Quality Blues: An Assessment of Cultural and Chemical Factors Used to Define Indigo Quality in China

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Research

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Abstract

Background: The appearance of synthetic indigo has caused most people to forget the past brilliance of natural indigo. However, in parts of southern China, the folk still use and trade in natural indigo paste. The aims of this study were to i) document the traditional knowledge and experience of how people identify the quality of indigo paste and ii) explore the rationality of the quality judgment index used by local people.

Method: We interviewed 283 traditional indigo paste artisans in 3 markets and 15 villages in Guizhou, Yunnan, and Fujian Provinces. Frequency of citation (FC), Mention index (QI), and Fidelity level (FL) of each indigo paste quality judgment index were calculated to determine the most commonly used, most recognized, and most important quality judgment index. A quantitative study was conducted on 21 indigo paste samples of different quality grades using high-performance liquid chromatography with diode-array detection (HPLC-DAD), an acidity meter, and particle size analyzer. The relationship between the content of the effective components, pH, and particle size of the indigo paste and quality was explored.

Results: The people divided indigo paste into five quality grades i.e., best, good, general, poor, and worst. Four main quality judgment indices were color, taste, touch, and dyeing ability. Among all study areas, color was the most commonly used, most important, and most recognized index. Effective ingredient content and pH differed significantly with different quality graded indigo pastes, but there was no significance difference between particle size and quality. In addition to indigo, indirubin played an important role in identifying the quality of indigo paste.

Conclusion: Our study confirmed that the quality of indigo paste could be evaluated using the four indices (color, taste, touch, and dyeing ability). The people used indigo and indirubin content together to determine the quality of the indigo paste, while synthetic indigo is determined by indigo content only. The simple knowledge and experience of traditional folk has guiding significance for the modern development of indigo paste production.

Background

Indigo is considered one of the oldest pigments known to humanity. Over thousands of years of use, people have had a deep affection for natural indigo. The color of indigo has also been endowed with a unique meaning, such as independence and individualism, and is called “the king of colors” and “the color of kings” [1]. In order to facilitate transportation, people make natural indigo into indigo paste, which is one of the most commonly used natural dyestuffs in the world, particularly in countries such as in Egypt, India, China, and Africa [2,3]. However, with the rise of industrialization, synthetic indigo now occupies almost the entire dyeing industry, with its advantages of high purity, low price, and better reproducibility [4-7]. These advantages are not possessed by indigo paste. Since then, the production and use of indigo paste has declined and gradually disappeared. It now only exists in a few remote areas in China, India, and a few other countries [8,9].

The disadvantages of using synthetic dyes are the high costs of the raw materials, their toxicity, and environmental pollution [4,5,10-14]. In recent years, there has been increased interest in indigo paste among consumers, which has attracted the attention of dyeing enterprises. Compared with synthetic indigo, indigo paste as a natural green dye has a harmonious and sustainable relationship with the environment and is biodegradable [8,12,15]. In terms of dyeing, indigo paste has many advantages over synthetic indigo. While synthetic indigo contains residual chemical impurities and only the indigo component, indigo paste is a mixture containing 7–45% indigo, as well as indirubin, dark brown indigo, and yellow indigo [1,16]. These components give fabric dyed using indigo paste a richer color and better colorfastness compared to fabric dyed with a chemically identical synthetic indigo [1,17,18]. In addition to its pleasant natural fragrance, dyed fabrics of indigo paste also have certain health benefits: they can act as an insect-repellent and as a disinfectant [15,19].

However, the quality of indigo paste currently on the market varies and it lacks a reasonable quality judgment method. The plant source and processing methods used in producing indigo paste significantly influence the quality of the paste, which in turn directly affects the quality of the dye [20-23]. Therefore, in the process of use and trade, quality is an unavoidable issue. The chemical industry standard of the People’s Republic of China stipulates five indicators used to judge the quality of synthetic indigo. These are the appearance (dark blue uniform powder or granule), the mass fraction of indigo (≥93%), the mass fraction of moisture (≤1.0%), the mass fraction of fineness (≤5.0% beyond 250 μm), the residue in the sieve (≤5.0%), and the iron content

(≤500 mg/kg). In contrast, there are no regulations or standards for indigo paste. There are also a limited number of research reports on the standards of quality judgment.

Through our investigations, we found that in parts of Guizhou, Yunnan, and Fujian Provinces in southern China, people still use and trade in indigo paste on a large scale, and the traditional knowledge of indigo paste is passed on from generation to generation. Moreover, the people separate indigo paste into different quality grades for use and trade without the use of laboratory equipment, and we did not understand how they classified indigo paste into different quality grades or how they made quality judgments. Based on this, the aims of this study were: i) to record the indigo paste quality grades and quality judgment indices used by the folk in detail; ii) to identify the most commonly used, most recognized, and most important quality judgment indices; and iii) to explore the rationality of the quality judgment indices used by the folk.

Methods

Ethnobotanical survey method

Study site

Information provided by local government and a preliminary survey played a decisive role in the selection of the research site. We selected villages and markets in Guizhou, Yunnan, and Fujian Provinces as survey sites, as the people frequently used indigo paste and had a good heritage of traditional knowledge in these areas (Table 1). Guizhou and Yunnan Provinces are located in southwest China, and 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 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. The annual average temperature is 18.5 °C and annual average precipitation is 1185.9 mm. Ninety-five percent of Congjiang County's population are ethnic minorities, such as the Miao and Dong people [25], providing an ideal environment for the study because of its 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 good indigo paste culture, and the daily dress of Miao women is still the traditional national costume. In the local area, every family, except for migrant families, cultivates indigo yielding plants every year to make indigo paste for dyeing cloth. The climate of Zhenfeng County is subtropical humid monsoon climate. The annual average temperature is 16.6 °C and average annual precipitation is 1276.9 mm [24]. In Zhenfeng County, indigo paste is produced and traded as a commodity and thus it is produced at scale. The main local trade time is market day and the location is the farmers' markets in each town. The sellers are mostly Han residents from nearby villages, and the buyers are local ethnic minorities and merchants. In Zhenfeng County, we conducted surveys in two main farmers' markets and three villages that producing 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. The annual average temperature is 24.4 °C and average annual rainfall is 899.5 mm. Yuanyang County is inhabited by seven ethnic groups including the Hani, Yi, and Han people who have lived there for generations. Ethnic minorities account for 89.44% of the total population [26]. Jinping County has a tropical monsoon climate. The annual average rainfall is 2358.6 mm and annual average temperature is 18 °C. Nine ethnic groups including the Miao and Yao groups have lived there for generations, and ethnic minorities account for 87.6% of the total population [27]. In the four villages surveyed in 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 in the middle of the southeast coast of Fujian Province. The climate is south subtropical maritime monsoon climate. The annual average temperature is 20.6 °C and annual average rainfall is 1300–2300 mm. Among them, the Shufeng Township we investigate has the reputation of being "the Hometown of Indigo Naturalis" because of its long history of making high quality Indigo Naturalis [28]. Indigo Naturalis is the powder processed to make indigo paste. Its active ingredients are indigo and indirubin. It is used to treat oral ulcers [29], ulcerative colitis [30], and psoriasis [31-33].

Table 1 Summary of the survey site.

Survey site	Province	county	national	latitude	longitude
Tingdong Town market	Guizhou	Congjiang	Miao	25°49′28.71″	108°37′01.22″
Jiuyue village				25°47′16.32″	108°35′25.13″
Jiugua village				25°46′45.49″	108°36′38.97″
Miaopeng village				25°47′35.90	108°36′38.77″
Jiali village				25°50′15.90″	108°38′30.65″
Lingwa village				25°50′27.15″	108°40′32.83″
Changzhai village				25°50′04.13″	108°37′42.98″
LongchangTown market		Zhenfeng	Han	25°27′43.05″	105°30′06.34″
Zhenfeng Town market				25°23′18.34″	105°38′53.50″
Weizhai village				25°27′16.00″	105°33′10.04″
Zikong village				25°31′49.34″	105°34′01.54″
Shuiyindong village				25°31′29.84″	105°33′11.23″
Sihuang village	Fujian	Xianyou	Han	25°26′52.61″	118°39′25.40″
Shufeng village				25°26′34.12″	118°39′16.95″
Gama village	Yunnan	Yuanyang	Hani	23°01′36.23″	103°01′13.84″
Zhetai village				23°01′59.18″	102°59′10.52″
Yaorenxiaozhai village			Yao	23°00′13.77″	103°02′11.23″
Gaoxing village		Jinping	Hani	22°54′52.74″	103°12′11.94″

Field survey and data collection

The first field survey was conducted in Congjiang County and Zhenfeng County of Guizhou Province in August and September 2019, for approximately ten days each. The second field survey was conducted in Xianyou County of Fujian Province in October 2019 for seven days. The third field survey was conducted in Yuanyang County and Jinping County of Yunnan Province in January 2020 for six days. During the field investigation, we invited local people who could speak the local language and Mandarin to serve as translators. The intentional sampling method [34], snowball sampling method [35], participatory observation method, and questionnaire survey method [36] were used to collect the data. The interviewee questionnaire is shown in Table 2. All interviewees possessed traditional knowledge relating to indigo paste. Before conducting interviews with them, we obtained informed consent from all interviewees orally. After obtaining permission, we took photographs [37], audio, video, and other forms of material to assist with our research.

As shown in Table 3, 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 informants was between 31 and 81 years. The age of most informants was concentrated between 30 and 69 years (88.0%). The number of female informants (n = 219) was almost 3.5 times that of males (n = 64).

Table 2 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 3 Gender and age of the interviewees.

	Number	Percentage
All		
Gender		
Male	64	22.6%
Female	219	77.4%
Age		
30-49	101	35.7%
50-69	148	52.3%
≥70	34	12.0%
Guizhou		
Gender		
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		
Gender		
Male	8	11.4%
Female	62	88.6%
Age		
30-49	18	25.7%
50-69	38	54.3%
≥70	14	20.0%
Fujian		
Gender		
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.

Survey data analysis

In order to screen out the most commonly used, most recognized, and most important quality judgment indicators, 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 judgment index, respectively. For each quality judgment index, the number of informants using the index was counted as the citation frequency of the index (FC). QI was used to test homogeneity of the knowledge, where QI = number of mentions/number of informants. FL was used to evaluate the importance of the different quality judgment indices, where FL = (total number of informants who provide one quality judgment index/ total number of informants who provide all quality judgment indicators) × 100%.

Material collection and pretreatment

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 areas. The 21 indigo paste samples were identified and categorized by 3 key informants using 5 quality levels, i.e., the best (3 samples), good (3 samples), general (7 samples), poor (5 samples), and worst (3 samples). We used the values 1, 2, 3, 4, and 5 to represent the five quality grades of indigo paste, respectively. During the field investigation, indigo paste samples were collected and sealed into 50 ml plastic tubes. At the laboratory, we used a blast-drying oven (Shanghai Chengshun Instruments Co., Ltd. Shanghai, China) to dry the samples to a constant weight at a constant temperature of 60 °C, which were then stored.

High-performance liquid chromatography (HPLC) analysis

Chemicals and reagents

Indigo standard product (≥98%) was purchased from Guangzhou Kehua Commercial and Trading Co. Ltd., Guangzhou, China. Indirubin standard product (≥98%) was purchased from Shanghai Macklin Biochemical Co. Ltd., Shanghai, China. Indole standard product (≥99%) was purchased from Aladdin Reagent (Shanghai) Co. Ltd., Shanghai, China. Indican standard product (≥98%) was purchased from Jiangsu Aikon Biopharmaceutical R&D Co. Ltd., Jiangsu, China. Methanol (≥99.9%) was purchased from Shanghai Xingkegaochun Solvent Co. Ltd., Shanghai, China. N-N dimethylformamide (≥99.5%) was purchased from Yunnan Shusen Biotechnology Co., Ltd., Yunnan, China. All chemical reagents were of analytical grade.

HPLC-DAD

We ground 1–2 g of the dried indigo paste sample in a 5 ml centrifuge tube (60 Hz for 80 s) using a high-throughput tissue grinder (Scientz-48, Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China). This was repeated four times. After grinding, we weighed 100–200 mg of the sample powder into a 10 ml centrifuge tube, added 6 ml of N, N-dimethylformamide (DMF), and performed extraction ultrasonically for 30 min. After cooling to room temperature, the final volume was adjusted to 10 ml using a 10ml volumetric flask. The solution was then passed through a 0.45 μm microporous membrane, ready for use.

The Agilent 1260 series equipment (Agilent Technologies, USA) was used to quantitatively analyze the active ingredients such as indigo and indirubin in the indigo paste samples [40]. Each indigo sample was subjected to three repeat experiments, and the final active ingredient content was the average of the three measured data. The standard curves of indigo, indirubin, indole, and indican were established using five different concentration gradients. The sample was separated using an Agilent ZORBAX Extend-C18 column (5 μm, 4.6 × 250 mm) at 30 °C. The mobile phase consisted of water (solvent A) and methanol (solvent B). The eluent flow rate was 1.0 ml/min, and the injection volume was 10 μl. The mobile phase elution gradient was 0–30 min, 10–80% B; 30–50 min, 80% B; 50–51 min, 80–10% B; and 51–55 min, 10% B. The detection wavelength was 280 nm.

pH analysis

We weighed 0.5 g (accurate to 0.0004 g) of ground indigo powder and ultrasonicated it for 30 min to completely disperse the dye in 50 ml of distilled water with a pH of 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.

Particle size analysis

An indigo paste suspension with a content of 0.1 g/L was prepared with distilled water, and the upper suspension was taken 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

In order to analyze whether there were significant differences in the effective ingredient content and pH (95% confidence interval) of the indigo paste samples in different quality grades, we performed an analysis of variance (ANOVA), using the percentage of active ingredient and pH as the variables ($p \leq 0.05$). In order to analyze whether there were differences in the particle size of indigo paste with different quality grades, we used 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

Quality grades and quality judgment indices

As shown in Table 4, five quality grades and four quality judgment indices for indigo paste were recorded. The five quality levels were best, good, general, poor, and worst. The four quality judgment indicators included color, taste, touch, and dyeing ability (Fig. 2). All four indicators were used in Yunnan and Guizhou Provinces, but Fujian Province only used two indicators, namely color and touch.

Because we found differences in the use and storage of indigo paste across the different regions, the detailed operation methods were also slightly different. 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. 2 a and 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 believed that high-quality indigo paste would appear dark blue and purple-red in both its wet and dry states. These are known as “water color” and “dry color” in Zhenfeng County, 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 use the touch method 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. If there is obvious graininess, the quality would be considered slightly worse.

In Guizhou and Yunnan Province, people thought that high-quality indigo paste had a “sweet” or “spicy” taste, and dyed cloth easily. It should be noted that there were differences in the description of the folk’s taste index, and this phenomenon was more obvious in Guizhou Province. Although more than a quarter of the informants in Guizhou used this index, different informants had different descriptions of taste. Some people thought that indigo paste was of good quality when it had a “sweet” taste, and a “spicy” or “bitter” taste indicated poor quality, while other people thought the opposite.

Table 4 Quality judgment indices used by folk to assess indigo paste.

Indices	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 index is in disagreement.

Quantitative evaluation of the quality judgment indices

The color index had the highest FC value, QI value (QI = 1), and FL value (100%) in all study areas. In contrast, the color index was the most commonly used and recognized index among the people (Table 4). The touch index was used in all study areas, but its frequency and importance differed across the regions. The touch index had the highest QI and FL values in Fujian Province (QI = 0.21/FL = 21%), followed by Guizhou Province (QI = 0.12/FL = 12%), and Yunnan Province had the lowest (QI = 0.07/FL = 7%). The taste index and dyeing ability index were only used in Guizhou and Yunnan Provinces. Similarly, there were differences in the frequency and importance of the two quality judgment indices in the two regions. In Guizhou Province, the taste index (FC = 47) was more frequently used than the dyeing ability index (FC = 11), while in Yunnan Province, people use the dyeing ability index (FC = 3) more than the taste index (FC = 2), despite the very small difference in the FC value of the two indices. The QI values of the taste index and dyeing ability index 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 indicators were 0.03 and 0.04, respectively and the FL values were 3% and 4%, respectively. This showed that the taste index and dyeing ability index were more important in Guizhou Province than in Yunnan Province, and were frequently used by the people, especially the taste index.

Overall, the most important and recognized evaluation index among the people was color, while the other three indices (taste, touch, and dyeing ability) were auxiliary methods only.

HPLC analysis

The main active ingredients in the indigo paste samples were indigo and indirubin and some samples contained insignificant amounts of indican or indole (Table 5). Since 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 a decrease in the quality grade. There was a positive correlation between the content of active ingredients and the quality grade of the indigo paste. The 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 of indigo paste.

Table 5 Active ingredients, pH, and particle size of indigo paste samples from Guizhou Province.

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%)
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.

Table 6 Active ingredients and pH of 21 indigo paste samples with differing quality, analyzed by one-way ANOVA (P ≤ 0.05).

content	P
Percentage of active ingredients (%)	
Indigo (Wet weight)	0.033
Indirubin (Wet weight)	0.000
Indigo (Dry weight)	0.000
Indirubin (Dry weight)	0.000
PH	0.000

pH analysis

The minimum pH value of 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. The one-way ANOVA showed that there were significant differences in the 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 good quality indigo paste was lower, while the pH value of poor quality indigo paste was higher.

Particle size analysis

The particle size distribution of the indigo paste samples ranged from 78.82 nm to 5560 nm, and the particle size of most samples were concentrated between 200 nm to 2600 nm (Table 5). 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 21 indigo paste samples. Different quality grades are indicated by different colors. As shown in the figure, the indigo paste of each quality level does not have an obvious independent distribution interval, and is randomly distributed across the whole area. Evidently, there was no correlation between quality level and particle size.

Discussion

Sociocultural characteristics of indigo paste artisans

In this study, we recorded the traditional knowledge and experience of 283 people to investigate how they assessed the quality of indigo paste, and then conducted a quantitative analysis to explore the rationality of the quality judgment index used by the people. Here, we must mention that indigo paste played a different role in the different regions, and thus we found some differences across the regions. In Zhenfeng County of Guizhou Province and Xianyou County of Fujian Province, indigo paste was the main source of income for local Han farmers. However, in Congjiang County of Guizhou Province, indigo paste served as a traditional model of national self-sufficiency. Both these models exist in Yuanyang County and Jinping County in Yunnan Province.

The different social roles of indigo paste lead to different social divisions of labor. Firstly, considering the gender of the indigo paste artisans, there were more men (81.3%/71.4%) engaged in indigo paste production than women (18.7%/28.6%) in Zhenfeng County of Guizhou Province and Xianyou County of Fujian Province, respectively. However, in the traditional self-sufficiency model of Congjiang County in Guizhou Province, only women (100%) were engaged in the production of indigo paste. Men did not understand traditional knowledge relating to indigo paste production (Table 3). 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 that of men (11.4%). These results are consistent with previous findings. Traditional dyeing knowledge is transmitted matrilineally, and this knowledge is mainly mastered and used by women [43,44]. Furthermore, activities such as dyeing are considered unfavorable to men [45]. However, when indigo paste becomes a tradable commodity and generates economic benefits, men also become involved in this work [44], even the main labor force will get involved.

Secondly, considering the age of the indigo paste artisans, in the Congjiang County, young women aged 30–49 years (50.4%) were mainly 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 (Zhenfeng 68.8%/Yunnan 54.3%/Fujian 76.2%), while young women aged 30–49 years (Zhenfeng 28.1%/Yunnan 25.7%/Fujian 9.5%) were relatively rare. Overall, young people aged 30–49 years (35.7%) and middle-aged and elderly people aged 50–69 years (52.3%) had extensive knowledge and artisanship of indigo paste production. However, previous published reports indicate that most traditional knowledge was usually held by the elderly, while young people did not understand [9,46].

Quality judgment indices

Generally, the blue hue in indigo paste comes from indigo, and the purple-red luster comes from indirubin [47]. Thus, the ratio of the indigo and indirubin content will determine 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, the people preferred purple-red indigo paste to pure blue indigo paste. In other words, the quality judgment indices revealed that the indirubin content was more important than the indigo content. Although these findings differ from existing synthetic indigo quality judgment standards, they agree with reports of indigo paste quality judgments in ancient Chinese books. For example, there is a record in "Liping Fuzhi"(Guizhou): "[illegible]", which means that the purple indigo paste is the best. There is also a descriptio in "Dyeing Sutra": "[illegible]" [50], which means that the best quality indigo paste should have dark blue and red lust In addition, the Hainan Li and Miao people think that dark blue and reddish indigo paste is of better quality [46]. Therefore, the indirubin content in the indigo paste is not only a by-product, but also has a decisive effect on the quality of the indigo paste.

In the indigo paste production process, many factors affect the quality [8,22,51], one of which is 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 was then rubbed in the soaking liquid to produce a fine lime slurry that flowed out of the bag. This method produced very delicate lime particles and greatly reduced 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 was added and mixed, and then poured directly into the soaking solution (Fig. 5); thus, they ignored large lime particles and impurities. This might be why folk rubbed their fingers together to check whether the indigo paste was smooth. The second factor is the amount of lime added. In the production process, adding too much or too little lime resulted in a low-quality indigo paste. In addition to the indigo yielding plants, the taste of indigo paste was mainly related to the amount of lime added during the production process, and the folk judged whether lime was added properly based on the taste. However, in the interviewing process, the interviewees' descriptions of taste were different and even opposite to one other. There are two possible reasons for this difference: one is that the taste description is mainly influenced by personal subjectivity, and the other is that interviewees have different perceptions and descriptions of taste due to cultural divergence. This phenomenon also occurred in the use of the color index. Informants described good quality indigo paste as being red in color, even though it was purplish red (Fig. 6).

Verification of traditional 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 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. The better the quality the indigo paste, the higher the content of indigo and indirubin, especially indirubin. This confirmed that the color of the indigo paste was a reasonable quality judgment index. However, due to the limitations of the experimental samples, the active ingredients range of the indigo and indirubin components and the color distribution range of different grades of indigo paste cannot be identified by this research which need to be further studied.

By drying the samples in the laboratory, we observed that there were no significant differences in the color and gloss of indigo paste in its wet state, except in the very good and very poor samples. However, the color difference was obvious after drying and removing water (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. Likewise, after drying different indigo pastes, the inside of the block had a different appearance. Some indigo pastes had a uniform internal color, and no obvious lime particles or impurities were observed. In other indigo pastes, however, there were varying amounts of white or other colored particles (Fig. 7). Thus, from an experimental perspective, the touch index was also necessary for assessing the quality of the indigo paste.

Conclusion

Although indigo paste has withdrawn from the stage of history, it is still frequently used in parts of China. Although indirubin has always been considered a by-product of indigo paste, our study revealed the importance of indirubin for assessing indigo paste quality by recording the various quality judgment indices used by traditional folk, which was then verified by current scientific methods. Our findings have guiding significance for the modern development of indigo paste production. Moreover, our research

proves that traditional knowledge has a scientific basis. Traditional knowledge that has accumulated thousands of years of wisdom is a huge treasure house for humankind.

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 analyzed the data and prepared the manuscript with assistance from SL. 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

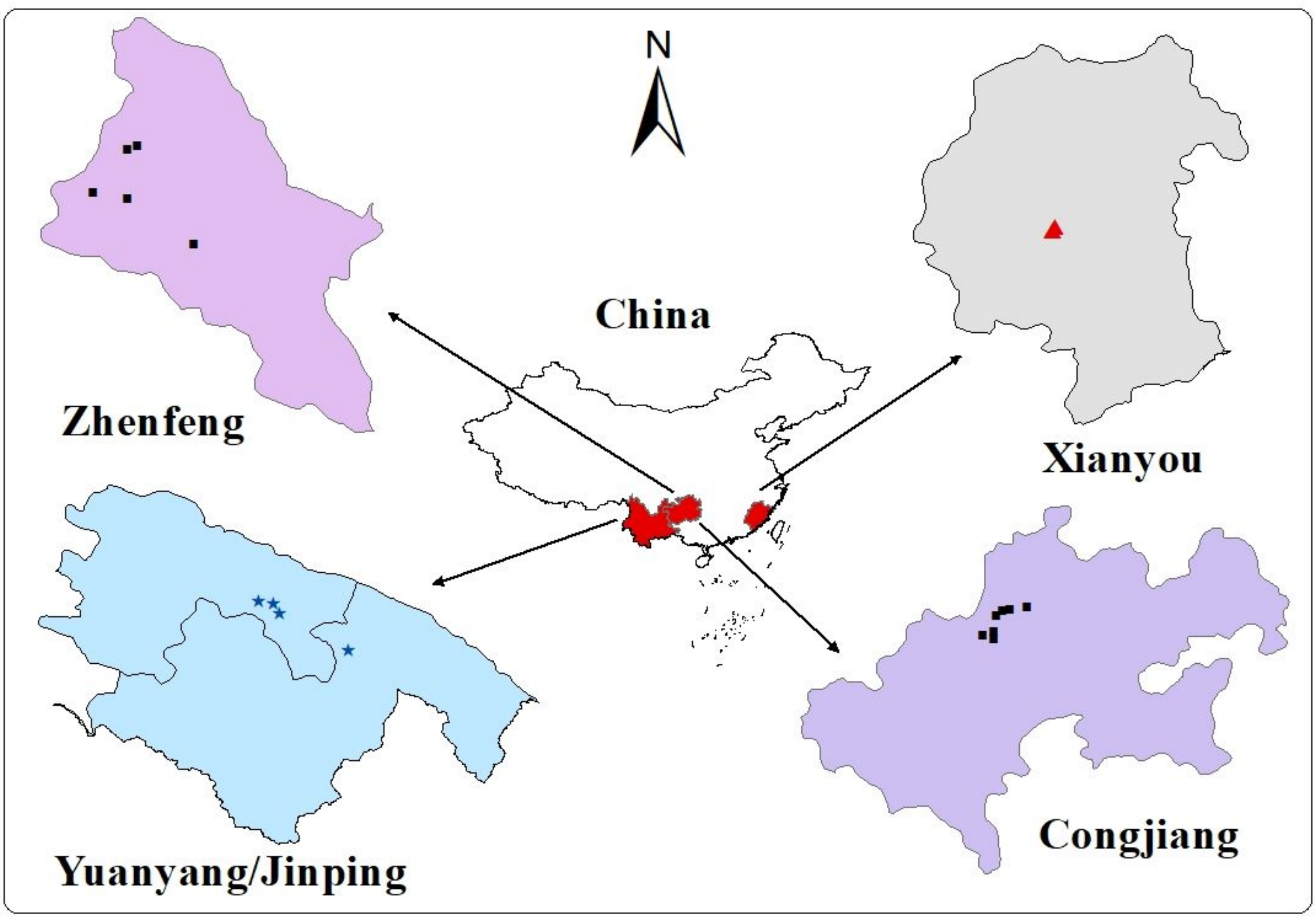
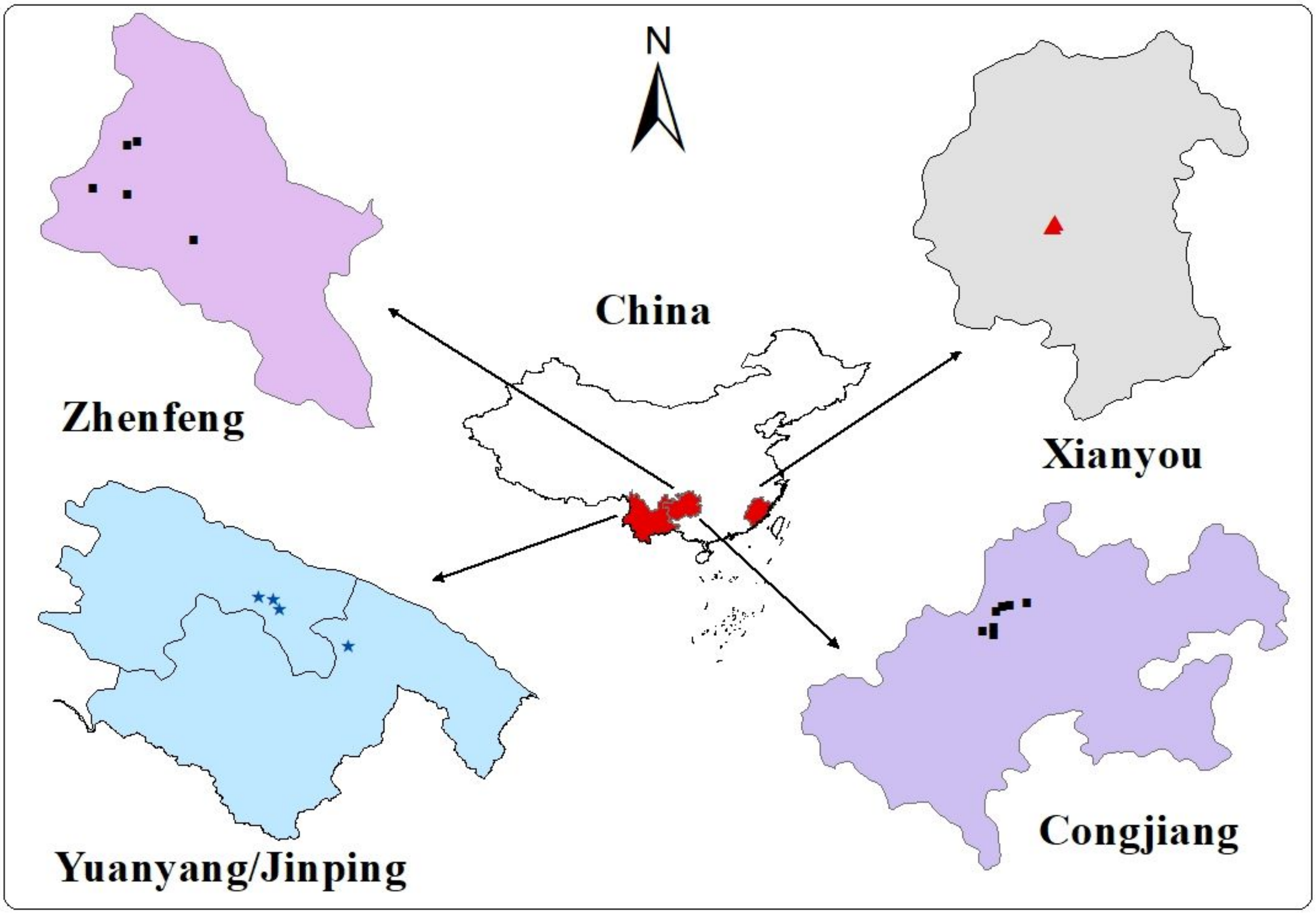


Figure 1

Three markets and fifteen 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.



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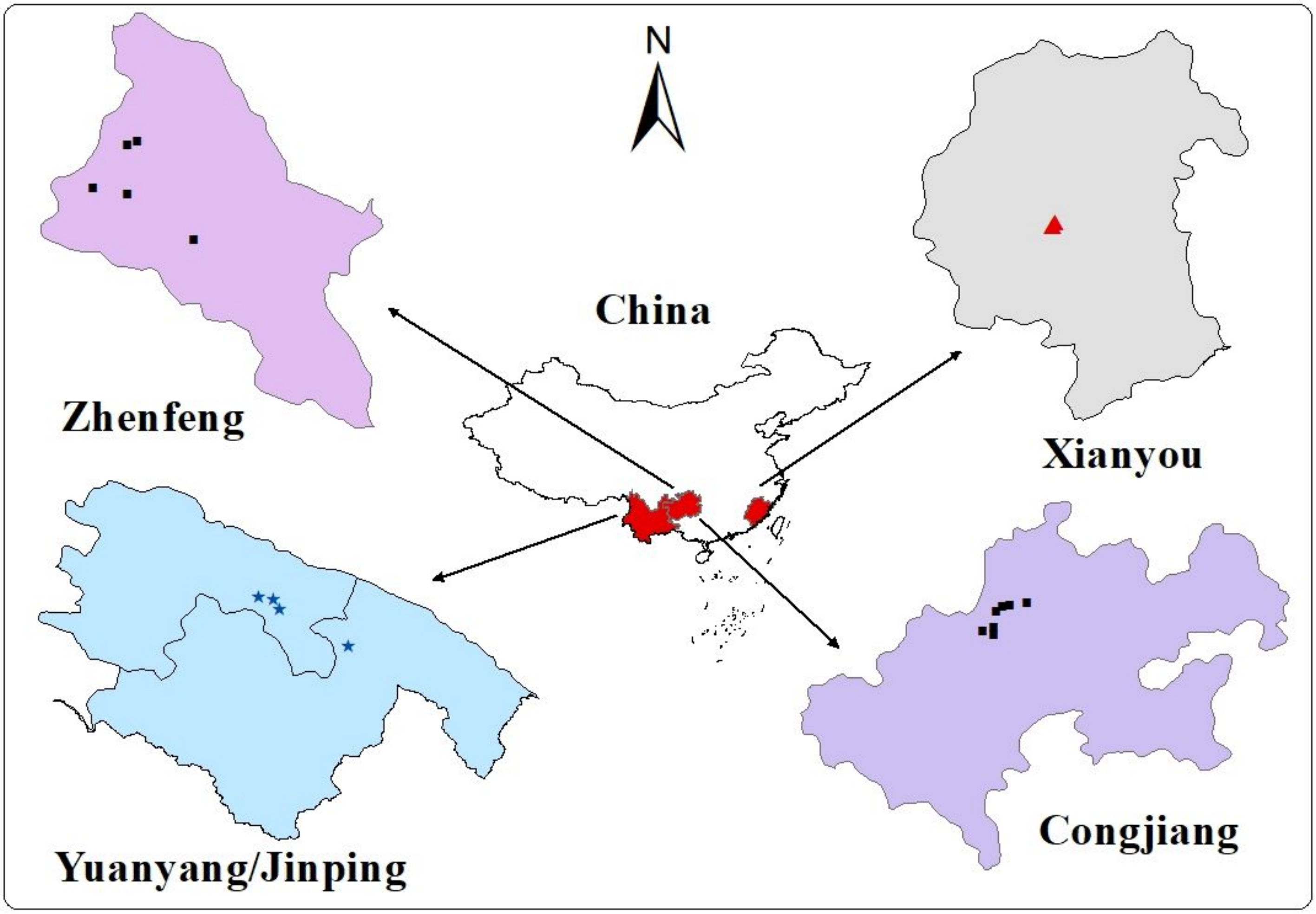

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Figure 2

Different folk quality evaluation methods. a b Methods of color. c d Methods of Tactile. e Methods of Taste. f Method of dyeing ability. 1,2,3,4 and 5 represent the five folk quality grades of indigo paste, which are best, good, general, poor and worst respectively.

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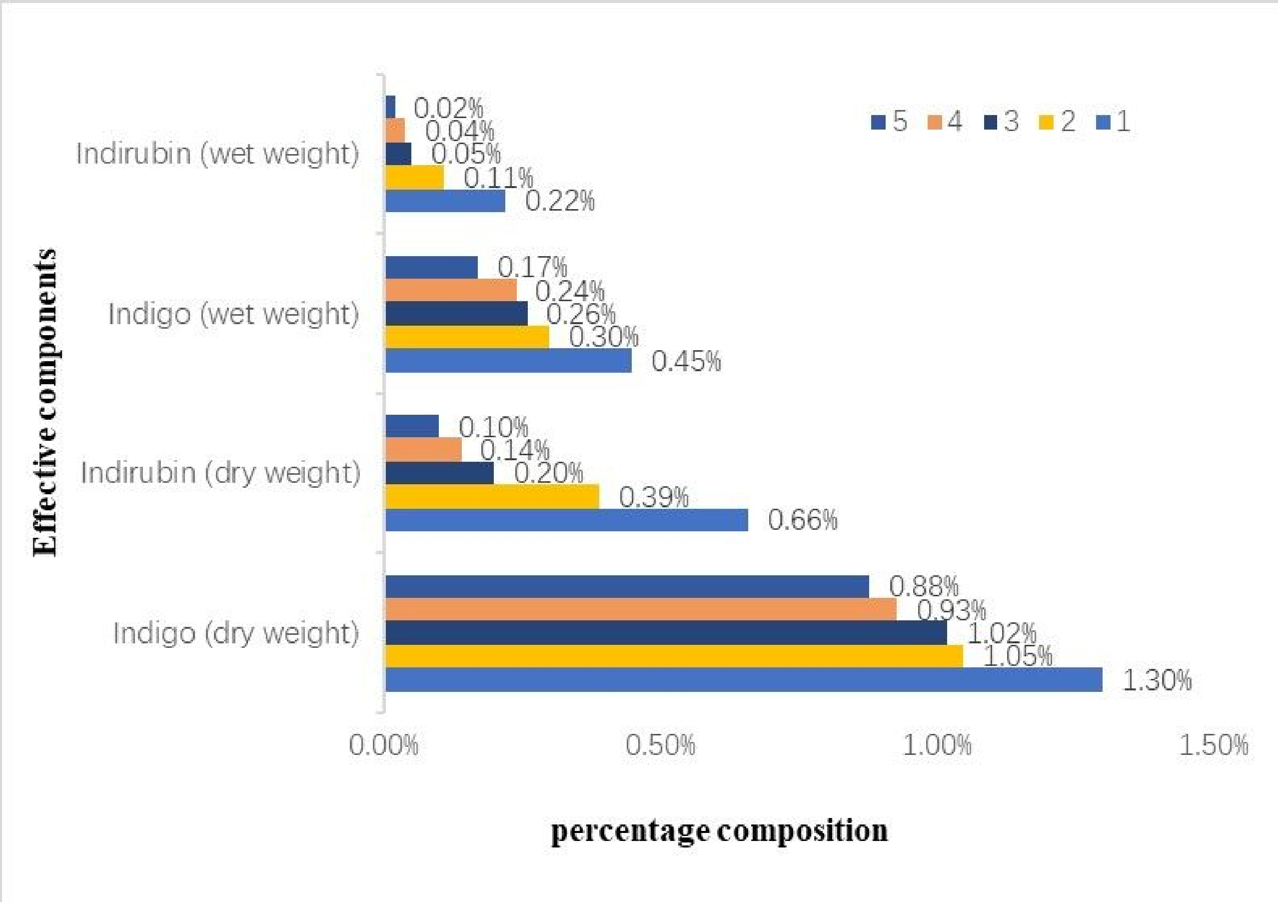
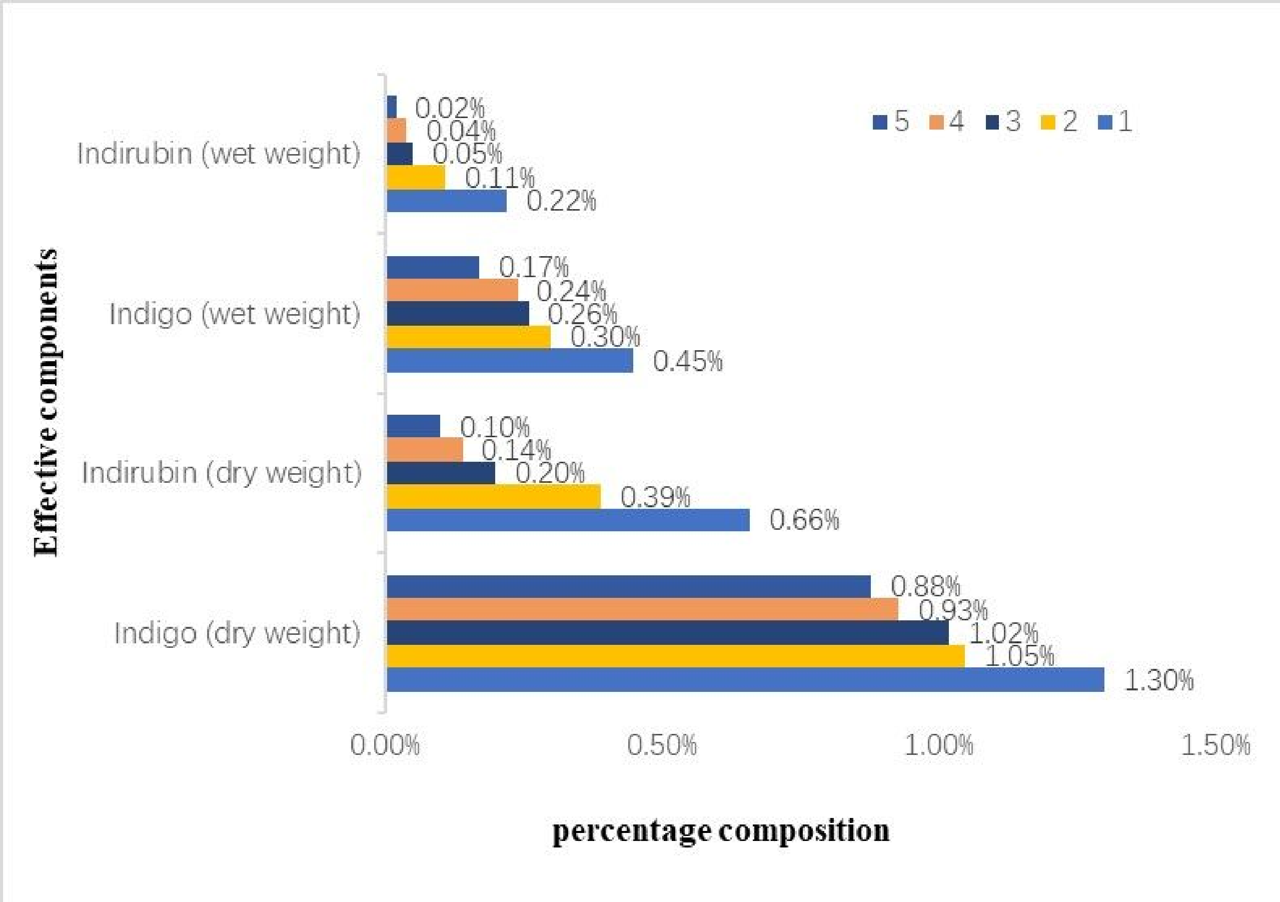


Figure 3

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



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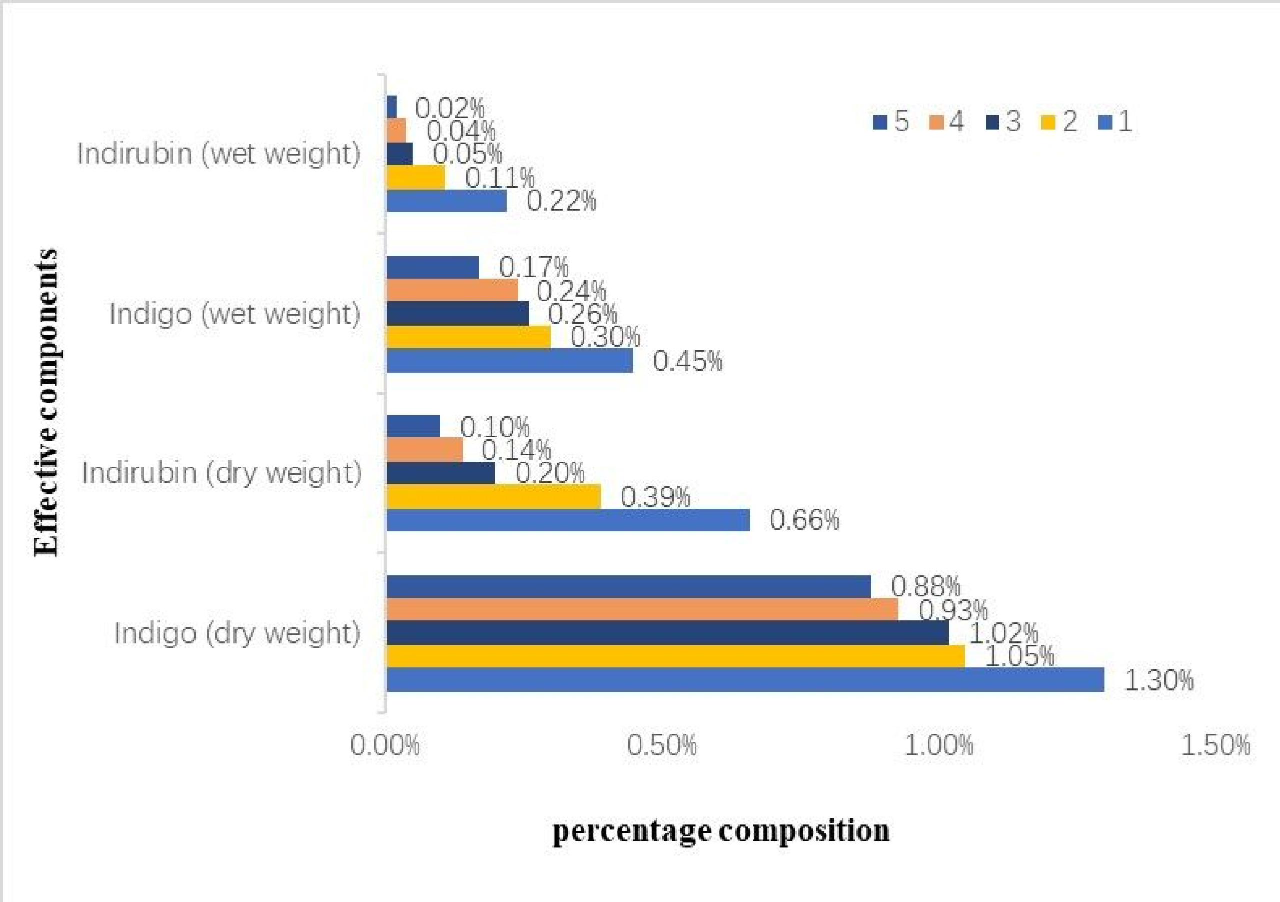

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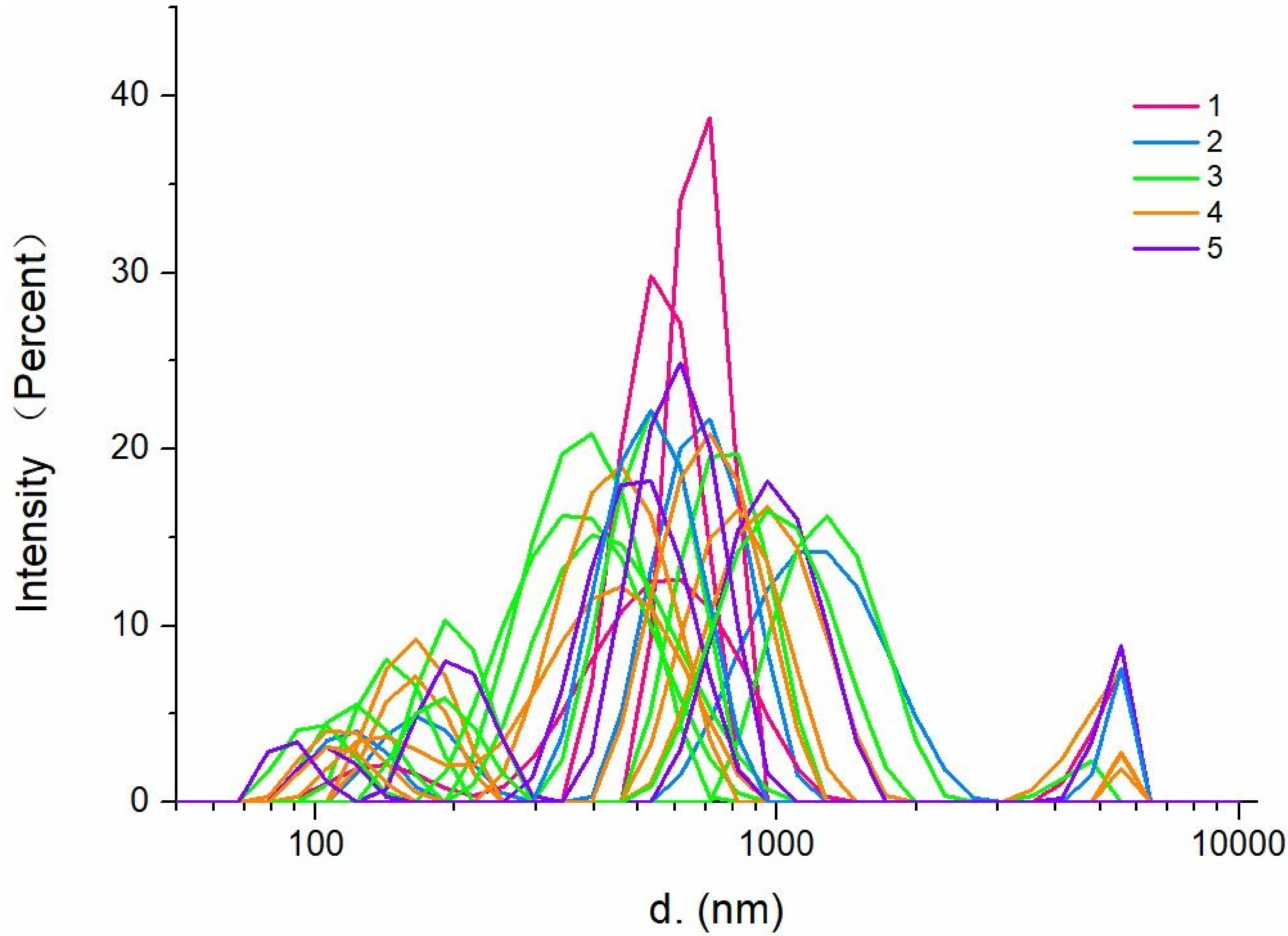
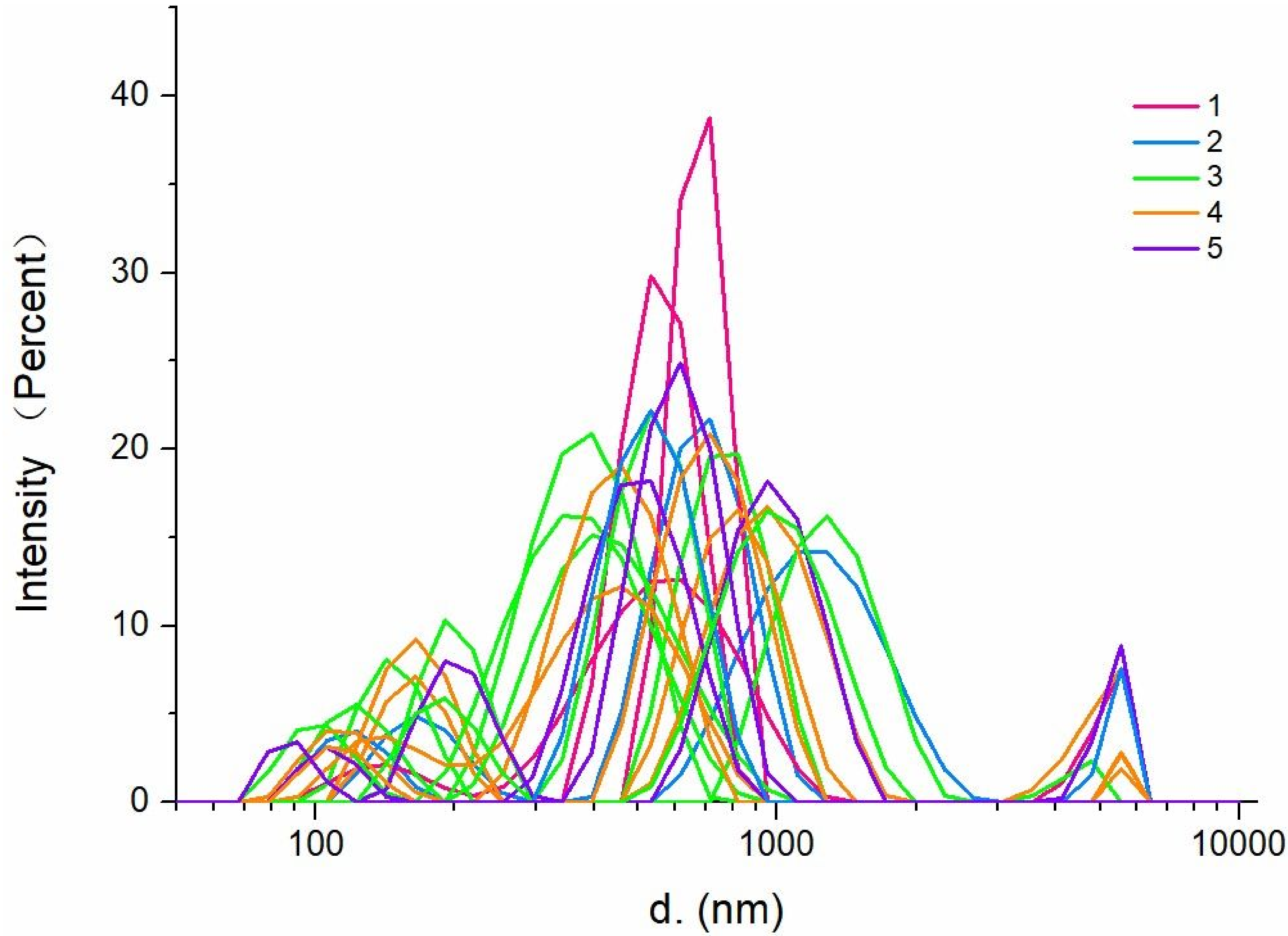


Figure 4

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



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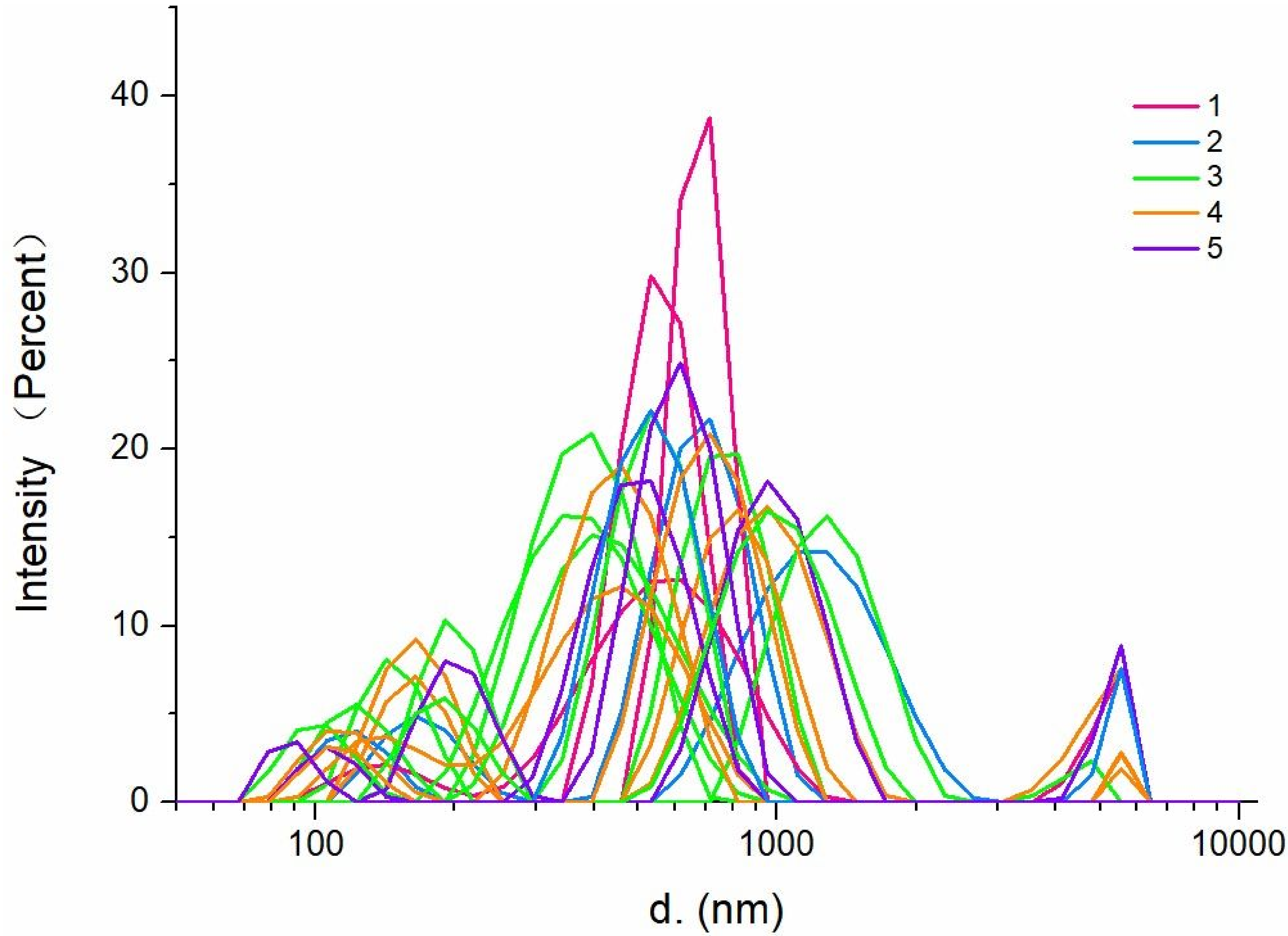


Figure 4

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

Figure 5

Different ways of adding lime.

Figure 5

Different ways of adding lime.

Figure 5

Different ways of adding lime.

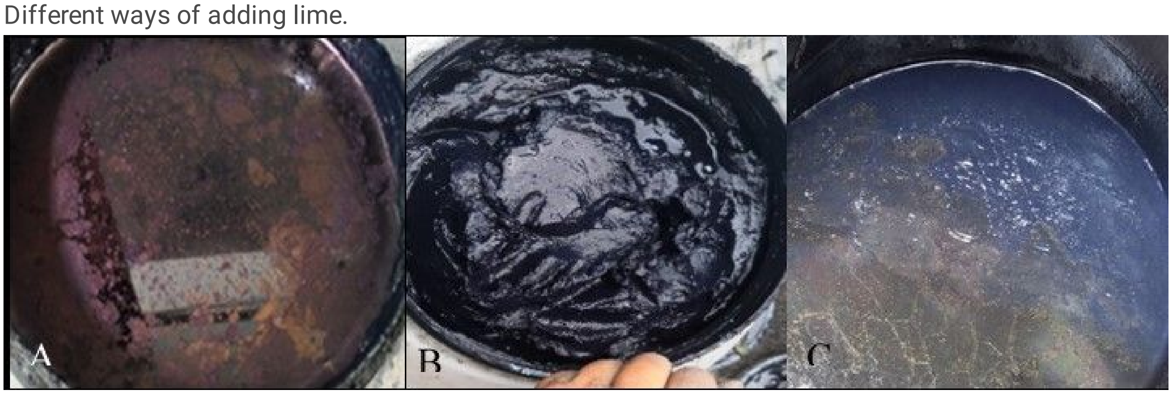


Figure 6

Different colors of the wetting Indigo paste.

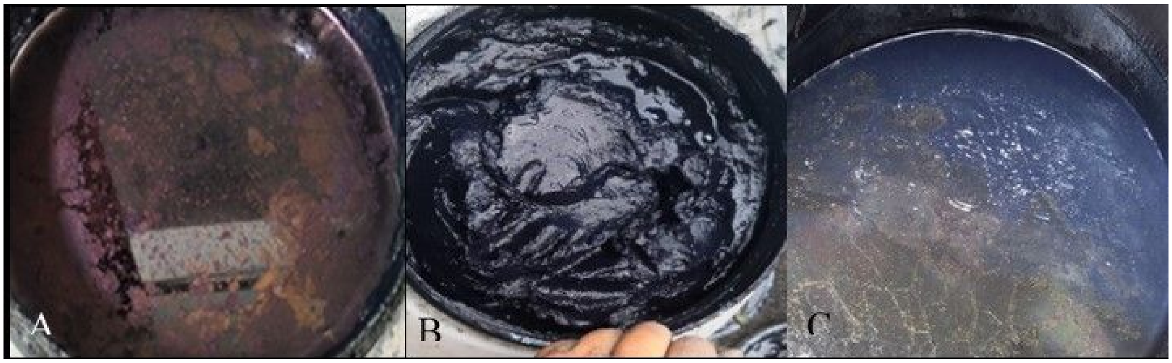


Figure 6

Different colors of the wetting Indigo paste.

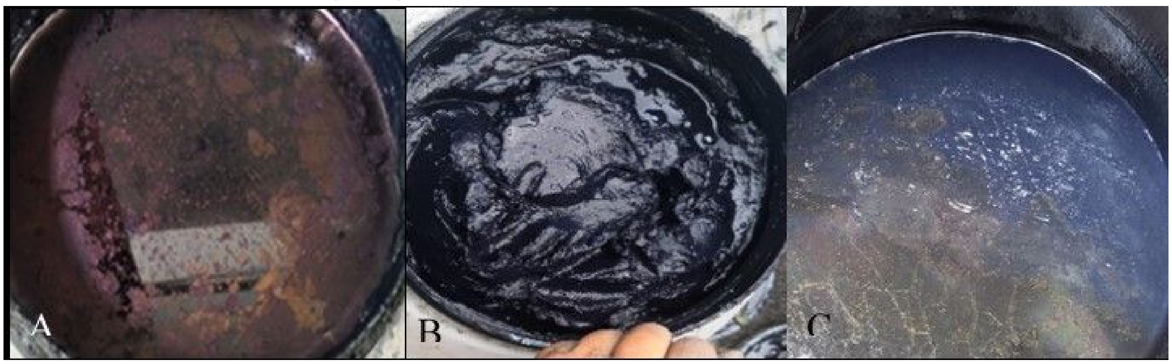


Figure 6

Different colors of the wetting Indigo paste.

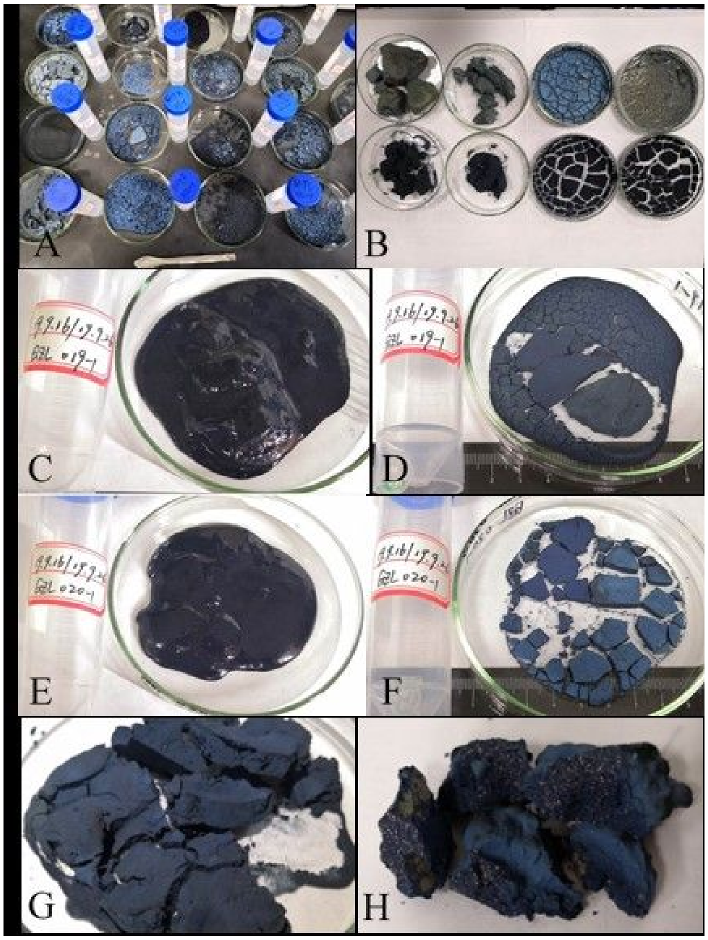
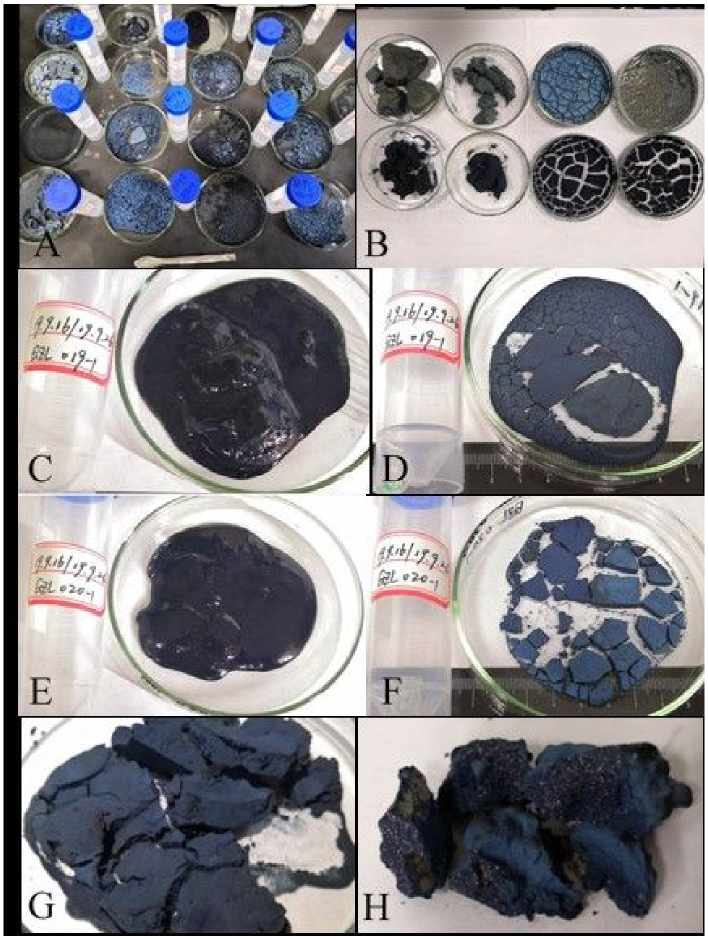


Figure 7

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



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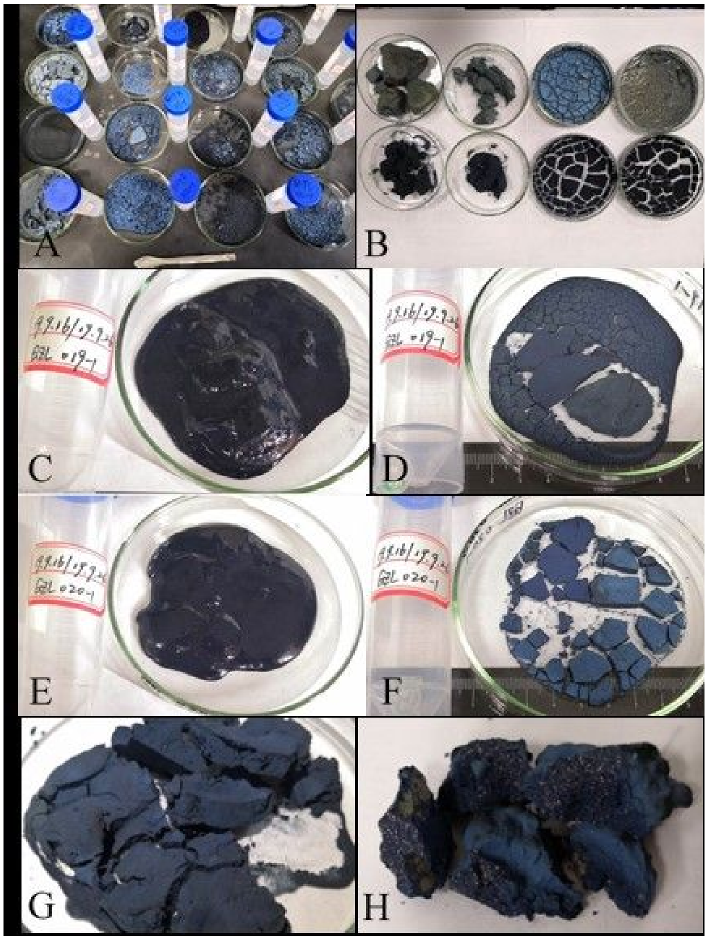

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Supplementary Files

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- Fig.31.jpg.pptx
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- Fig.32.xls
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