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Black, Red and White: Characterization of Painting Materials on a Group of Bwa Masks from Burkina Faso

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Abstract: The distinctive and visually striking wooden masks associated with the Bwa culture in Burkina Faso, West Africa, are carved from a soft wood into different shapes and display various geometrical patterns and symbols according to their purpose. One of their characteristic features is the use of the colors black, red, and white, which evoke the three major rivers crossing the country: the Black, Red, and White Voltas. According to published accounts of scholars who have worked directly with the artists, the materials used to obtain these colors include reptile excrement for the white, iron-rich stones powdered and mixed with egg or plant gums for the red, and boiled Acacia seed pods for the black, as well as modern materials such as enamel paint in some cases. A group of four Bwa masks in the Arts of Africa collection of the Art Institute of Chicago was investigated using a complement of analytical techniques including Fourier transform infrared spectroscopy, scanning electron microscopy with energy dispersive spectroscopy, pyrolysis gas chromatography–mass spectrometry, and mass spectrometry-based proteomics to characterize their painting materials. The results obtained corroborate the published accounts, while also providing new insights into the nature of the coloring materials and the selection and substitution of pigments and binders. These findings highlight the complementary value of scientific research, in combination with fieldwork and artists' accounts, to generate a fuller understanding and appreciation of this traditional artistic practice.

Keywords: Bwa; wooden mask; Burkina Faso; paint; mass spectrometry; proteomics; artists' materials

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1. Introduction

Burkina Faso in West Africa, known as The Republic of Upper Volta until 1984, has been described as the land of masks since many people in this country make and use them. Masks are used during the year for diverse occasions such as family members' initiation, cults, funerals, burials, annual rites associated with harvesting cycles, introduction in the village of a new mask, as well as during performances for the villagers, and nowadays tourists, during market days [1]. Masks can be made with different materials, such as leaves and grass, carved wood, and decorated with various patterns and colors. The several peoples living in this country belong to two main language families, mainly Mande in the west and Voltaic in the east. This distinction in language is also observed in the style of the masks produced: colorful three-dimensional masks for the Mande people, such as the Bobo, and primarily two-dimensional masks characterized by geometrical patterns and painted black, red and white, among the Voltaic people, such as the Mossi and Bwa [2].

Fundamental information about the production, materials, and meaning of masks within the different groups in Burkina Faso has been provided by Dr. Christopher Roy (1947–2019), former professor of African art history at the University of Iowa and curator at the Stanley Museum of Art (Iowa), who worked in Burkina Faso for over 40 years. With respect to the Bwa culture, while masks made of leaves and other plant materials represent nature, wooden masks are connected to individual families and village activities. These masks can resemble animals (such as antelope, hawk, snake, etc.) or human characters, or

can be more abstract; however, they are not meant to represent a particular natural being but to embody a spirit or supernatural force called to intervene for the family or clan on a specific occasion [3,4]. Bwa masks are made to be secured to the dancer's face with a fiber rope and attached to a body costume made of plant fibers such as hemp. The masks are usually carved during the dry season when less work is required in the fields. According to Roy's descriptions, most are made of wood from the *Ceiba pentandra* tree, also known as Kapok or silk-cotton tree, which is native to West Africa. This wood is very soft and light, which makes it suitable for carving and easier to wear, but at the same time, it is susceptible to insect attack. While the symbols used to decorate the masks and their meanings vary according to the family, village, and occasion of usage, Bwa masks are typically painted with the colors black, red, and white, which evoke the three major rivers crossing Burkina Faso: the Black, Red, and White Voltas. As stated by scholars who worked in Burkina Faso with Bwa carvers and artists, masks are traditionally painted with natural materials. Roy describes the use of a thick black paint prepared by boiling the seed pods of the *Acacia nilotica* tree, and also mentions a thinner black made by mixing powdered charcoal with an egg binder. Red pigment is obtained by grinding iron-rich stones, such as hematite, which is then mixed with a binder such as egg or Acacia plant gum. White is traditionally obtained in the form of excrement of lizards or snakes, but the use of schoolroom chalk is also mentioned. The use of enamel paint in place of the more traditional materials, both for newly made masks and to repaint older ones, has been reported [1,5]. The repainting process takes place usually every year and is preceded by immersion of the mask in a river or swamp for several weeks in order to kill the insects that easily attack the soft wood. This process also removes much of the red and white paint, while the black paint, according to Roy's description, is water-insoluble and remains on the surface [1].

The aim of the research presented in this paper was to characterize the painting materials of four Bwa masks in the Arts of Africa collection of the Art Institute of Chicago (AIC): a Fish mask (AIC #1958.116) from the late 19th-early 20th century; a Plank mask, also called Luruya (AIC #2000.313), from the same time period [6]; a butterfly-shaped Plank mask (AIC #2008.190) and a Hawk mask (AIC #1970.103), both dated early-mid 20th century (Figure 1). All four masks show the characteristic geometric pattern of wooden Bwa masks and most of their surface is still covered with the typical black, red, and white paint. The paint materials were investigated using spectroscopic and mass spectrometric analyses. The nature of the inorganic compounds was characterized by scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) and Fourier transform infrared spectroscopy (FTIR) which, together with pyrolysis gas chromatography–mass spectrometry (Py-GCMS), allowed also to characterize the organic compounds. Bottom-up proteomics using liquid chromatography–tandem mass spectrometry (LC-MS/MS) was performed to further characterize the nature and biological origin of the protein-based materials detected in some of the paints. Although micro-invasive, requiring microscopic samples for analysis, this strategy was selected in order to achieve a high degree of specificity in the materials' characterization, especially with regard to the organic components.

During this research we benefited in particular from discussions with Abdoulaye Ouédraogo, an artist from Boni, Burkina Faso, with extensive experience in the preparation of the masks following traditional methods; his detailed comments on the painting materials are provided as an appendix to this paper. While this information, and the accounts of scholars working in Burkina Faso, was crucial to inform this project, to our knowledge no complementary scientific studies have been conducted so far to characterize the painting materials. In addition to corroborating artists' accounts and observations made in the field, this research was undertaken with the aim to provide more precise insights into the nature and composition of the pigments and media, and the choices made in their selection. An additional impetus for scientific analysis to document the traditional materials and techniques is the increased interest and market for Bwa masks in Western museums and collections, which may influence the methods used for their production, leading to greater use of more readily available or modern materials [7].

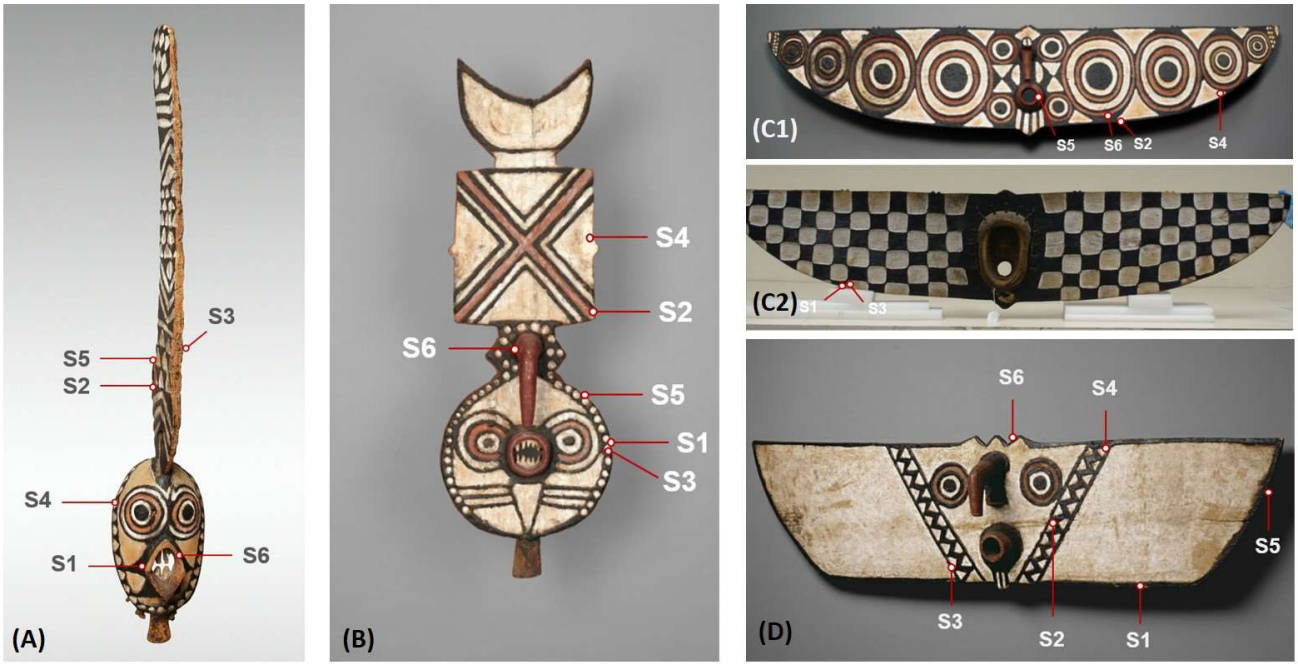

Figure 1. (**A**) Fish mask, AIC #1958.116, late 19th/early 20th century, 114.3 cm (45 in.) height, Mrs. Chauncey B. Borland Fund; (**B**) Plank Mask (Luruya), AIC #2000.313, late 19th or early 20th century, 63.5 cm (25 in.) height, gift of Joan Faletti Scottberg, the Faletti Family Collection; (**C1**) front and (**C2**) back of a Plank Mask, AIC #2008.190, early/mid 20th century, 238.8 cm (94 in.) length, Charles H. and Mary F. Worcester Collection Fund; (**D**) Hawk mask, AIC #1970.103. early/mid 20th century, 127.6 cm (10 1/4 in.) length, African and Amerindian Art Purchase Fund. All masks are from Burkina Faso. Sampling locations are indicated.

2. Materials and Methods

2.1. Samples from Masks

Samples in the order of tens to hundreds of micrograms were taken from the painted surfaces of the objects, under magnification, using a surgical scalpel. The micro-samples were taken from inconspicuous areas, such as those showing pre-existing damage, and after careful examination using UV illumination to avoid non-original materials possibly added during conservation treatments. A list of samples is reported in Table 1, and sampling locations for the four masks are shown in Figure 1.

Table 1. List of samples collected from the two objects. PR = proper right, PL = proper left.

Object, Accession No.	Sample	Description
Fish mask, 1958.116	S1	Black paint, front, PR side of the mouth
	S2	Black paint, PR side, headdress, bottom
	S3	White paint, PL side, headdress, geometrical decoration
	S4	White paint, PR side, head, geometrical decoration
	S5	Red paint, headdress, back,
	S6	Red paint, PL side of the mouth
Plank mask, 2000.313	S1	Black paint, front, PL edge
	S2	Black paint, front, PL edge
	S3	White paint, front, PL edge
	S4	White paint, front, PL edge
	S5	Red paint, PL side
	S6	Red paint, front, PR side protuberance

Table 1. *Cont.*

Object, Accession No.	Sample	Description
Plank mask, 2008.190	S1	Black paint, back, PL bottom edge
	S2	Black paint, front, PL bottom edge
	S3	White paint, back, PL, bottom edge
	S4	White paint, front, PL, bottom edge
	S5	Red paint, front, center
	S6	Red paint, front, PL, geometrical decoration
Hawk mask, 1970.103	S1	Black paint, front, PL, bottom edge
	S2	Black paint, front, center, geometrical decoration
	S3	White paint, front, bottom edge
	S4	White paint front, top edge
	S5	Red paint, front, PL side edge
	S6	Red paint, top center

2.2. Reference Materials

Seed pods from *Acacia nilotica* (collection no. 273504 and 620990) were provided by The Field Museum of Natural History (Chicago, IL, USA). Other *A. nilotica* seed pods were purchased from a private seller in India. A reference sample of excrement from *Calumma parsoni* (Parson's chameleon) was provided by Adriana Rizzo, Scientific Research Department, The Metropolitan Museum of Art (New York, NY, USA).

2.3. SEM-EDS

Portions of selected samples were deposited on carbon tape on an aluminum pin stub mount. A Hitachi S-3400N Variable Pressure SEM, equipped with an Oxford x-act silicon drift detector, was used. The parameters were the following: 25–30 kV accelerating voltage, 50 Pa pressure, and 50–80 probe current for EDS. SEM-EDS analyses were performed in the EPIC facility of the NUANCE Center at Northwestern University (Evanston, IL, USA).

2.4. FTIR

A portion of each sample was mounted on a Specac diamond compression cell. Analysis was performed with a Bruker Hyperion 3000 FTIR microscope with a mercury cadmium telluride D315 detector interfaced to a Tensor 27 spectrometer bench. Data were collected in transmission mode between 4000 and 400 cm^{-1}, resolution was 4 cm^{-1}, and 256–512 scans per spectrum were collected according to the sample response. Data were interpreted by comparison with published literature and databases.

2.5. Py-GCMS

A portion of each sample (a few μg) was placed in a micro vial, approximately 1.5 μL of a 2.5% solution of tetramethylammonium hydroxide (TMAH) in methanol was added, and the vial placed in an Agilent Thermal Separation Probe (TSP). A separate portion of the white sample S4 from mask 1950.116 was treated with approximately 1.5 μL of Hexamethyldisilazane (HMDS). The gas chromatograph is an Agilent 7890B, equipped with an Agilent HP-5ms Ultra Inert column (30 m, 0.25 mm i.d., 0.25 μm film), and interfaced to a 5977B MS. Helium was set at a flow of 1.2 mL/min. The TSP was inserted into the multimode inlet of the GC, operated in splitless mode, and ramped from 50 °C to 450 °C at a rate of 900 °C/min. The final temperature was held for 3 min and then decreased to 250 °C at a rate of 25 °C/min. The GC oven was programmed from 40 °C to 200 °C at 10 °C/min, then to 310 °C at 6 °C/min, and held isothermally for 20 min; for a total run time of 54.33 min. The MS transfer line temperature was 280 °C. The MS was run in scan mode (m/z 35–550 from 5–25 min, and m/z 50–700 from 25 min) with the ion source at 300 °C and the quadrupole at 150 °C.

2.6. Proteomics

2.6.1. Sample Preparation and LC-MS/MS Analysis

Each sample was incubated in 100 µL of an aqueous buffer containing guanidinium chloride (GuHCl) 2 M, tris(2-carboxyethyl)phosphine 10 mM, chloroacetamide 20 mM, and Tris 100 mM, for 3 h at 80 °C. A first digestion was performed by incubating the samples for 2 h at 37 °C with 0.2 µg rLysC (Promega, Sweden). The solution was diluted to a final concentration of 0.6 M by adding a Tris 25 mM in 10% acetonitrile (ACN) solution, and samples were digested with 0.8 µg trypsin (Promega, Sweden) overnight at 37 °C. The supernatant was recovered after centrifugation, dried in a vacuum centrifuge, and resuspended in 100 µL of 5% ACN/0.1% formic acid (FA) and the pH was adjusted to 2 by adding FA. Peptides were desalted on C18 spin columns (Pierce), dried in a vacuum centrifuge, and resuspended in 30 µL of 5% ACN/0.1% FA prior to analysis.

The extracted peptides were analyzed by LC-MS/MS using a DionexUltiMate 3000 Rapid Separation nano-LC coupled to a Q Exactive HF (QE) Quadrupole Orbitrap mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA). 2 µL of the peptide solution was loaded onto a trap column, 150 µm × 3 cm in-house packed with 3 µm ReproSil-Pur® beads (New Objective, Inc., Woburn, MA, USA), followed by the analytical column (75 µm × 10.5 cm PicoChip column packed with 3 µm ReproSil-Pur® beads). The flow rate was 300 nL min^{-1}, solvent A was 0.1% FA in water and solvent B was 0.1% FA in ACN. Peptides were separated on a 120 min analytical gradient from 5% ACN/0.1% FA to 40% ACN/0.1% FA. The mass spectrometer was operated in data-dependent mode, the source voltage was 2.40 kV and the capillary temperature was kept at 275 °C. MS1 scans were acquired from 375–2000 m/z at 60,000 resolving power and automatic gain control (AGC) set to 1×10^6. The top fifteen most abundant precursor ions in each MS1 scan were selected for fragmentation in the ion trap. Precursors were selected with an isolation width of 1 m/z and fragmented by collision-induced dissociation at 30% normalized collision energy. Previously selected ions were dynamically excluded from re-selection for 60 s. The MS2 AGC was set to 3×10^5.

2.6.2. Data Analysis

Raw data were processed with the MaxQuant software [8] version 2.0.3.0. Peptide spectra were first searched against a wide database (SwissProt from Uniprot, January 2017) [9]. Following these results, MS/MS spectra were searched against more restricted databases: peptides from the red paint samples were searched against a database including all the birds under the order Galliformes (downloaded from Uniprot, April 2023), while peptides from the white paint sample S3 from mask #1958.116 were searched against different databases containing protein sequences from birds (infraclass Neognathae), snakes (infraorder Serpentes) and iguana lizards (family Agamidae) (downloaded from Uniprot, February 2023). The database search was performed by setting the following modifications: carbamidomethylation (C) as fixed modification; methionine (C) oxidation, deamidation of glutamine (Q) and asparagine (N), conversion of N-terminal Q and glutamic acid (E) to pyroglutamic acid (pyro-E) as variable modifications. Trypsin was set as the digestion enzyme. Peptides were searched with a maximum of two missed cleavages, the minimum peptide length was set at 7, the minimum score for both modified and unmodified peptides was set to 40, and the protein false discovery rate (FDR) was set at 1. The contamination.fasta provided by MaxQuant, which includes common laboratory contaminants ("MaxQuant Downloads -contaminants.fasta"—http://www.coxdocs.org/doku.php?id=maxquant:start_downloads.htm (accessed on 4 June 2018)), was used to assess contaminant proteins, which were omitted from further analysis. Proteins were considered as confidently identified when two unique and non-overlapping peptides were at least observed unless differently specified. Each peptide was searched against the entire NCBI protein database using the pBLAST alignment tool [10] and considered species-specific only when it could be assigned to a single species, or a limited number of species among which only one could be probable, depending on the

geographical origin and date of the material investigated. A different taxonomic category above the species level (e.g., genus, family, etc.) was reported when species specificity was not available.

3. Results

Direct observation of the masks' painted surfaces showed that the three colors were likely applied in different manners. The white paint layers appear thin, underbound, and of powdery consistency, with the wooden support often visible beneath (Figure 2, left). The red paint also appears matte and thinly applied (Figure 2, right). The black paint, in contrast, was thickly applied, creating a raised texture. It appears dense and heavy-bodied, and either matte or glossy depending on the mask and area of application (Figure 2). Residues of what look like plant fibers are often visible embedded in the paint (Figure 2, left). Inspection of the masks' surfaces under magnification, together with observations made during sampling and investigation of the samples under the microscope, suggested that the painting material, for all three colors, was applied directly on the wooden support without any preparation layer.

Figure 2. (**left**) Detail of white and black paint, lower PR front side (1970.103), with plant fibers indicated by an arrow, and (**right**) detail of red and black paint, front side (2008.190).

All paint samples were first screened by FTIR for the characterization of both the inorganic fraction, which was further investigated by SEM-EDS, and the organic component. Py-GCMS was used to better elucidate the composition of the organic material, and bottom-up mass spectrometry-based proteomics was specifically used to determine the nature and biological origin of any proteinaceous materials detected by FTIR or Py-GCMS. The results obtained for each color are reported below, and discussed in more detail in Section 4.

3.1. Black

Under microscopic examination, samples taken from the black paint areas of all four masks appeared instead a homogeneous, translucent brown color, and without any discernible black particles (Figure 3A). FTIR analysis showed very similar profiles, and comparison with published databases suggested the black material to be a type of tannin, a plant-derived polyphenolic compound. Most bands in the spectra could be related to the FTIR profile of tannic acid, including the OH stretching of hydrogen bonds at 3300 cm^{-1},

the C=C stretching of the benzene ring at 1709 cm^{-1} (less intense in the samples' spectra than in the reference spectrum for tannic acid), the C–C stretching and C–H deformation of benzene ring at 1612 and 1535 cm^{-1}, a sharp band at 1447 cm^{-1} from the C–C stretching of the benzene ring, two intense and broad bands at 1348 and 1221 cm^{-1}, and another sharp band at 1033 cm^{-1} (Figure 3B) [11]. The spectra did not exhibit any additional bands attributable to the common classes of natural (e.g., protein, polysaccharide, or plant oil) or synthetic organic materials that might function as paint binders, suggesting that the paint is a relatively pure tannin-based material. Results of Py-GCMS analysis supported the FTIR data, showing a series of compounds consistent with plant tannins. A representative chromatogram, of sample S1 from the Hawk mask (1970.103), is dominated by the methyl derivatives of phenolic and polyphenolic compounds including gallic and ellagic acids, together with the methyl derivative of the cyclic polyalcohol myo-inositol (Figure 3C).

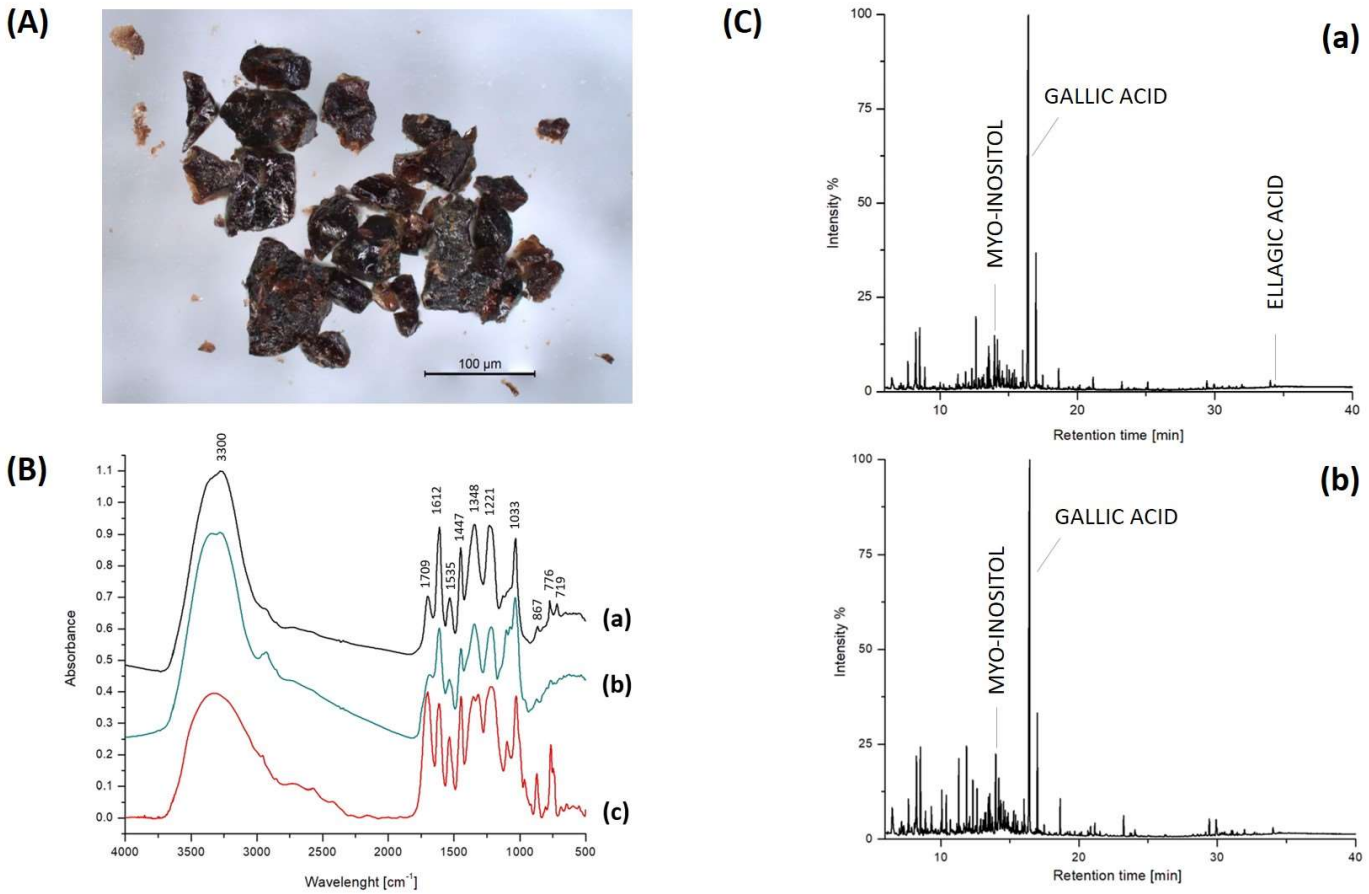

Figure 3. (**A**) microphotograph of black sample S1 from the Fish mask (1958.116) under visible light; (**B**) FTIR spectra of (a) black sample S1 from the Hawk mask (1970.103), (b) *Acacia nilotica* seed pod dried brown extract (8 h), (c) reference spectrum of a tannic acid (IRUG database, OD00186 [12]); (**C**) Py-GC/MS data (total ion chromatogram) of (a) sample S1 from the Hawk mask (1970.103), (b) *Acacia nilotica* seed pod dried brown extract (8 h).

While these analyses allowed us to determine the nature of the coloring material in the black paint, further experiments were undertaken to corroborate its source as reported in the literature. Following Roy's descriptions of the preparation of the so-called "thick black" paint [1], seed pods from *Acacia nilotica* were placed in a beaker, covered with distilled water, and boiled on a hot plate for 8 h (Figure 4A–C).

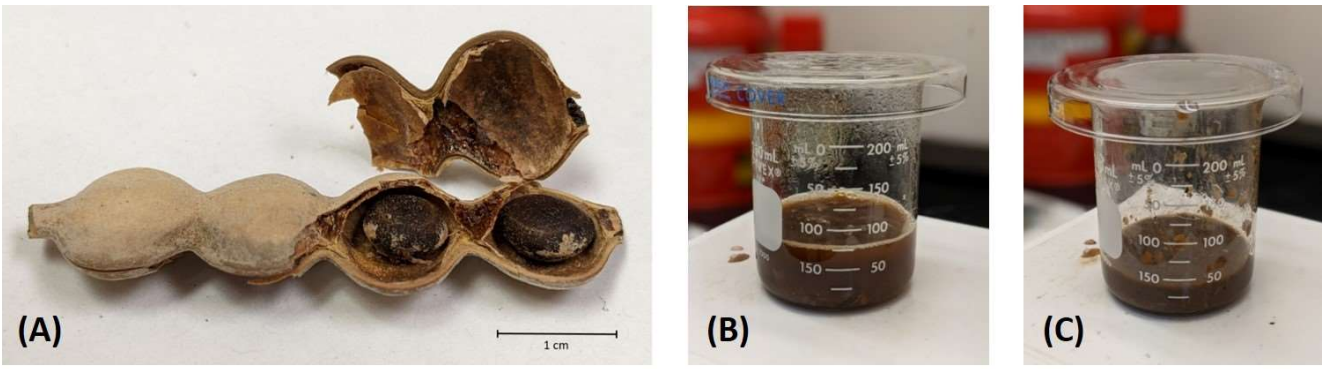

Figure 4. (**A**) *Acacia nilotica* seed pod from a private seller in India, (**B**) and (**C**) seed pods in water after 2 and 8 h of boiling, respectively.

An aliquot of the brown extract was then dried and analyzed by FTIR and Py-GCMS. Results were very similar to those obtained for samples from the masks, with the FTIR profile (Figure 3B) closely resembling that of tannic acid, and the methyl derivatives of gallic acid, myo-inositol and other hydroxybenzoic acids identified by Py-GCMS (Figure 3C). While these results do not allow us to specifically determine seed pods from *Acacia nilotica* to be the source of the black paint, since tannins are widespread in various types of plants, they nonetheless support the accounts of the source of the paint and its manner of preparation.

3.2. Red

In a red paint sample (S5) from the Fish mask (1958.116), aluminum (Al), silicon (Si), sulfur (S), phosphorus (P), and iron (Fe) were detected as major elements by EDS analysis, along with minor to trace amounts of sodium (Na), magnesium (Mg), chlorine (Cl), potassium (K), calcium (Ca) and titanium (Ti) (Supplementary Figure S1). This elemental composition suggests an iron-based pigment of natural origin, as indicated by the simultaneous presence of aluminosilicates with other minerals and salts. The phosphorus may be associated with the binder, as detailed below. FTIR analysis provided complementary information on the inorganic fraction and confirmed the presence of kaolinite ($Al_2Si_2O_5(OH)_4$), as indicated by bands including the characteristic doublet from OH stretching at 3694 and 3620 cm^{-1}, and hematite (Fe_2O_3) as highlighted by the two bands around 537 and 460 cm^{-1}. FTIR provided further information about the organic component: all spectra from the red samples showed characteristic bands of a protein-based material, such as the band at 1650 cm^{-1} (amide I), 1540 cm^{-1} (amide II) and 3075 cm^{-1} (amide II overtone), as well as features indicating a lipidic component, including two bands at 2928 and 2856 cm^{-1} from the asymmetric and symmetric CH stretching respectively, and a shoulder around 1733 cm^{-1} from the C=O stretching (see Figure 5A). The lipidic compound could indicate the presence of a plant oil or, due to the identification of a proteinaceous material in the same sample, egg yolk, which has a lipid content of about 30% [13].

Mass spectrometric techniques were used to further investigate the nature of the binding medium. Methyl derivatives of fatty and dicarboxylic acids, principally palmitic, stearic, azelaic, and oleic, were detected at different intensities by Py-GCMS in all red paint samples analyzed (Figure 5B). The ratio of palmitic to stearic acid (1.9), together with the low level of azelaic acid detected, is consistent with the presence of egg yolk [14], as hypothesized from the FTIR results.

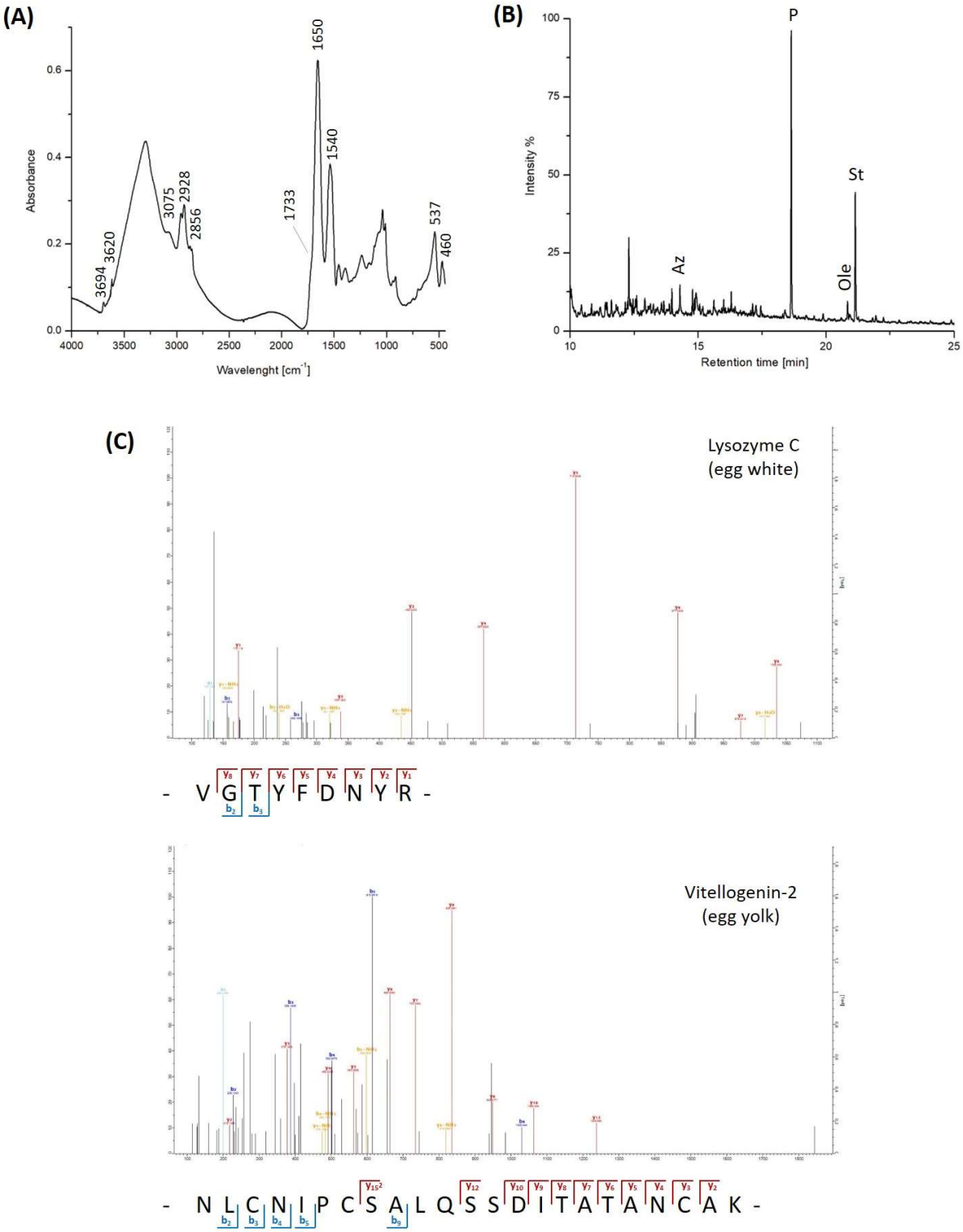

Figure 5. Data from the red sample S6 from the Hawk mask (1970.103) showing an increased level of specificity: (**A**) FTIR spectrum showing the presence of a proteinaceous and lipid material; (**B**) Py-GC/MS total ion chromatogram (Az = azelaic acid, P = palmitic acid, Ole = oleic acid, St = stearic acid) suggesting the presence of egg; (**C**) MS/MS spectra of peptides from vitellogenin-2 protein (top), indicative of egg yolk, and Lysozyme C protein (bottom), indicative of egg white, from *Numida meleagris* (helmeted guinea fowl).

The two S5 red paint samples from the Fish (1958.116) and Hawk mask (1970.103) were investigated by mass spectrometry-based proteomics to better characterize the nature of the proteinaceous material. MS/MS spectra were searched against a database including all birds under the order Galliformes (see Section 2.6.2). The full list of proteins and diagnostic peptides, supporting the identification of the proteinaceous material and species of origin, is provided in Supplementary Tables S1 and S2 respectively. In both samples, the most abundant proteins present in egg yolk, such as vitellogenin-1, vitellogenin-2, and apolipoprotein B [15]; and in egg white, namely ovalbumin, ovotransferrin, and lysozyme [16], were detected, together with several other less abundant ones. Investigation of each tryptic peptide by using the pBLAST alignment tool clearly indicated the taxonomic source of the whole egg proteins in the Hawk mask sample as *Numida meleagris* (helmeted guinea fowl) (Figure 5C). For proteins lacking in species-specific peptides, a higher classification was provided (e.g., *Neognathae*) (Supplementary Table S1). However, the sequences of these non-species-specific peptides still matched the helmeted guinea fowl ones and could therefore be assigned to this bird following the parsimony rule. Similar results were also obtained for the red sample from the Fish mask. Interestingly, in this sample, besides whole egg proteins from the helmeted guinea fowl, egg yolk and egg white proteins specific to the species *Meleagris gallopavo* (wild turkey), were also identified, although only the most abundant proteins and with a lower number of peptides than those of *N. meleagris*. Only one egg white protein (Ovalbumin-partial) specific to *Gallus gallus* (chicken) was detected. The absence of other abundant egg proteins from the same species suggested the finding to be most likely a contamination.

3.3. White

FTIR results for both white samples from the Hawk mask (1970.103) showed the use of gypsum ($CaSO_4 \cdot 2H_2O$), indicated by the band at 1130 cm^{-1} and a weaker band at 672 cm^{-1} from the sulfate group, together with the OH stretching vibrations at 3550 and 3410 cm^{-1}. Of the white samples from the two Plank masks (2008.190 and 2000.313), two were identified as gypsum (samples S3), and the other two as kaolinite (samples S4). The gypsum and kaolinite did not seem to be mixed together, suggesting that they may correspond to different campaigns of painting.

In contrast, FTIR spectra of the two white samples from the Fish mask (1958.11) showed the use of a different material: comparison with published spectra indicated the samples to be uric acid, or urate salts, as suggested by the N-H stretching bands in the 3600–2600 cm^{-1} range, the intense band at 1670 cm^{-1} corresponding to the carbonyl of urea groups with a less intense band at 1590 cm^{-1} from the carbonyl of conjugated amide, together with C-N vibration bands at 1120 and 1028 cm^{-1} [17,18] (Figure 6, left). This identification was supported by Py-GCMS analysis using both TMAH and HMDS as derivatizing reagents. When using TMAH, the chromatogram was dominated by the tetramethyl derivative of uric acid (m/z 82, 139, 224), together with an unidentified reaction product (m/z 70, 155, 211, 240) and a smaller amount of methoxy caffeine (m/z 209, 224), which derives from uric acid [19] (Figure 6, right). The tetra-TMS (trimethylsilyl) derivative of uric acid appeared as the most intense peak in the chromatogram obtained using HMDS (Figure 6, right). Furthermore, the backscattered electron (BSE) image of a portion of the Fish mask's white sample S4 showed the characteristic spherical shape of urate salts (Figure 7), and EDS analysis indicated the presence of potassium. Uric acid and its salts are characteristic of the excrement of reptiles and birds, animals that excrete nitrogen in this form rather than as urea, which is excreted by mammals [20,21]. Further supporting this attribution, FTIR analysis of a reference sample of excrement from the reptile species *Calumma parsoni* (Parson's chameleon) also showed a close resemblance to the spectra from the white pigment sample from the Fish mask and the uric acid reference (Figure 6, left).

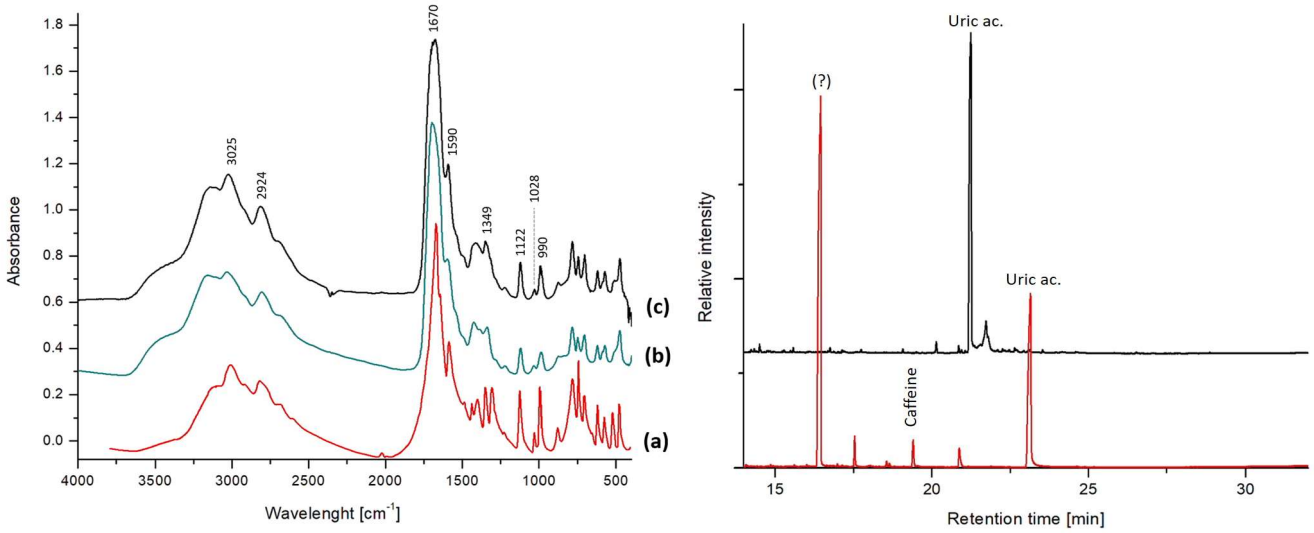

Figure 6. Data from the white samples from the Fish mask (1958.116): (**left**) FTIR spectra of sample S3 (c), compared with a spectrum of uric acid from the NIST database [18] (a), and of an excrement sample from species *Calumma parsoni* (Parson's chameleon) (b); (**right**) Py-GC/MS data (total ion chromatograms) for sample S4 obtained using TMAH (red) and HMDS (black); (?) indicates an unidentified reaction product.

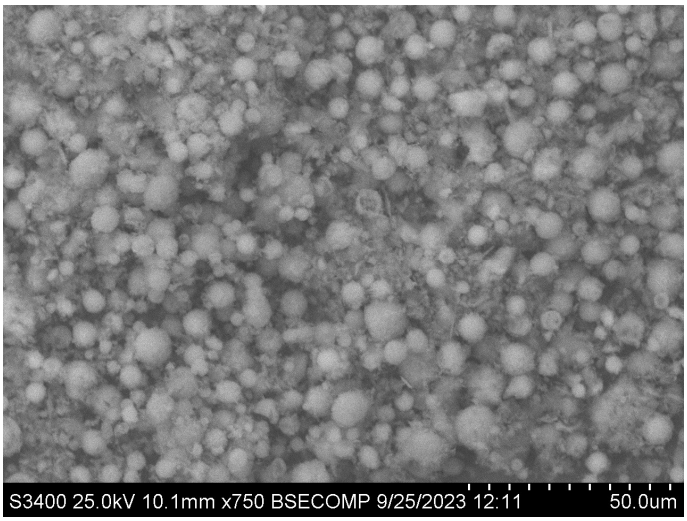

Figure 7. BSE image, sample S3 from the Fish mask (1958.116).

One of the white samples from the Fish mask containing uric acid (sample S3) was investigated by mass spectrometry-based proteomics to possibly determine the animal of origin. MaxQuant searches were performed against different databases containing all publicly available protein sequences from birds and reptiles (see Section 2.6.2). While no species-specific proteins were observed when searching the detected peptides against databases containing sequences from birds and snakes, results from the search against the Agamidae (iguanian lizards) database showed a match with two proteins of the species *Pogona vitticeps* (central bearded dragon), a lizard native of Australia, together with other non-species-specific proteins (see Supplementary Tables S3 and S4). The lack of a more geographically appropriate match is most likely due to the absence of sequenced proteins from African species in the databases, but the result nonetheless supports the interpretation that the pigment is derived from lizard excrement.

With regard to the characterization of the binding medium in the white samples, neither FTIR nor Py-GCMS analysis provided evidence for the presence of an organic material that could have been used as a paint binder, indicating that the pigments were applied directly in a dry form.

4. Discussion

The difference in appearance and thickness of the black, white, and red paint, observed in the first investigation of the four masks, is associated with the distinct properties of the pigments and plant products mixed, or not, with organic binders, and applied directly to the wooden support, without any preparation layer. Scientific analysis, informed by a conversation with artist Abdoulaye Ouédraogo (see Appendix A), corroborated and complemented the information reported by scholars on the use of different painting techniques for each of the three colors, and provided a deeper insight into the materials' selection and application.

According to Roy's descriptions, two different kinds of black paint are used on Bwa masks: what he called a 'thick black', obtained by boiling the seed pods from the *Acacia nilotica* tree, a synonym of *Vachellia nilotica* (L.) P.J.H.Hurter & Mabb. [22], a plant native to North Africa; and a 'thin black', prepared by mixing charcoal with a binder, such as egg [1]. The exclusive detection in the samples investigated in this study of tannins, and of no other organic binding media or black particles, indicated the black paint could be ascribed to the 'thick black' category. Tannins are polyphenolic secondary metabolites of higher plants, both gymnosperms and angiosperms. They are mainly present in bark, seeds, roots, and leaves, with the primary function of protecting the plant from microbial and fungal attacks [23]. Due to their property of binding and precipitating proteins, specifically collagen, they have been widely used since ancient times to prepare leather from animal skin [24,25]. In the art and archeological fields, tannins have also been used as writing and drawing material (iron gall ink) and natural dyes, often combined with metallic mordants for better stability [26]. From a chemical standpoint, tannins can be generally categorized into either condensed or hydrolyzable types, the former made of flavonoids linked together, and the latter characterized by a sugar core with ellagic and gallic acids linked to it [23]. The identification by Py-GCMS of both gallic and ellagic acid in the black paint samples (Figure 3C), and the absence of catechins, suggested the tannins present in the black paint to be hydrolyzable tannins and, in particular, the sub-category containing both gallotannins and ellagitannins.

While the chemical composition of the black paint and the seed pod extract prepared in our laboratory was comparable, the consistency and color of the two were significantly different, with the extract obtained after 8 h of boiling in water having a dark brown color instead of black. Information provided by Ouédraogo (Appendix A), and collected by Gaash during his time in Burkina Faso [5], helped to account for this difference and provided greater insight into the paint preparation process described by Roy. The *Acacia nilotica* seed pods are collected and crushed in their entirety, water is added in a 2:1 ratio, and the mixture is boiled over fire for several hours, or until the liquid turns a dark brown color. The mixture is filtered, and the brown extract is recovered and boiled again for several hours until it is reduced to a thick black liquid. This prolonged boiling and concentration of the extract allows the tannin-based liquid to turn into a thick black material usable as painting material. This product can be kept for up to 2 years and it is heated up again before use. The fact that the tannin-based extract is further concentrated to reach a tar-like consistency explains why the black paint appears textured and not flat, as in the red and white painted areas.

All red paint samples showed a consistent composition, characterized mainly by a mixture of an iron-oxide-based pigment and a protein-based binding medium. The leanly bound nature of the red paint explains its matte appearance. Iron-based stones and clays have been reported in the literature as a common source of red pigments in African sculptures and objects [27–30]. Ouédraogo confirmed that the red pigment is

obtained by grinding laterite, an iron-oxide-rich clay that can be locally found as both rock and soil. In the masks studied at AIC, no enamel paint was identified, even in the most recent examples. Ouédraogo himself mentioned that modern paints are used only if requested (Appendix A). With regard to the binding medium, complementary analytical techniques with different levels of specificity were used for its identification. The application of untargeted bottom-up proteomics was necessary to achieve a high degree of protein specificity, which is valuable when investigating artworks that have, until now, received little scientific attention. Proteomics suggested the use of whole eggs from the helmeted guinea fowl (*Numida meleagris*) and wild turkey (*Meleagris gallopavo*), which were found together in the red sample from the Fish mask. The helmeted guinea fowl is native to Burkina Faso (Appendix A) and genomic studies showed that it was domesticated in West Africa around 1300–5500 years ago [31]. The turkey is native to central Mexico, but during the Spanish colonization, it was imported to Europe and then distributed in the 17th century across other continents, including Africa [32]. Ouédraogo verified that, besides guinea fowls, eggs from other birds including turkey are also used for painting. The presence of whole egg proteins from both the helmeted guinea fowl and turkey on one of the masks might be evidence for two different painting campaigns, or could alternatively indicate the use of a mixture of eggs from the two birds.

Investigation of the white paints showed a greater variety of materials in the four masks. Gypsum, most likely from schoolroom chalk, as mentioned by Roy [1], was used to paint white areas of the Hawk (1970.103) and the two Plank masks (2008.190 and 2000.313). Kaolinite was also identified in one of the two samples taken from each Plank mask, suggesting the use of kaolin clay, which is widely available and used as a pigment in different regions of Africa [29,30,33,34]. The two inorganic compounds were not identified as a mixture, which might indicate that they represent different painting campaigns. In the white paint of the Fish mask (1958.116), FTIR, Py-GCMS, and SEM analysis instead showed the presence of uric acid and/or urates, consistent with the use of reptile or bird excrement. Both uric acid and its salts are found in the excrement of these animals, varying in relative quantity according to the animal diet and other factors [20]. The application of such material as a pigment on the surface of African sculptures and other objects, and for other purposes, has been reported in previous studies. Abraham recorded the use of snake excrement among the Tiv people in Nigeria, mixed with herbs, cooked and powdered, and used to bless, for example, hunters before a hunt [35]. Griaule described the use of a mixture of snake or lizard excrement, rice, and limestone, as a white pigment among the Dogon in Mali [36]. More recently, urate salts have been scientifically identified by time-of-flight secondary ion mass spectrometry (ToF-SIMS) in micro-samples from the accumulative surface of a wooden statuette from the Dogon [37]. This analytical technique was also used by the same authors to characterize the white pigment applied on a Dogon rock painting; ToF-SIMS results in this case showed the white pigment to be composed of uric acid [21]. In our research, mass spectrometry-based proteomics was applied to provide a more certain determination of the animal of origin. Proteomics allowed us to confidently exclude a bird's origin, and data suggested the excrement to come from a lizard species, based on the detection of peptides specific to *Pogona vitticeps*, a lizard native of Australia. The absence of a more geographically appropriate match is not surprising since only a few proteins have been sequenced up to now for indigenous, or lately introduced, reptiles of Burkina Faso [38], but is nevertheless convincing evidence of the use of excrement from lizards. Ouédraogo confirmed the traditional use of reptile excrement, and that materials such as kaolin and chalk are now commonly used as substitutes (Appendix A). The Fish mask (1958.116), which shows the presence of lizard excrement, is indeed one of the oldest masks of the group, dating late 19th–early 20th century. However, one of the two Plank masks (2000.313), which also dates from the same period, was found to be painted with gypsum and kaolin. It is possible that the inorganic materials were applied in subsequent painting campaigns of this Plank mask, representing a detachment from the traditional materials. It is notable that all of the areas of white paint on the four masks had a similar

visual appearance when examined for sampling, and that scientific analysis was essential for their discrimination, in order to shed light on these questions of the use of traditional materials and their substitutes.

Furthermore, the scientific analysis did not indicate the presence of additional organic materials in the white paints, suggesting that the pigment was applied 'dry', without any binding medium. Ouédraogo confirmed that the white pigment is not mixed with a binder: the white powder is wetted with water and applied to the wooden surface with the fingertip or a feather (Appendix A). The soft nature of the wood selected for the masks might promote the adhesion of the dry pigment to the surface, but the absence of a binding medium might also facilitate its removal during the yearly soaking of the masks in water. The absence of a binding medium explains the powdery consistency first noticed when observing the masks' surface and the fact that the wooden support is often visible beneath the paint.

5. Conclusions

The characteristic black, red, and white painting materials of four Bwa masks, made in Burkina Faso between the late 19th and 20th century, were characterized by micro-invasive spectroscopic and mass spectrometric analyses, with data interpretation informed by accounts in the published literature and by a discussion with artist Abdoulaye Ouédraogo. Scientific results showed that in most cases the paint materials on these masks are consistent with the accounts of artists and scholars, but they additionally provided a greater level of specificity, an improved understanding of the nature of the colored materials, evidence for different campaigns of paint of individual masks, and insights into the selection and substitution of materials.

A combined analytical approach including proteomics has proven again to be a crucial strategy when working with unfamiliar materials, since it provides us with precise information to better understand the availability of materials and the artwork's history, supplementing accounts derived from fieldwork.

While the process of making and painting the masks has been reported in some detail, our scientific analyses have corroborated the information and provided additional insights into the materials' nature and composition, which explained the unique appearance of the three colors. The black, red, and white paint is prepared using mostly traditional, locally available materials—derived from plant, animal, and mineral sources—and no modern paints, such as enamel, were identified in the examples studied. With the increased interest in the Western market for Bwa masks, we might expect a change in the material choices of artists; therefore the application of scientific analysis becomes even more significant for the ongoing study of these artworks, in order to better understand the persistence or divergence from traditional techniques.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app132212240/s1, Figure S1: EDS spectrum of red paint sample (S5) from the Fish mask (1958.116); Table S1: List of proteins identified in a red sample (S5) from Hawk mask (1970.103) and Fish (1958.116): protein name, taxonomy source, number of total and unique peptides, percentage of total and unique sequence coverage, sequence length and total number of identified MS/MS spectra; Table S2; List of species-diagnostic peptides identified in the red sample (S5) from Hawk (1970.103) and Fish (1958.116) masks: protein name, peptide sequence, species matching on the NCBI database, peptide length and mass, MaxQuant score, and total number of identified MS/MS spectra. Species in parentheses are unlikely according to the geographical origin of the artworks; Table S3: List of proteins identified in the white sample (S3) from the Fish mask (1958.116): protein name, taxonomy source, number of total and unique peptides, percentage of total and unique sequence coverage, sequence length and total number of identified MS/MS spectra; Table S4: List of species-diagnostic peptides identified in the white sample (S3) from the Fish mask (1958.116): protein name, peptide sequence, species matching on the NCBI database, peptide length and mass, MaxQuant score, and total number of identified MS/MS spectra.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Information from artist Abdoulaye Ouédraogo (Boni, Burkina Faso), summarized, with permission, from email exchanges with Clara Granzotto, April–September 2023.

The acacia used to make the black paint is the acacia nilotica. It is a very beneficial plant with several uses and is very important in tradition and in art. The acacia pods are boiled together with the seeds. Before boiling they are pounded or crushed to transform into powder and put in a large or small container depending on the quantity needed. The level of the powder must be at least 1/3 of the container used to boil it, the rest will be filled with water. The container and its contents are put on a fire. The mixture must be boiled for several hours (3 to 4 h if not more) until the liquid takes the color of coffee with milk (brown). We filter the boiled acacia to recover only the brown liquid. This liquid will be boiled again for hours. The time to boil the seed pods depends on the quantity of black paint needed, the average time is two days. As the liquid boils, it decreases in quantity and gradually changes color. It turns black. We will put the black liquid on a low heat to be very heavily concentrated. Once we prepare the black paint we do not have to use it quickly. It stays in liquid form for some time. The black paint can be kept for two years, it will be heated each time for use. It is certainly a very long and laborious activity, but there is a lot of symbolism during the preparation.

We commonly use white kaolin or white chalk for the white paint. The white pigment is applied with the end of the finger or with a feather. The pigment is wetted first before being applied on the wood surface. Generally the Bwa do not mix a binder with white

pigment. In the past, excrement from snakes and lizards was used. This is not a common practice nowadays.

There is a specific native lizard and snake we collect the excrement from. It is collected in nature (in caves).

The red pigment is obtained from a piece of laterite. It is crushed with fresh egg in a broken pottery. There are minerals available in our region. We do not buy mineral powder in shops.

The bird guinea fowl is native to the region. We have other birds, such as turkey, whose eggs are used.

These pigments and paints are chosen mainly for their practical properties and they also have cultural significance to the artists and community.

We don't use any modern paints, such as enamel, acrylic, etc., except on order.

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