	0.18	0.02	1.03	0.14	11.36	122.4-220.2 (30.1%) / 531.2–1281 (67.2%) / 5560 (2.8%)
4–4	4	9251.13	1480.47	0.20	0.03	0.93	0.15	11.34	78.82–164.2 (13.2%) / 255-712.4 (86.8%)
4–5	4	9691.15	1316.95	0.29	0.04	0.97	0.13	11.53	91.28–825 (84.4%) / 3580–5560 (15.6%)
5 – 1	5	7834.44	1103.13	0.10	0.01	0.78	0.11	11.22	78.82–105.7 (7.4%) / 396.1-955.4 (92.6%)
5 – 2	5	8106.15	1062.89	0.14	0.02	0.81	0.11	11.64	91.28–141.8 (7.5%) / 295.3–825 (80%) / 4145–5560 (12.5%)
5 – 3	5	10369.98	894.61	0.26	0.02	1.04	0.09	11.43	141.8-295.3 (24.9%) / 712.4–1484 (75.1%)
Note: W Wet weight, D Dry weight.									

The minimum pH value among the 21 indigo-paste samples was 9.10; the maximum value was 11.64, and the average value was 10.67. As the quality of the indigo paste deteriorated, the pH tended to increase. One-way ANOVA showed that there were significant differences in pH of the indigo paste with different quality levels (P = 0.000). Within a certain range ($9 \leq pH \leq 12$), the pH value of high-quality indigo paste was lower, while the pH value of poor-quality indigo-paste was higher.

The particle size distribution of the indigo-paste samples ranged from 78.82 nm to 5560 nm, with the particle size of most samples ranging between 200 nm to 2600 nm (Table 4). All samples had two or three distribution intervals, except for three samples (1–1/1–2/3 – 2) distributed at continuous intervals. Figure 4 shows a broken line graph of the particle size distribution of the 21 indigo-paste samples analyzed. Different quality grades are indicated by different colors. As shown in Fig. 4, the indigo paste of each quality level did not have an obvious independent distribution interval and was randomly distributed across the whole area. Evidently, there was no correlation between quality level and particle size.

Discussion

Sociocultural characteristics of indigo paste artisans

Here, we documented the traditional knowledge and experience of 283 people to analyze how they assess the quality of indigo paste, and then conducted a quantitative analysis to explore the characteristics and material basis of such traditional knowledge. Indigo paste played different roles in different regions. Thus, in Zhenfeng County of Guizhou Province and Xianyou County of Fujian Province, indigo paste is the main source of income for local Han farmers. On the other hand, in Congjiang County of Guizhou Province, indigo paste serves as a traditional model of national self-sufficiency. Both these models exist in Yuanyang County and Jinping County of Yunnan Province.

The different social roles of indigo paste lead to different social divisions of labor. Firstly, with regard to the sex of indigo paste artisans, there were more men engaged in indigo paste production than women in Zhenfeng County of Guizhou Province (81.3% versus 18.7%) and in Xianyou County of Fujian Province (71.4% versus 28.6%). However, in the traditional self-sufficiency model of Congjiang County in Guizhou Province, only women were engaged in the production of indigo paste (Table 2). Although both production models exist in Yunnan, the scope and quantity of the trade was relatively small, which was mainly the traditional model of national self-sufficiency; therefore, the number of women (88.6%) engaged in indigo paste production was almost eight times the number of men (11.4%). These results are consistent with previous findings, namely, traditional dyeing knowledge is reportedly transmitted matrilineally, and it is mainly mastered and used by women [43, 44]. Furthermore, activities such as dyeing are considered inappropriate for men [45]. However, when indigo paste becomes a tradable commodity and generates economic benefits, men readily become involved in this work [44]; indeed, they may even participate as the main labor force.

Secondly, as for the age of the indigo-paste artisans, in the Congjiang County, mainly young women aged 30–49 years (50.4%) were engaged in the production and use of indigo paste, 40.3% were 50–69 years old, and only 9.3% were over 70 years old. In the local area, making indigo paste and dyeing cloth seemed to be the daily work of minority women. In other regions, it was mainly old women aged 50–69 years, namely, 68.8% in Zhenfeng 54.3% in Yunnan, and 76.2% in Fujian, while young women aged 30–49 years were relatively rare, i.e., 28.1% in Zhenfeng, 25.7% in Yunnan, and 9.5% in Fujian. Overall, young people aged 30–49 years (35.7%) and middle-aged and elderly people aged 50–69 years (52.3%) showed extensive knowledge and artisanship of indigo-paste production. However, previous reports indicate that most traditional knowledge was usually held by the elderly, whereas young folk did not prove very knowledgeable of the trade [9, 46].

Traditional quality criteria of indigo paste

Generally, the blue hue in indigo paste derives from indigo content, while the purple-red luster derives from indirubin content [47]. Thus, the ratio of the indigo and indirubin content determines the color of the indigo paste. However, indirubin, which is an isomer of indigo, has always been regarded as a by-product in the production of indigo paste [48, 49]. Interestingly, in our survey, people preferred purple-red indigo paste to pure blue indigo paste. In other words, the quality criteria used revealed that indirubin was judged to be more important than indigo. Although these findings differ from existing synthetic indigo-quality standards, they agree with reports of indigo paste quality assessment in ancient Chinese books. For example, there is a document in “Liping Fuzhi”(Guizhou): “[illegible]”, which means that the purple indigo paste is the best. Similarly, there is a description in “Dyeing Sutra”: “[illegible]” [50], which means that the best quality indigo-paste should have dark blue and red luster. addition, the Hainan Li and Miao people think that dark blue and reddish indigo paste is of better quality [46]. Therefore, the indirubin in indigo paste is not considered as a by-product but as a critical determinant of the quality of indigo paste.

In the process of indigo-paste production, many factors affect the final quality [8, 22, 51], one of them being lime. The first factor is the way in which lime is added. Before adding lime, some folk would put lime into a cloth or gauze bag. The cloth or gauze bag is then rubbed in the soaking liquid to produce a fine lime slurry that flows out of the bag. This method produces very delicate lime particles and greatly reduces the number of impurities in the lime. However, other folk would place the lime in a water scoop or bucket, and a small amount of soaking liquid is added and mixed, and then poured directly into the soaking solution (Fig. 5), thus, ignoring large lime particles and impurities. This might be why folk rub their fingers together to check the smoothness of the indigo paste obtained. The second factor is the amount of lime added. Indeed, adding too much or too little lime results in a low-quality indigo paste. In addition to indigo-yielding plants, the taste of indigo paste is mainly related to the amount of lime added during the production process and people judge whether lime is added properly based on the taste. However, during the interviews, the descriptions of taste provided by informants differed and were even contradictory. There are two possible reasons for this difference: one is that taste description is mainly influenced by personal subjectivity and the other is that informants have different perceptions and descriptions of taste due to cultural divergence. This phenomenon also occurred with respect to the use of the color criterion. Thus, for example, some people might describe high-quality indigo paste as being red in color, despite an apparent purplish-red (Fig. 6).

Verification of indigenous knowledge by modern scientific methods

The folk in the study area used indigo and indirubin together to determine the quality of the indigo paste. Compared to a pure dark-blue color, people preferred indigo paste with purple-red luster. The HPLC-DAD quantitative analysis confirmed that the quality of indigo paste is related to the content of indigo and indirubin. Thus, the higher the content of indigo and indirubin -especially indirubin -the better the quality of the indigo paste. This confirmed that the color of the indigo paste is an adequate quality criterion. However, due to the limited number of the experimental samples, the range of indigo and indirubin contents and the color distribution range of different quality grades of indigo paste could not be identified in this study and certainly warrants further research.

Informants in Guizhou Province offered inconsistent descriptions of taste criterion. Through pH quantitative experiments, we found that within a certain range ($9 \leq$ pH ≤ 12), the pH of high-quality indigo paste was lower, while that of low-quality indigo paste was higher. The level of alkalinity stimulates taste buds differentially among people. Local people judge the quality of indigo paste based on simple taste sensation.

By drying the samples in the laboratory, we observed that there were no significant differences in color or gloss of indigo paste in its wet state, except in the very high and the very low-quality samples. However, the color difference was obvious after drying (Fig. 7). This indicates a degree of rationality associated with the simultaneous observation of “water color” or “dry color” in Zhenfeng County, and the importance of color in assessing indigo-paste quality. Similarly, after drying different indigo pastes, the inner part of the block had a different appearance. Some indigo-paste blocks had a uniform internal color; no obvious lime particles or impurities were observed. However, there were varying amounts of white or other colored particles in other indigo paste blocks (Fig. 7). Thus, from an experimental perspective, the touch criterion is also necessary for the assessment of indigo-paste quality.

Conclusion

Although modernization and urbanization continue to change the traditional ways in which people produce goods, some local people still maintain the traditional culture and methods used for indigo extraction and indigo-paste preparation. After thousands of years, the ancient methods used by the local people for identifying natural indigo are comprehensive and unique. The traditional method for indigo-paste quality assessment is seemingly backward, but it is advantageous in its simplicity and ease of use, as well as in its environmental friendliness and high energy efficiency.

This study revealed the importance of indirubin and pH for assessing the quality of indigo paste by documenting the various folk quality criteria. Furthermore, these empiric criteria were verified by quantitative experiments. Simple traditional knowledge also can be inspiring for the development of modern industrial technology, maybe the invention of modern detecting equipment and the exploitation of novel blue dyes. Traditional knowledge remains an invaluable cultural heritage of humanity that we need to actively preserve and transmit to the new generations.

Abbreviations

FC: Frequency of citation; QI: Mention Index；FL: Fidelity level

Declarations

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Authors' contributions

YRS and YHW conceived and designed the research. YRS, LBZ, LW, ZCQ and XYD carried out the field surveys, collected ethnobotanical data and voucher samples. YRS completed the verification experiments and analyzed the data, and then prepared the manuscript with assistance from SL and LBZ. YRS, LBZ, LW, ZCQ and XYD took the photographs, and YHW reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study is purely based on filled survey instead of human or animal trials. Ethical guidelines of the International Society of Ethnobiology (http://www.ethnobiology.net) were strictly followed. Permissions were verbally informed by all participants in this study.

Consent for publication

The people interviewed were informed about the study's objectives and the eventual publication of the information gathered, and they were assured that the informants' identities would remain undisclosed. Moreover, the portraits we used have been agreed by the owner.

Competing interests

The authors declare no conflict of interest.

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Figures

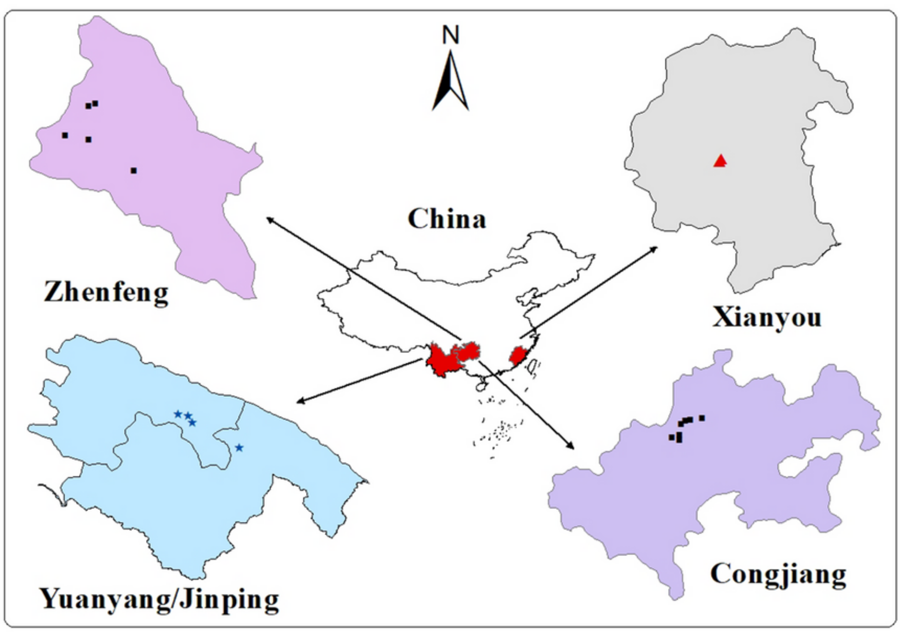
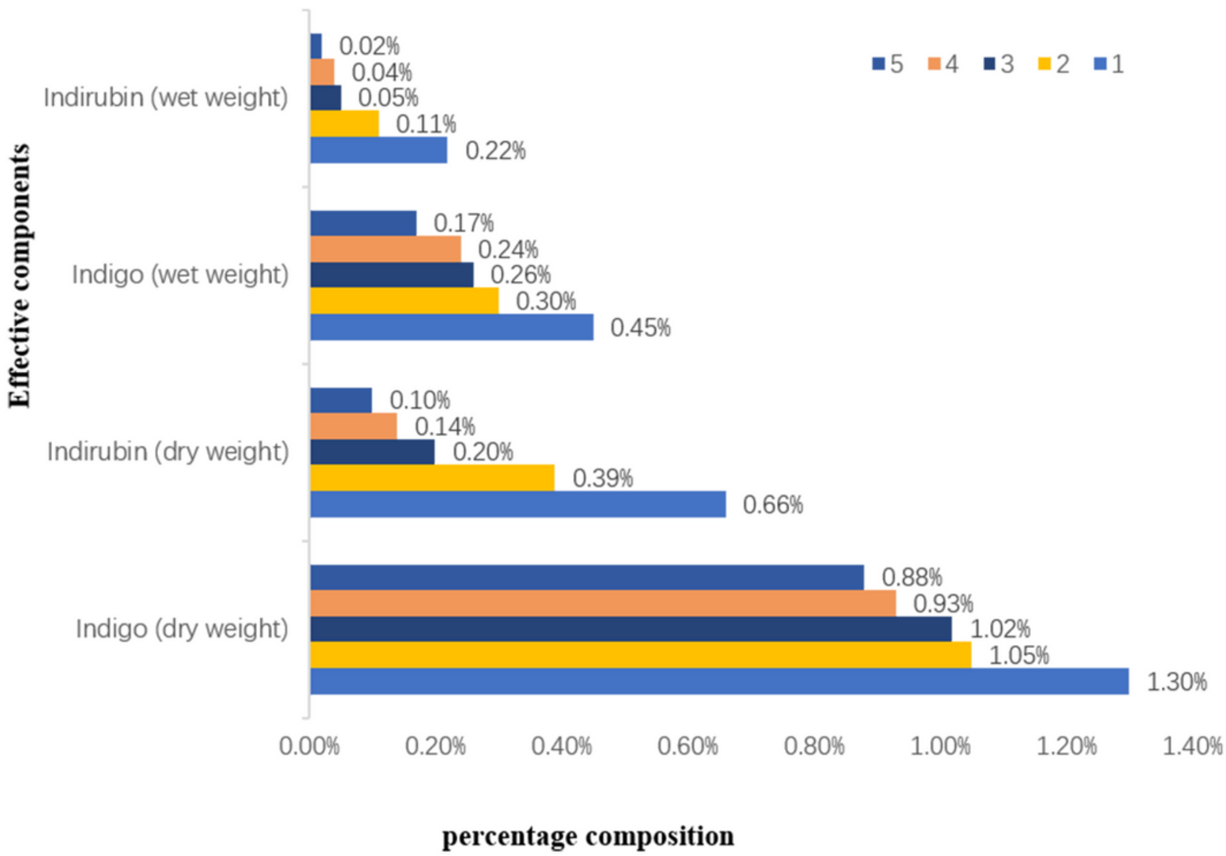


Figure 1

Three markets and 15 villages in Guizhou, Yunnan and Fujian were selected as the survey sites. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Different folk quality criteria. a-b Method of color criterion. c-d Method of touch criterion. e Method of taste criterion. f Method of dyeing ability criterion. 1- 5 represent the five folk quality grades of indigo paste, which are best, good, general, poor and worst respectively.



quality grades	Indigo (dry weight)	Indirubin (dry weight)	Indigo (wet weight)	Indirubin (wet weight)
1	1.30%	0.66%	0.45%	0.22%
2	1.05%	0.39%	0.30%	0.11%
3	1.02%	0.20%	0.26%	0.05%
4	0.93%	0.14%	0.24%	0.04%
5	0.88%	0.10%	0.17%	0.02%

Figure 3

The average content of the active ingredients in each quality grade of indigo paste.

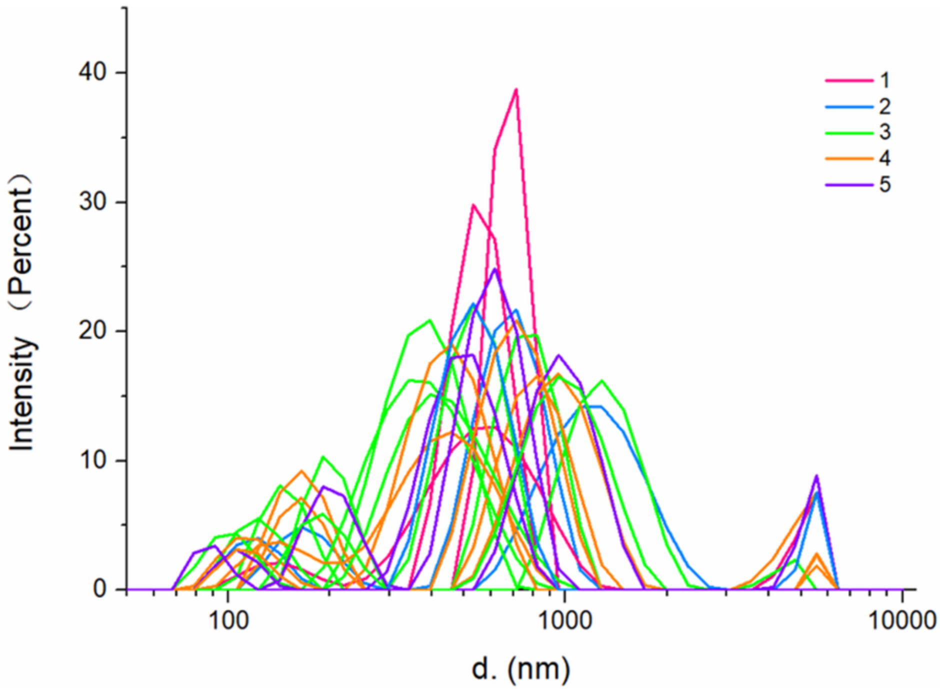


Figure 4

Broken line graph of the particle size distribution of 21 indigo paste samples.

Figure 5

Different ways of adding lime. a-c put lime into a cloth or gauze bag d-i put lime in a water scoop or bucket

Figure 6

Different colors of the wetting indigo paste.

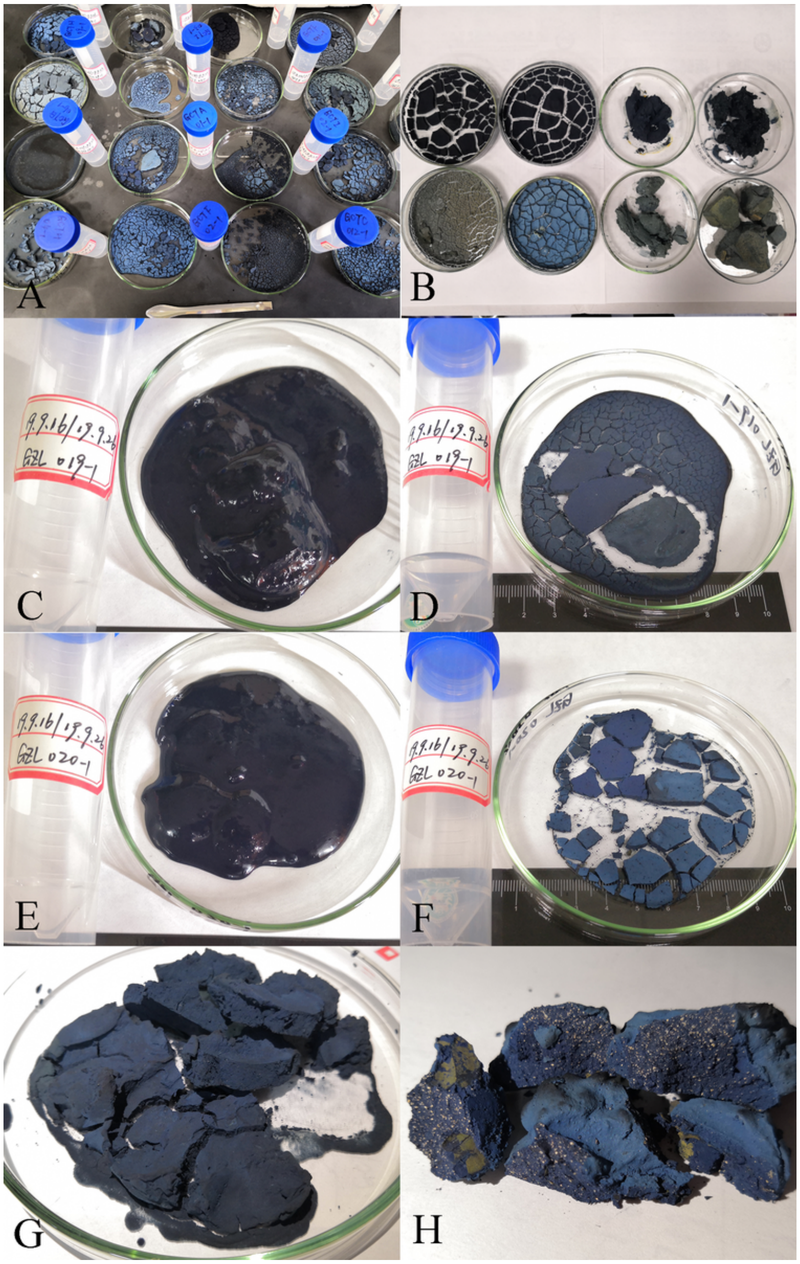


Figure 7

The status of indigo paste of different quality grades after drying. a b d f Different colors of the indigo paste after drying. g-h After drying, the lime particles and impurities in different indigo paste can be observed.