Pozzi *et al. Herit Sci* (2020) 8:85
https://doi.org/10.1186/s40494-020-00431-x

Heritage Science

RESEARCH ARTICLE Open Access

Mixing, dipping, and fixing: the experimental drawing techniques of Thomas Gainsborough

Federica Pozzi[1*], Julie Arslanoglu[1], Francesca Galluzzi[2], Caroline Tokarski[2] and Reba Snyder[3]

Abstract

The Morgan Library & Museum, New York, owns twenty-five works on paper by the 18th-century English artist Thomas Gainsborough. Scholarly publications over the past 20 years, as well as Gainsborough's own writings, have highlighted his proclivity toward innovative methods and experimentation. In particular, a letter that the artist wrote in 1773 reveals details of his secret recipe for making oils on paper, such as his recommended use of lead white and the unorthodox practice of dipping his works in skim milk, possibly to prevent the pigments from discoloring. About a dozen of Gainsborough's creations were included in a 2018 exhibition at The Morgan entitled *Thomas Gainsborough: Experiments in Drawing*. On this occasion, an in-depth scientific study aimed to explore the artist's work as a draftsman, with a special focus on his mastery of materials, his technical innovations, and his development of an original approach to drawing. Initially, a selection of artworks was examined using magnification along with transmitted and raking light to improve surface visualization and to investigate the structure of each piece. Further photographic documentation with ultraviolet and infrared light was performed to gather preliminary information on the variety of white pigments employed, on the wet and dry chalk techniques used in certain works, as well as on the possible presence of coatings and underdrawings. Subsequently, scientific analysis by means of X-ray fluorescence (XRF) and Raman spectroscopies aimed at characterizing the white pigments present in The Morgan's drawings, which mostly consisted of calcite and lead white. Moreover, a combination of advanced micro-sampling tools, i.e. polyvinyl chloride (PVC)-free erasers and fine polishing films, *ad-hoc* sample preparation methods, highly sensitive proteomics analysis via nano-liquid chromatography/mass spectrometry (nano-LC/MS), and sophisticated bioinformatics data processing was employed to assess Gainsborough's use of skim milk as a "secret fixative" on some of his works. Results have revealed the presence of specifically bovine milk in all of the samples evaluated to date. Notably, only through the combined use of such advanced technical resources can the interrogation of all milk proteins retrieved from the samples provide evidence for the presence of a milk fixative and open the discussion about milk processing methods in the 18th century. In addition to granting conservators and art historians a deeper understanding of the complexity of Gainsborough's drawing techniques, this study paves the way for further investigations to probe the use of casein-based fixatives by other artists working on paper such as Degas and Van Gogh.

Keywords: Gainsborough, Drawing, Pigments, Fixative, Milk, Minimally invasive sampling, Proteomics, Nano-LC/MS, Bioinformatics

Introduction

Celebrated in his lifetime as an outstanding artist and renowned painter of portrait and rural landscapes, Thomas Gainsborough (1727–1788) is a central figure in the history of British art and, more generally, of 18th-century art. Although most famous for his portraits and romantic landscape paintings, Gainsborough was also a prolific draftsman, with over 900 of his

*Correspondence: federica.pozzi@metmuseum.org
[1] Department of Scientific Research, The Metropolitan Museum of Art, 1000 Fifth Avenue, New York, NY 10028, USA
Full list of author information is available at the end of the article

drawings presently known. As most artists of his generation, Gainsborough did not draw directly from nature; instead, he composed landscapes in his studio, relying on his sketches but also laying out stones, branches, leaves, and soil of various colors on his work table to recreate an ideal scene. This practice allowed him to experiment with materials and create dramatic scenes using multiple media on paper, such as graphite, chalk, charcoal, oil, watercolor, and pastel.

The Morgan Library & Museum, New York, owns twenty-five works on paper by Gainsborough, including highly completed landscape studies, an unfinished oil sketch, and a rare preparatory study for a painting (Fig. 1). About a dozen of these artworks were included in an exhibition entitled *Thomas Gainsborough: Experiments in Drawing*, which was held from May 11th to August 19th, 2018. A small catalogue that contains updated descriptions of The Morgan's works, as well as a technical study of Gainsborough's materials and techniques as observed in The Morgan's collection, accompanied the exhibition [1].

Conservation and art historical publications over the past 20 years have clearly demonstrated that Gainsborough was an innovative and experimental artist, having developed some unique drawing and painting methods during the decades of his career. By disrupting the traditional canons of drawing, he created real "experiments on paper" in which the landscape is key to creating original visual effects, each time different, to refine new techniques, and to investigate a new approach to art on paper. A crucial source of information about Gainsborough's singular working process is a letter, held at the Yale Center for British Art, which the artist addressed to his friend William Jackson in January 1773. In this letter, Gainsborough shared a brief account of his complex, extremely secret recipe for making oils on paper, using unorthodox technical solutions that subverted the academic principles of contemporary practice. The significance of this document for The Morgan drawings lies in the artist's documented use of certain white pigments and his process of intricate layering of various media and fixatives. According to Gainsborough's recipe, this type of drawing is created using lead white (*"your lights of Bristol-made white lead which you buy in lumps at any house painters"*). The paper sheet is then allegedly immersed multiple times in milk (*"dip it all over in skim'd milk"*), possibly to protect the pigments from discoloring, and adhered while wet to a temporary wooden frame or stretcher lined with paper. After the addition of supplementary light and dark layers as necessary, the drawing is dipped again in milk. Colors are then added, and gum Arabic glazing reported to be applied (*"float it all over with Gum water, 3 ounces of Gum Arabic to a pint of water"*). Finally, the piece is varnished on both sides to keep it flat (*"let that dry & varnish it 3 times with Spirit Varnish"*), after which the paper is removed from the strainer and mounted. Gainsborough's description of his secret recipe for making these works ends with a theatrical sign off: *"Swear now never to impart my secret to anyone living"*.

Fig. 1 Example of Gainsborough drawing analyzed in this study. Thomas Gainsborough (1727–1788). *Hilly Landscape with Cows on the Road*, The Morgan Library & Museum, III, 62. Photography by Steven H. Crossot, 2014

The Morgan's 2018 exhibition provided an impetus to undertake an in-depth technical study of Gainsborough's works for inclusion in the associated catalogue. Initially, a selection of his drawings housed at The Morgan Library were inspected using magnification along with different types of illumination, including transmitted and raking light, in order to better visualize the artworks' surface and appreciate the structure of each piece. Further photographic documentation and examination with ultraviolet (UV) and infrared (IR) light were carried out in an attempt to gain insight into Gainsborough's working methods, with a special focus on the possible application of coatings and the presence of underdrawings. In a second phase of this project, scientific analysis with both non-invasive and micro-invasive instrumental techniques aimed at characterizing the different white pigments found on the artist's works, as well as his potential use of fixatives. Identification of the white pigments was performed non-invasively by means of X-ray fluorescence (XRF) spectroscopy and, upon careful removal of a few microscopic samples, with a benchtop Raman spectrometer. On the other hand, the detection and characterization of milk-based fixatives on Gainsborough's drawings was accomplished using minimally invasive sampling techniques, such as polyvinyl chloride (PVC)-free erasers and fine polishing films, combined with proteomics

analysis via nano-liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry (nano-LC-ESI-MS/MS, henceforth referred to as nano-LC/MS).

Typically, the very thin layers of nearly invisible coatings or fixatives that might be present on a drawing cannot be identified using traditional analytical approaches such as Fourier-transform infrared (FTIR) spectroscopy, because a clean sample would be extremely difficult to isolate and the residues of paper substrate that are carried along would likely mask any signal from the target analytes. Similarly, protein analysis via gas chromatography/mass spectrometry (GC/MS) poses a great challenge, as the extensive sampling required in order to gather a sufficient amount of material would not be considered safe for the artwork. The present study, on the other hand, demonstrates that the combined use of minimally destructive micro-sampling methods [2], allowing safe sampling of the drawings, with trace-compatible sample preparation protocols, highly sensitive analytical techniques, and sophisticated bioinformatics data processing enables to achieve high levels of sensitivity and accuracy in the collection and interpretation of results [3]. Only thanks to the implementation of such multi-step methodology can the interrogation of all milk proteins retrieved from the samples provide evidence for the presence of a milk fixative and open the discussion about milk processing methods in the 18th century. In addition to granting conservators and art historians a deeper understanding of the complexity of Gainsborough's drawing techniques, this work paves the way for further investigations aiming to probe the use of casein-based fixatives by other artists working on paper such as Degas and Van Gogh.

Experimental

XRF Analysis was performed using a handheld Bruker Tracer III-V^{TM} energy dispersive XRF analyzer, with Peltier-cooled advanced high-resolution silver-free Si-PIN detector with a 0.2-μm beryllium (Be) window and average resolution of approximately 142 eV for the full width at half maximum of the manganese (Mn) Kα line. The system is equipped with changeable filters, and a rhodium (Rh) transmission target with maximum voltage of 45 kV and tunable beam current of 2–30 μA. The size of the spot analyzed is approximately 3×4 mm. Analysis was performed using 40 kV, 30 μA, 180-s acquisition time, and a titanium (Ti)-aluminum (Al) filter, by positioning the instrument at a $\approx$1-mm distance from the artwork's surface.

Raman Analysis was conducted using a Bruker Senterra Raman spectrometer equipped with Olympus 20× and 50× long working distance microscope objectives and a charge-coupled device (CCD) detector. A continuous wave diode laser, emitting light at 785 nm, was used as the excitation source, and two holographic gratings (1800 and 1200 rulings/mm) provided a spectral resolution of 3–5 cm^{-1}. The output laser power was kept between 10 and 25 mW, while the number of scans and integration time were adjusted to prevent damage from overheating and according to the Raman response of the samples examined. Spectra were interpreted by comparison with published literature and library databases available at The Met.

Visual examination Visual examination of the artworks was performed using a Nikon SMZ-1270 stereo zoom microscope with 6.3–80× zoom range, equipped with stops and 0.5× lens. Examination under UV light was carried out by means of light model Q-22B, with peak intensity at 6"—1200 μW/cm^2. A Fuji IS Pro Digital camera with Forensic Zeiss lens kit, Peca #916 and Wrattan 2E filters, was used to collect UVA-induced visible fluorescence photographs. Illumination was provided by CFL BLB bulbs working at 13 W and 120 V. IR photography was performed using a Pixelteq Spectrocam SWIR camera system including RAPTOR 320×256, a filter wheel, and a Coastal Optic 60-mm lens; all IR images were captured using a 1332-nm filter.

Sampling and sample preparation for nano-LC/MS Sampling of the Gainsborough drawings was performed following a protocol described by Kirby and coauthors, in which the use of both PVC-free erasers and fine polishing films was first demonstrated on photographs [2]. In detail, PVC-free erasers (Staedtler, 525 B) were cut into small blocks of approximately 3–5 mm and held with tweezers for rubbing under the microscope. Small discs of 5–6 mm in diameter were cut from fiber optic polishing film discs with aluminum oxide (Precision Fiber Products) of 6- and 15-μm grits. These discs were adhered to polystyrene rods of varying diameter (Walther) and held in a nickel Starette Pin Vise (Grainger) for rubbing under the microscope (Fig. 2). Rubbing duration and pressure varied for each sample until the deposition of material could be observed on the eraser or fine polishing film (appearing as surface grime or as a grayish/whitish residue, respectively). Microscopic examination of the works' surface after sampling did not show any evidence of surface disruption or media loss. Based on a treatment method previously developed by some of the authors of this article [3], including the adoption of filter-aided sample preparation [4], erasers and fine polishing films were handled as follows prior to nano-LC/MS analysis: upon sampling, these protein-trapping supports were placed in a lysis buffer containing 4% sodium dodecyl sulfate, 8 M urea, 0.2% deoxycholic acid, and 50 mM dithiothreitol. After washing, alkylation was induced by adding 55 mM iodoacetamide. Buffer was then exchanged to 50 mM ammonium bicarbonate and

Fig. 2 Sampling of a drawing for proteomics analysis via nano-LC/MS using fine polishing films

0.2% deoxycholic acid (pH 8.8) for digestion with trypsin and lysC enzymes. Liquid-liquid extraction was performed using ethyl acetate, while trifluoroacetic acid was employed for acidification. Several washing steps were carried out before and after phase transfer. The aqueous phase was then entirely evaporated by means of a speed vacuum system, after which a minimal volume of 0.1% formic acid was added. A list of the artworks' locations from which samples were retrieved is provided in Table 1.

Nano-LC/MS The peptide mixture was analyzed by means of an UltiMate 3000 nano-LC system coupled with an electrospray orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer (Thermo Fisher Scientific, San Jose, California), using (A) 0.1% formic acid and (B) 0.1% formic acid in 80% acetonitrile. 1 µL of peptide digest was initially loaded onto a 5-mm C18 PepMap™ trap column with 300-µm inner diameter (Thermo Fisher Scientific) at a flow rate of 10 µL/min with 0.1% formic acid. Peptides were then eluted from the trap column onto an analytical 50-cm C18 PepMap™ column with 75-mm internal diameter (Thermo Fisher Scientific) with a 4–40% linear gradient of solvent B in 235 min. The elution flow rate for the latter step was set at 300 nL/min. The mass spectrometer operated in positive ion mode at a 1.9-kV needle voltage. Data were acquired using Xcalibur 4.1 software in a data-dependent mode. MS scans were recorded in the m/z 375–1500 range at a resolution of R = 120,000 (at m/z 200), while MS/MS scans were collected in the orbitrap at a resolution of R = 30,000 (at m/z 200). Ions between +2 and +7 charge were selected for high-energy collisional dissociation (HCD) fragmentation. Reference samples, blanks, as well as positive and negative controls were injected and tested alongside the historic samples to prevent contamination and carryover.

MS data processing Raw data files generated from nano-LC/MS spectral acquisition were searched using PEAKS 8.5 software against UniProtKB/Swiss-Prot 2020_02 database and, then, against a restricted UniProt *Bos taurus* database (Proteome ID UP000009136). Parameters for data analysis were set as follows: 3 missed cleavages maximum; precursor ion error tolerance 10.0 ppm; fragment ion error tolerance 0.02 Da. Carbamidomethylation was set as a fixed modification, while oxidation (methionine, proline), deamidation (asparagine, glutamine), phosphorylation (serine, threonine), and lactosylation (lysine, arginine) were set as variable modifications. Protein false discovery rate (FDR) was set to 0.1% with -10lgP ≥ 18 score threshold for peptides. A manual filter of at least two different non-overlapping peptides was set for protein identification. Data resulting from the injection of positive and negative controls, as well as blanks, were carefully examined prior to injecting the historic samples to verify the possible presence of contaminants or residues from carryover. Reference samples were also used to verify retention times, peak shapes, peptide signal to noise ratio, the occurrence of contaminants resistant to washing, as well as the overall validity of the procedure adopted. For the historic samples, MS/MS spectra of each peptide of interest were inspected manually, and the presence of various peptide species was investigated by searching unknown spectra against the whole nrNCBI database via Basic Local Alignment Search Tool (BLAST).

Results

A summary of the results obtained from visual examination, as well as XRF and Raman analysis of a selection of Gainsborough drawings, is provided in Table 1, along with an indication of the sampling locations for casein-based fixative identification with nano-LC/MS.

Results of XRF and Raman spectroscopy

Identification of the white pigments in a selection of Gainsborough drawings from The Morgan Library holdings was carried out using a combination of in situ, non-invasive XRF analysis and micro-invasive Raman measurements on microscopic samples. Results have shown that, in addition to a prominent use of lead white as described in Gainsborough's 1773 letter, calcite is also present in white areas and highlights of several works among those examined. The data collected strongly suggest that, in all drawings selected for analysis, these two pigments were used individually. In addition to lead and calcium, indicative of the use of lead white or calcite pigments, XRF spectra display a series of trace elements that are attributable to the paper substrate. In detail, as also summarized in Table 1, lead white was detected in five

Table 1 Summary of the results of visual examination, XRF and Raman analysis, as well as sampling locations for casein-based fixative identification for a selection of Gainsborough drawings. NA indicates samples or areas either not removed or not analyzed

Accession number	Title	Date	Medium (from object label)	White pigments (confirmed with XRF and/or Raman)	Coatings (inferred from visual examination)	Sampling locations for casein identification with nano-LC/MS
2005.82	*Study of Trees*	1750	Graphite, with fixative, on laid paper	NA	Coating broadly applied with brush over media	White wash in background
III, 60	*Landscape with Group of Figures Resting on a Hillside*	1770s	Brown chalk, lead white and blue oil paint, varnished, on laid paper	Lead white (area of cloud and sky near top edge at center)	Probable natural resin varnish	NA
III, 55	*Landscape with Horse and Cart, and Ruin*	1770–1775	Oil paint, lead white chalk and watercolor over black chalk, varnished, on laid paper	Lead white (area of building at right and cloud at center)	Probable natural resin varnish	NA
2014.32	*Open Landscape with Drover and Packhorses*	1775–1780	Watercolor and opaque watercolor, on brown laid paper	Lead white (area of cloud to the left of tree, opaque background, and all highlights)	No visible coating, bright lead white	Background with possible white wash
III, 63	*Landscape with Horse and Cart Descending a Hill*	ca. 1780	Black chalk with smudging, on wove paper	NA	Deteriorated overall coating and probable selective use of wet media or binder to make chalk washes	Bare spot of upper right corner where G. may have held the paper while dipping in fixative Background, middle of sky, right of tree (no paint apparent) Background, sky above trees, right side (no paint apparent)
2017.89	*Coastal Scene with Shipping, Figures, and Cows*	ca. 1780	Black watercolor and white opaque watercolor over black chalk with smudging	NA	Probable overall coating, bright lead white	Background, left portion of sky (no paint apparent)
III, 61	*Wooded Landscape with Horseman, Figures, and Bridge*	ca. 1780	Black chalk with smudging, opaque lead white, on laid paper	Lead white (areas of river bank at proper left and cloud at center)	Locally applied, deteriorated coating	Center of pond (no paint apparent)
III, 62	*Hilly Landscape with Cows on the Road*	ca. 1780	Black chalk with smudging, white chalk applied wet, on wove paper	Calcite (area of sheep on proper left at bottom, and white spots on tree at center)	Probable overall coating and selective use of wet media or binder to make chalk washes	Background, above trees (no paint apparent) Foreground, shaded road
III, 63a	*Wooded Landscape with Cows in a Pool*	1780–1785	Black wash, lead white watercolor and red chalk over traces of black chalk, on laid paper	Lead white (highlights of cow)	Deteriorated overall coating	Front shoulder of light colored cow at center
III, 59	*A Woman with Three Children*	1780–1785	Black chalk with smudging, and white chalk, some applied wet, on tan laid paper	Calcite (areas from girl's dress and baby's arm)	No visible coating and selective use of wet media or binder to make chalk washes	NA
III, 63b	*Lady Walking in a Garden*	ca. 1785	Black and white chalks with smudging, worked wet and dry, watercolor, on laid paper washed with watercolor	Calcite (area of lady's gown)	No visible coating and selective use of wet media or binder to make chalk washes	NA

drawings that span two decades of the artist's life. On the oil sketch *Landscape with Horse and Cart, and Ruin* (1770-75; III, 55) this pigment was likely applied in stick form, while on *Landscape with Group of Figures Resting on a Hillside* (1770s; III, 60) it is probably present as oil paint. A significant amount of lead white was also identified in the black chalk drawing entitled *Wooded Landscape with Horseman, Figures, and Bridge* (ca. 1780; III, 61), where it was applied dry and appears to have been covered locally with a liquid material using a brush, perhaps to protect the lead from oxidation. On *Wooded Landscape with Cows in a Pool* (1780-85; III, 63a) lead white was found in the tiny highlights of the cow, whereas this pigment forms much of the opaque background and all of the highlights in *Open Landscape with Drover and Packhorses* (1775-80; 2014.32). On the other hand, calcite was mostly detected in works created during the last decade of Gainsborough's artistic production, for instance, *Lady Walking in a Garden* (ca. 1785; III, 63b), *Hilly Landscape with Cows on the Road* (ca. 1780; III, 62), and *A Woman with Three Children* (1780-85; III, 59). The limited occurrence of calcite in pieces dated to a decade after Gainsborough's letter of 1773 may attest to an intrinsic evolution in the artist's choice and use of materials over the course of his career.

Results of visual examination

All the whites, both lead- and calcium-based, appear bright upon visual inspection with normal light. Close examination with transmitted and raking light, as well as UV photography, indicated that overall and selectively applied coatings or fixatives might be present on many of the drawings under study. Both the oil sketch *Landscape with Horse and Cart, and Ruin* (1770-75; III, 55) and *Landscape with Group of Figures Resting on a Hillside* (1770s; III, 60) appear to be varnished with a natural resin, which displays fluorescence under UV illumination as frequently observed in traditional paintings, and are in an excellent state of preservation. Interestingly, on *Landscape with Horse and Cart Descending a Hill* (ca. 1780; III, 63), normal light and UV photography suggested the presence of a deteriorated overall coating with a clean corner, at the upper left, possibly corresponding to the location where the work might have been held by the artist when dipping it in a fixative bath (Fig. 3). In *Hilly Landscape with Cows on the Road* (ca. 1780; III, 62) an overall yellowing might be indicative of the presence of a coating. Careful study of the road in the lower left portion of this drawing, in which only calcite was detected, revealed a complex overlapping of dry black and white chalks, with layers of black and white chalk wash and chalks worked wet. Similar discoloration issues are observed in *Wooded Landscape with Cows in a Pool* (1780-85; III, 63a), a drawing that contains very limited lead white highlights, while a thin wash, now slightly degraded, was applied locally over the white pigments in *Wooded Landscape with Horseman, Figures, and Bridge* (ca. 1780; III, 61). None of the latter coatings appeared shiny or reflective, thus the presence of gum Arabic and varnish was not considered likely. In a few works, such as *Study of Trees* (1750; 2005.82), a more broadly brush-applied liquid material seems to be localized in the image area. Here, the liquid material, characterized by a yellow discoloration, may have been used to deliberately solubilize some of the graphite to create a gray wash and fix it on the surface; while this is not numbered among the traditional uses of graphite, it would be consistent with Gainsborough's experimental approach to media. In another drawing with extensive lead white watercolor, *Open Landscape with Drover and Packhorses* (1775-80; 2014.32), no coating was visible under UV or raking light, although the darker paper tone and its rough texture may have masked discoloration. The lack of any conversion of the lead white to lead sulfide, in this piece as well as on *Coastal Scene with Figures and Cows* (ca. 1780; 2017.89), may suggest the presence of a protective coating. These observations led to formulate a theory according to which Gainsborough may have used his "secret ingredient", skim milk, on many of his drawings, as a fixative or working medium to manipulate chalk, graphite, and opaque white pigments more easily [5].

Results of proteomics analysis

Verification of reported fixatives, i.e. very thin coatings applied to protect or "fix" friable or sensitive artists' media, is relevant to Gainsborough as well as to many other artists working on paper. While Gainsborough's innovative methods for making drawings have been of great interest to scholars, obtaining a sufficient amount of sample from the many thin and delicate layers of such fixatives found on his artworks has posed a significant hurdle when using traditional analytical approaches. Indeed, in most cases, well-established instrumental techniques such as FTIR, GC/MS, and pyrolysis-GC/MS are not adequately sensitive for this type of investigations unless a sample of inappropriately large size for cultural heritage artifacts is removed. In the present study, high levels of sensitivity and accuracy in the collection and interpretation of results could only be achieved thanks to the combined use of minimally destructive micro-sampling tools (PVC-free erasers and fine polishing films), advanced sample preparation methods, and highly sensitive analytical techniques (nano-LC/MS), in association with sophisticated bioinformatics data processing. In the literature, the use of erasers for mass spectrometry analysis was first successfully demonstrated in the removal of

specimens from parchment [6]. Kirby and coworkers further expanded the use of erasers for sampling of cultural heritage and introduced polishing films to sample coatings on photographs and works of art on paper [2].

Proteomic analysis by nano-LC/MS is the only way to manage extremely low sample amounts and, at the same time, address the likelihood of complex sample mixtures. As stated previously, Gainsborough reportedly used a wide range of media, including oil paint, chalks, watercolor, and possible gum Arabic isolating layers, as well as various fixatives and varnishes. As a result, every sample removed from any of his drawings may potentially contain more than one medium, and the amount of material relevant to protein analysis is only a fraction of the total sample collected. Therefore, the use of special sample preparation methodologies tailored to the treatment of the minute quantities of material gathered upon sampling by PVC-free erasers or fine polishing films represents a first, necessary step to isolate the protein fraction contained in the sample and ensure successful analysis. The subsequent application of a proteomic approach, i.e. the digestion of such proteins into peptides, enables all of the proteins to be detected and identified as each peptide sequence can be assigned to a protein by means of bioinformatics softwares.

Methodologically, proteomics, and in particular the so-called "bottom-up" strategy used in this study, is based on four main steps, as follows: (i) Protein extraction from the sample matrix containing organic and/or inorganic components; here, this step was conducted using an adapted filter-aided procedure, as described in the Experimental section. (ii) Controlled hydrolysis of proteins into peptides using one or more enzymes; in the present work, the digestion was performed using trypsin and lysC, i.e. endoproteases that cleave proteins at the carboxyl side of lysine amino acid for both enzymes and of arginine amino acid for trypsin. (iii) Analysis of the resulting peptide mixture by means of an analytical workflow adapted to very low sample amounts; here, through the coupling of nano-LC and high-resolution tandem MS, peptides are purified, separated, amino acid sequences are obtained by the elucidation of fragment ions resulting from the peptide fragmentation spectra and, then, coded to represent their origin (N-terminal charged fragment ions are classed as a, b or c, while the C-terminal charged ones as x, y or z) and length (for example, a 10 amino acid peptide could create fragments y_1^+ through y_{10}^+ with the corresponding b ions) [7]. (iv) MS data processing by means of bioinformatics tools that integrate genomic or protein databases, allowing for the identification of proteins and their modifications.

In the present case, despite the extremely low sample amounts investigated, remarkably high protein sequence coverages were achieved, leading to the unequivocal identification of different milk proteins in all of the samples analyzed, as shown in Table 2. As a representative example, the results obtained for sample S2C2 from *Coastal Scene with Shipping, Figures, and Cows* (2017.89) are presented in the following. For this specimen, proteomics analysis revealed high sequence coverage (over 50%) for alpha S1 casein. Spectra display good signal to noise ratios, as well as a well-defined fragmentation pattern characterized by an almost complete y and b ions series from the protein peptides, ensuring unambiguous sequence assignments. The data shown in Fig. 4, for instance, enables identification of the HQGLPqEVLNENLLR sequence corresponding to the 23–37 peptide of alpha S1 casein. In this spectrum, y_1^+ to y_{10}^+ and b_2^+ to b_{11}^+ fragment ions are detected; Q28, i.e. glutamine at position 28 of the sequence, referenced in lower case, is deamidated; and variants of deamidation were also identified on positions Q24, N32, and N34, consistently with the fact that this is a common modification in old proteins.

Along with alpha S1 casein, ten milk proteins belonging to three main types have been successfully characterized in sample S2C2 [8, 9]: (i) caseins, (ii) whey proteins, and (iii) milk fat globule membrane proteins (MFGM), representing about 78%, 17%, and 1–2% of the total bovine milk proteins present, respectively. Belonging to the first group, the alpha S1, alpha S2, beta, and kappa caseins are low molecular weight proteins of 20–25 kDa and are found in cow's milk in 38%, 10%, 35%, and 12% respective proportions [10]. Several of these proteins exhibit variants and they are heavily post-translationally modified in protein biosynthesis with varying level of phosphorylation and glycosylation [11]. As displayed in Table 2, these four caseins were successfully identified in sample S2C2 from drawing 2017.89 with high sequence coverages. Several of their post-translational modifications such as phosphorylation were also detected and localized, while no lactosylation, a non-enzymatic modification indicating the potential heating treatment of milk, was identified with the current experimental design [12–14]. As an example of these results, Fig. 5 illustrates the 118–134 sequence of alpha S1 casein, exhibiting a phosphorylation on the serine at position S130 in both reference and historic samples, whereas only a peptide from a reference heated milk sample shows lactosylated K128 and K120 lysine residues; among these graphs, spectrum C corresponds to the 121–134 sequence, indicating a trypsin cleavage of the K120 lysine from sequence 118–134.

The second type of milk proteins found in sample S2C2 from drawing 2017.89 is whey proteins. Among them, several of the most abundant ones, such as beta-lactoglobulin (LGB), bovine serum albumin (BSA), and

Fig. 3 Normal light (left) and UV (right) photographs of a detail from *Landscape with Horse and Cart Descending a Hill* (ca. 1780; III, 63), suggesting the presence of a deteriorated overall coating with a clean corner, at the upper left, possibly corresponding to the location where the work might have been held by Gainsborough when dipping it in a fixative bath

immunoglobulins (IgG), representing approximately 60%, 10%, and 10% of total whey proteins, respectively [10], were identified with sequence coverages over 50% for LGB and between 20 and 50% for BSA and IgG. In addition to those, other whey proteins such as serotransferrin and lactoferrin/lactotransferrin were also found in the matching data. Remarkably, one of the typically most abundant whey proteins, namely alpha-lactalbumin, accounting for 20% of total whey proteins, was not detected in the historic samples analyzed. This protein, however, was identified in all the liquid and dry reference samples of modern milk examined with high sequence coverage (over 80%); analysis of reference milk-dipped slides and papers upon micro-sampling with PVC-free erasers and polishing films also yielded positive identification, albeit with lower sequence coverages (between 30 and 40%) compared to what was expected based on the scores obtained for other milk proteins identified. Alpha-lactalbumin is a metal-binding protein and its ability to interact with other organic compounds such as lipids has been the topic of extensive accounts in the literature [15]. At this stage, the reason for the failed or poor detection of this protein in the Gainsborough samples, compared to other proteins that appear to have similar physicochemical properties or action mechanisms, remains unclear [16, 17]. Ongoing research by the authors of this article is currently investigating drawing replicas in an attempt to shed light on the possible mechanisms involved.

The last type of protein identified in sample S2C2 from drawing 2017.89 is represented by MFGM. The structure of MFGM consists of lipid (40%), protein (60%), and cholesterol [18, 19]. More accurately, a phospholipid monolayer surrounds the triacylglycerol core, followed by a proteinaceous coat connecting the monolayer to the outer phospholipid bilayer. Glycoproteins such as butyrophilin and lactadherin (milk fat globule-EGF factor 8) are distributed over the external membrane surface. Lactadherin, a 47-kDa protein, was identified in the sample discussed with sequence coverage below 20%. Despite such low score value, in this case, a particularly good spectral quality enabled the unambiguous attribution of five characteristic peptides. Xanthine dehydrogenase/oxidase, a 146-kDa high molecular weight protein located between the outer glycerolphospholipd bilayer and the inner glycerolphospholipid monolayer that surrounds the triacylgrycerol core, was also identified with very low sequence coverage (below 5%) in another sample, namely S2B1, from the same drawing.

The presence of MFGM proteins in all of the historic samples analyzed is difficult to interpret in relation to Gainsborough's 1773 letter, in which the artist describes his own practice of dipping his drawings specifically in skim milk. In the mid-17th into the 18th century, English dairy and cheese production was distinctly artisanal in character, with very low levels of mechanization. Farmers in the most favored dairying regions, which included the Plain, Somerset, and North Wiltshire, began to commercialize and produce a milk surplus, which, in combination with the advances in the English transportation system, caused farmers to seek non-local markets. London was one of the largest beneficiaries of these developments. Farmers also took advantage of the combination of milk and cheese production. For example, the county of Suffolk had a reputation for high-yielding dairy herds and, since the 16th century, much of the capital's cheese and butter was supplied via a relatively short coastal passage from ports such as Yarmouth, Woodbridge, and Ipswich. However, since cream from the milk was required for making cheese, and farm cheese dairies were researching ways to utilize the remaining heavily skimmed milk, they employed it to produce an inferior "flet" (i.e. skim) milk cheese [20].

Due to industrialization and process improvement, modern skim milk is much more efficiently defatted than milk in 18th-century England and, consequently, what was considered to be skim milk three centuries ago may still have contained a certain amount of MFGM [21]. On the other hand, milk treatment such as cooling, heating, and centrifugation can damage or alter MFGM, causing the release of some membrane-associated proteins from MFGM into the aqueous phase (i.e. skim milk) [22]. In addition to the factors described above, the size of MFGM also induces differences in protein composition [23–26]. Hence, the designation of "skim" milk in the context of 18th-century dairy production is very complex. Further studies are currently underway, including replicating 18th-century milk defatting, to gain knowledge of

Table 2 List of the main milk proteins identified in the Gainsborough drawings for selected locations. Dark gray, medium gray, and light gray colors represent protein sequence coverages respectively over 50%, between 20 and 50%, and below 20%; asterisks indicate sequence coverages below 5%; blank rectangles mean lack of identification

Proteins identified[a]	Proteins accession number	Drawings	2005.82		2017.89		2014.32		III, 61		III, 62		III, 63a		III, 63		
		Samples	B	S1C2	S2B1	S2C2	S1B2	S1C2	S2B2	S2C2	S1C2	S3B2	S1C	S4B	S3B2	S2C2	S1A
		Sampling method	6-µm film	PVC-free eraser	6-µm film	PVC-free eraser	6-µm film	PVC-free eraser	6-µm film	PVC-free eraser	PVC-free eraser	6-µm film	PVC-free eraser	6-µm film	6-µm film	PVC-free eraser	15-µm film
Alpha S1 casein	P02662	Casein proteins															
Alpha S2 casein	P02663																
Beta casein	P02666																
Kappa casein	P02668																
Beta-lactoglobulin	P02754	Whey proteins[b]															
Serum albumin	P02769																
Lactotransferrin	P24627									*							
Lactoferrin	B9VPZ5																
Serotransferrin	Q29443			*								*					
Poly-immunoglobulin	P81265		*		*						*						
Lactadherin	Q95114	MFGM proteins									*					*	*
Xanthine dehydrogenase /oxidase	P80457				*											*	
Butyrophilin subfamily 1, member A1	P18892				*	*					*					*	*

[a] 10lgP protein score is above 40 for all the proteins listed

[b] Alpha-lactalbumin (accession number P00711) is not mentioned due to its absence or very poor detection in the samples analyzed

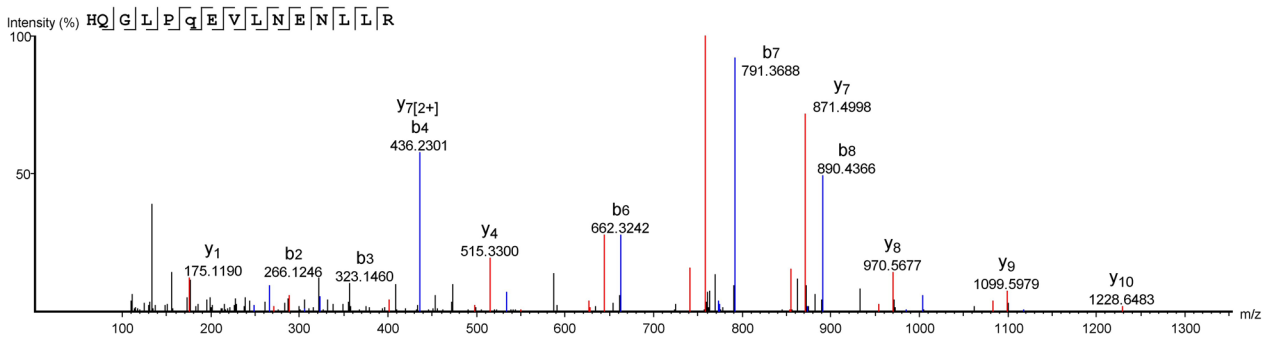

Fig. 4 MS/MS spectrum of peptides identified in sample S2C2 from *Coastal Scene with Shipping, Figures, and Cows* (ca. 1780; 2017.89). The data shows a triply charged ion at m/z 587.6483 (error Δm 0.7 ppm), enabling identification of the 23-37 peptide of alpha S1 casein, which includes one deamidation (Q28). This peptide is indicative of Bovinae subfamily biological origin

the milk protein composition that may most closely correspond to 18th-century skim milk.

Most of the peptide sequences detected in the present study are common to several animal species; however, some are indicative of the biological origin of the proteinaceous material under investigation and, therefore, may be used to draw conclusions in that regard. For example, in the case of sample S2C2 from drawing 2017.89, both the 23–37 peptide of the alpha S1 casein displayed in Fig. 4 and the 19–37 peptide with a missed cleavage listed in Table 3 belong to the Bovinae subfamily (*Bos taurus, Bos mutus, Bos indicus, Bos grunniens, Bison bison bison*). Other discriminant peptides from the most abundant proteins identified in this sample, namely alpha S1, alpha S2, and kappa caseins, as well as beta-lactoglobulin, are also shown in Table 3. Based on the documentary information available on Gainsborough's working

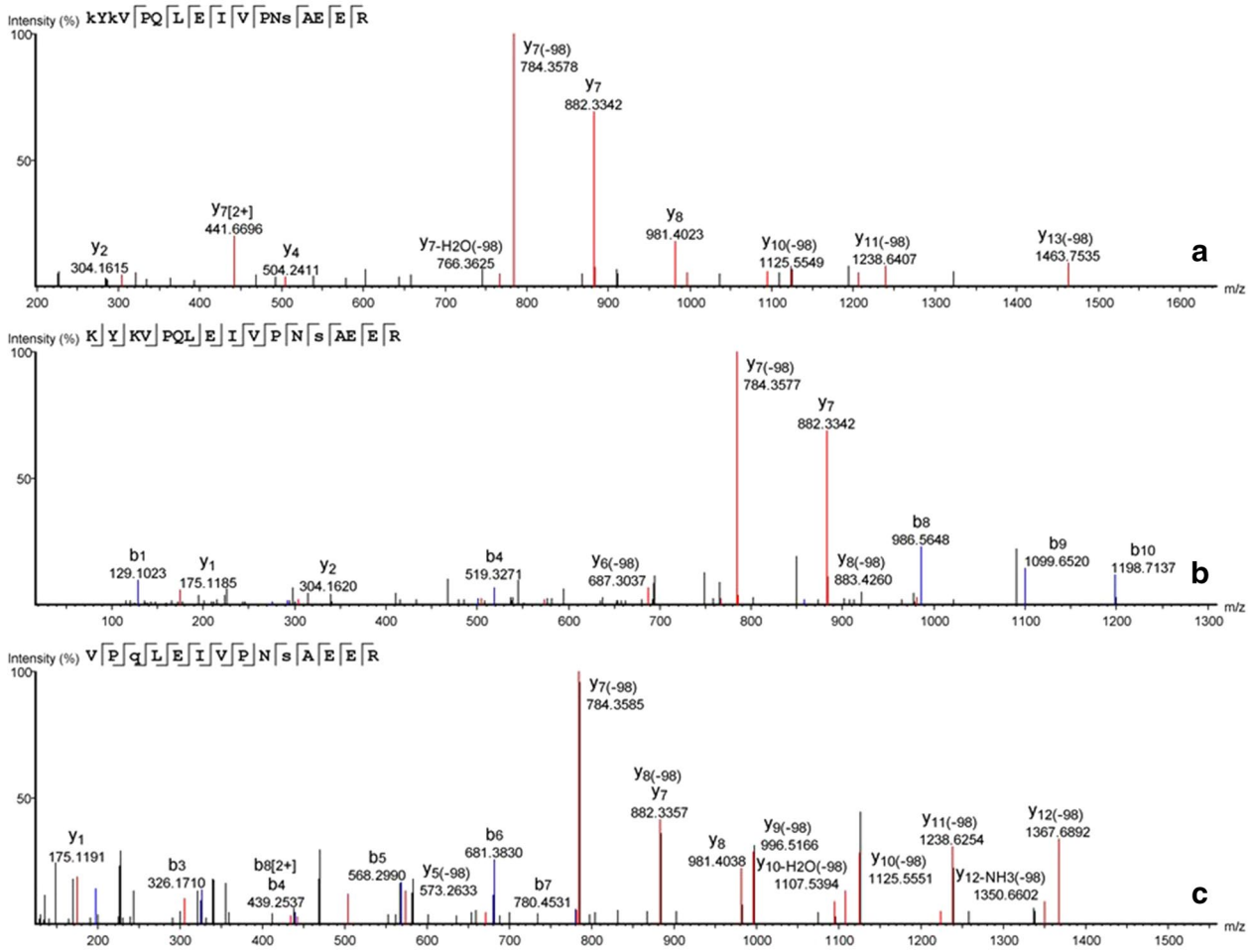

Fig. 5 **a** MS/MS spectrum of peptides identified in a reference sample of modern milk. The data shows a triply charged ion at m/z 910.0919 (error Δm 0.9 ppm), enabling identification of the 118–134 peptide of alpha S1 casein, which includes two lactosylations (lower case K118 and K120) and one phosphorylation (S130). **b**, **c** MS/MS spectra of peptides identified in sample S2C2 from *Coastal Scene with Shipping, Figures, and Cows* (ca. 1780; 2017.89). The data shows (B) a triply charged ion at m/z 694.0209 (error Δm 0.5 ppm), enabling identification of the 118-134 peptide of alpha S1 casein, which includes one phosphorylation (S130); and (C) a doubly charged ion at m/z 831.3934 (error Δm 0.8 ppm), enabling identification of the 121-134 peptide of alpha S1 casein, which includes one phosphorylation (S130) and one deamidation (Q123)

environment, among the members of the Bovinae subfamily, *Bos taurus* (domesticated cattle) is the most probable source of the milk-based fixative, while it seems unlikely that other species such as *Bos mutus* (wild yak), *Bos indicus* (domestic cattle native to South Asia), *Bos grunniens* (domestic yak), or *Bison bison bison* (American bison) may have been employed. Gainsborough's use of milk originating from *Bos taurus*, as opposed to other animal species such as goat or sheep, would appear to reflect the urban setting in which he is known to have created his drawings, in the cities of Bath (1759–1774) and London (1774–1788), during the four decades of his artistic career that are relevant to the present study.

Discussion

The various coating materials described by Gainsborough in his 1773 letter to Jackson include skim milk, gum Arabic, and varnish, all of which, however, are mentioned only in relation to his multi-layered oils on paper. The presence of yellowed coatings, both overall and selectively applied, on some of The Morgan's drawings clearly suggests that the artist likely used different procedures and materials to fix or protect his various creations.

Fixatives are materials used to enhance the attachment of friable pigments onto a surface, and have been in use for centuries. They may be applied by brush, by spraying with an atomizer, or by dipping. In the 18th century, discussions about fixing pastels, the most

friable of media, were taking place. Research in this area conducted by Shelley and other authors indicates that most fixatives employed consisted of natural resins or some form of animal glue [27–29]. These materials, however, would significantly alter the color, saturation, and texture of the pastels, and, because of this reason, they were not well liked by professional artists. Gainsborough's desire for an invisible fixative is understandable in light of the fact that he worked in various wet and dry media and was reportedly fearless in his experimentation.

Since the Renaissance, artists have known that lead white would darken under certain conditions and, by the 18th century, using this pigment in watercolors was considered a risky choice, as sulfur in the air would prompt the conversion of the white basic lead carbonate into gray lead sulfide. Writing on the need for careful preparation of lead white, Edward Norgate, English miniature painter and court musician to James I, states: *"You cannot be too carefull of this, the neglect whereof hath bene the spoile of many in the Vatican Library at Rome, as alsoe in the curious Villa of Cardinall Burghese and elsewhere. For these being heightened with white, are in the heigthninge become soe black, rusty and dis-coloured as I told you of before"* [30]. An unpublished technical study by Sophie Crombie, an undergraduate student at the Courtauld Institute of Art, in the 1980s attempted to re-create Gainsborough's oils on paper, as discussed in his 1773 letter. Interestingly, the author noted that varnishing lead white chalk without a fixative does not work, because the pigment is simply brushed away, indicating that it was poorly bound to the paper support and required being worked with a substance that would improve adhesion. According to Crombie, dipping in milk does appear to act as a fixative, adhering the chalk securely to the paper and allowing for a varnish (or other coatings) to be subsequently applied [5].

The general protein hydrophilicity promotes a good compatibility to polar surfaces, such as paper [31], and an effective barrier to apolar gases, such as oxygen and carbon dioxide. In light of these observations, coating industries are manipulating proteins' physicochemical properties in order to modulate selectively their barrier, mechanical, and surface properties for use in coatings and packaging [32]. A parallel is possible with protein-based coatings on artworks, as reported in a work exploring the protective properties of egg white as a coating for paintings [33], and, as in the present case of study, with milk proteins used as a protective coating for works of art on paper.

Observations of discolored coatings and subtle surface phenomena on many of The Morgan's drawings, as well as the artist's detailed letter of 1773, strongly suggested that a milk fixative might be present on Gainsborough's works on paper containing lead white. As explained above, results of non-invasive analysis and examination of microscopic samples revealed that both calcite and lead white were used in many of his artworks, which led to further questions about the possible presence of a fixative or coating that might protect other pigments, in addition to lead white, from darkening. All of the lead white-containing areas analyzed appeared to be in excellent condition, with no apparent cracking, flaking or darkening, although these phenomena have been observed in Gainsborough drawings housed in other collections [5, 34].

Micro-sampling in association with qualitative proteomics provided important new information. As seen in Tables 1, 2, and 3, all of the drawings tested by this method, regardless of the sampling location (front or back, background or painted area), the date of the artworks, or the white pigment used in them, were found to contain casein and other milk proteins specific to *Bos taurus*. This result was unanticipated, since it was expected that only the works with lead white might show milk proteins. These data would strongly suggest the following: (i) Gainsborough applied a milk fixative on his drawings whether the white pigment employed was calcite or lead white, or the medium was graphite, chalk, watercolor, or oil paint; (ii) in all cases examined, the milk fixative was applied to both sides of the paper support; (iii) Gainsborough could have been using milk fixatives as early as 1750. The discovery that milk was present on chalk, graphite, and wash drawings prompts further in-depth research to investigate all drawing types and styles in the artist's oeuvre not only to understand the extent of his use of milk fixatives, but also to evaluate the possible presence of other materials such as gums and natural resins. The present study indicates that dipping in milk was part of Gainsborough's process of making drawings over a long period of time. Furthermore, based on the data collected thus far, this practice does not appear to relate only to drawings with multiple layers (i.e. III, 61; III, 62; III, 63a), but even to purely graphite works (2005.82) and simple watercolors made with lead white wash (2014.32).

The question of what is yellowing in these works remains unclear and must be further studied. Because to date only the analysis of proteins was undertaken, future research must also consider whether polysaccharides or natural resins may be present, and if unexpected interactions with casein-based coatings leading to yellowing may occur. While Gainsborough noted that he used specifically skim milk, it is likely that at least some milk fat remained in his 18th-century processed milk, and that the amounts might vary in each fixative bath used. The interaction of the fat with the milk proteins and with

Table 3 List of peptides that are characteristic of the Bovinae subfamily, with indication of their position in the corresponding proteins, mass to charge ratio (m/z), charge (z), measurement error (Δm), identification score (reported as -10lgP, i.e. converted P value; higher numbers indicate higher-confidence matches), retention time (RT), and animal species that contain that specific peptide. Deamidation variants of these peptides are not referenced in the table. Lower case letters in peptide sequences indicate the following modifications: *n* asparagine deamidation, *m* methionine oxidation, *s* serine phosphorylation, *c* cysteine carbamidomethylation (induced by sample treatment), *w* tryptophan oxidation, *q* glutamine deamidation or N-terminal glutamate conversion to pyroglutamate

Proteins	Accession number	Peptides	Position	m/z (exp)	z	Δm (ppm)	-10lgP	RT (min)	Animal species
Alpha S1 casein	P02662	HPIKHqGLPQEVLNEN-LLR	19-37	559.8104	4	0.1	40.64	103.31	*Bos taurus, Bos mutus, Bos indicus, Bos grunniens, Bison bison bison*
		YYVPLGTQYTDAPSFS-DIPNPIGSENSEK	180-208	1063.836	3	1.1	47.31	184.3	*Bos taurus*
		YKVPqLEIVPNsAEER	119-134	976.9728	2	0.9	33.98	132.66	*Bos taurus, Bos mutus, Bos indicus, Bos grunniens, Bison bison bison*
Alpha S2 casein	P02663	nAVPITPTLNR	130-140	598.8367	2	2.4	36.27	111.8	*Bos taurus, Bos indicus x Bos taurus, Bubalus bubalis, Bos mutus, Bos grunniens, Bison bison bison*
		AmKPWIQPK	204-212	557.808	2	0.9	31.44	46.2	*Bos taurus, Bos indicus x Bos taurus, Bos mutus, Bison bison bison*
		NmAINPsKENLcSTFcK	40-56	709.2982	3	2.2	24.82	88.4	*Bos taurus, Bos indicus x Bos taurus, Bos grunniens, Bison bison bison*
		TKVIPYVR	213-220	488.3035	2	1.2	22.53	58.3	*Bos taurus, Bubalus bubalis, Bos mutus, Bos grunniens, Bison bison bison*
Kappa casein	P02668	SPAQILQwQVLSNT-VPAK	90-107	998.5488	2	2.1	64.79	161.5	*Bos taurus, Bos mutus, Bos indicus, Bos grunniens, Bison bison bison, Bos indicus x Bos taurus, Bos gaurus,Bison bison athabascae,Boselaphus tragocamelus*
		qEQNqEqPIRcEKDER	22-37	691.3049	3	0.9	28.41	47.71	*Bos taurus, Bos mutus, bos grunniens, Bos indicus, Bison bison bison*
		QVLSnTVPAK	98-107	529.2989	2	1.6	25.62	58.0	*Bos taurus, Bos mutus, Bos indicus, Bison bison bison, Bos grunniens, Bos indicus x Bos taurus, Bos frontalis, Bison bonasus, Boselaphus tragocamelus, Tetracerus quadricornis, Bos javanicus, Bison bison athabascae, Boselaphus tragocamelus*
Beta-lactoglobulin	P0275	TPEVDDEALEKFDK	141-154	818.3918	2	1.0	43.07	99.8	*Bos taurus, Bos mutus, Bubalus bubalis, Bos indicus, Bison bison bison*
		LIVTqTmK	17-24	475.7655	2	1.1	21.81	48.54	*Bos taurus, Bos mutus, Bos indicus, Bison bison bison*

other possible coatings also prompts for further studies. Scientific investigation, beyond The Morgan's collection, of potential milk fixatives from a larger group of paper artworks by both Gainsborough and his contemporaries would solidify the observations gathered in the present research and enable a deeper understanding of the working methods of the time. In addition to this, it would be crucial to investigate whether milk actually does protect lead white from pollution and conversion to lead sulfide, or if another coating was present that preserved the lead white films in The Morgan's drawings.

Conclusions

The present article describes the first, comprehensive scientific study of a selection of drawings by 18th-century English artist Thomas Gainsborough in the collection of The Morgan Library & Museum, New York. Visual examination of the artworks combined with in-depth analysis by means of a variety of instrumental techniques have been carried out in order to explore the artist's mastery of materials and innovative practices for making drawings, as described in a letter that he wrote to his friend William Jackson in January 1773. Among the main topics investigated are Gainsborough's choice of white pigments and, most interestingly, his reported use of milk-based fixatives to protect the media and prevent pigments from discoloring. Results of this study have shown that, in addition to lead white, calcite was also employed in several works created during the last decade of Gainsborough's artistic production, which may attest to an intrinsic evolution in the artist's choice of materials over the course of his career. Moreover, the artist appears to have applied milk from domestic cows as casein-based fixative on his drawings from 1750–1785, regardless of the media and white pigments used. The data collected indicate that visual examination does not correlate to the actual presence of fixative, which can only be unambiguously confirmed through scientific analysis. Gainsborough's documented practice of dipping his artworks in a fixative bath was initially supported by normal light and UV photography of one of the drawings examined, namely *Landscape with Horse and Cart Descending a Hill* (ca. 1780; III, 63). In this work, a clean corner at the upper left, very distinct from the overall deteriorated coating, possibly corresponds to the location where the work might have been held by the artist when dipping it in milk. The consistent qualitative identification of the milk proteins sampled on all the drawing surfaces would appear to indicate a similar deposition across both the front and reverse of the drawings. This likely supports an overall dipping of the paper rather than a localized application of a fixative by brush or random non-specific transfer from storage or handling.

In conclusion, the present study clearly demonstrates the necessity of a multifaceted approach combining art historical research, examination of the available documentary sources, careful inspection of the artworks, as well as advanced micro-sampling tools, *ad-hoc* sample preparation methods, highly sensitive analytical techniques, and sophisticated bioinformatics data processing to shed light on artists and their practices. In addition to granting conservators and art historians a deeper understanding of the complexity of Gainsborough's materials and innovative drawing techniques, this work paves the way for further investigations aiming to probe the use of milk-based fixatives by other artists working on paper such as Degas and Van Gogh. Additional work will be also required to assess more accurately the milk fixative identified on Gainsborough's drawings as representative of 18th-century skim milk. In the near future, the cutting-edge methodologies and techniques combined here may similarly provide useful insight into different classes of materials, such as paper sizing as well as gum Arabic coatings, among others.

Abbreviations

XRF: X-ray fluorescence spectroscopy; nano-LC-ESI-MS/MS or nano-LC/MS: Nano-liquid chromatography-electrospray ionization-mass spectrometry/mass spectrometry.

Acknowledgements

The authors would like to acknowledge the European Union (FEDER, Marie Skłodowska Curie European Training Network), the CNRS, the University of Bordeaux and IDEX (Excellence Initiative of University of Bordeaux), the Nouvelle-Aquitaine Region (France), the Institut Universitaire de France and the IBISA network (Infrastructures en Biologie Santé et Agronomie) for supporting this work. The authors are also grateful to Dr. Dan Kirby for his guidance and for generously sharing his sampling protocols. Additionally, FP acknowledges Dr. Anna Cesaratto for her initial contribution to this project during her time at The Met working in the NICS program, while RS would also like to thank Abigail Merritt and Justine Provino for performing UVA photography of the artworks studied.

Authors' contributions

FP coordinated the study, carried out XRF and Raman analysis and data interpretation, collected all results, and wrote an initial draft of the manuscript. JA removed samples for nano-LC/MS analysis and interpreted the proteomic results with CT. FG and CT carried out nano-LC/MS sample preparation, data analysis, interpretation and description of results. RS provided art historical context, performed visual examination, supported the scientific work, and helped with drafting the article's Introduction. All authors read and approved the final manuscript.

Funding

This research was made possible by the Network Initiative for Conservation Science (NICS), a Metropolitan Museum of Art program. Support for NICS was provided by a grant (31500630) from The Andrew W. Mellon Foundation. FG is supported by the Marie Skłodowska Curie European Training Network (ETN) "TEMPERA", a project funded by the European Union's EU Framework Programme for Research and Innovation Horizon 2020 (Grant Agreement number 722606). This project benefits from the support of the CNRS International Laboratory ARCHE ARt and Cultural HEritage: Natural Organic Polymers by Mass Spectrometry.

Availability of data and materials
All data generated during this study are included in this published article.

Competing interests
The authors declare that they have no competing interests.

Author details
[1] Department of Scientific Research, The Metropolitan Museum of Art, 1000 Fifth Avenue, New York, NY 10028, USA. [2] Institute of Chemistry & Biology of Membranes & Nano-Objects, University of Bordeaux, UMR CNRS 5248, Proteome Platform, 146 rue Léo Saignat, Bordeaux 33000, France. [3] Thaw Conservation Center, The Morgan Library & Museum, 225 Madison Avenue, New York, NY 10016, USA.

Received: 18 June 2020 Accepted: 10 August 2020
Published online: 24 August 2020

References

1. Snyder RF. Thomas Gainsborough: a master's techniques revealed. In: Thomas Gainsborough: experiments in drawing. London: The Morgan Library & Museum in association with Paul Holberton Publishing; 2018. p. 27–37.
2. Kirby DP, Manick A, Newman R. Minimally invasive sampling of surface coatings for protein identification by peptide mass fingerprinting: a case study with photographs. J Am Inst Conserv. 2019. https://doi.org/10.1080/01971360.2019.1656446.
3. Dallongeville S, Garnier N, Rolando C, Tokarski C. Proteins in art, archaeology, and paleontology: from detection to identification. Chem Rev. 2016;116(1):2–79.
4. Erde J, Ogorzalek Loo RR, Loo JA. Enhanced FASP (eFASP) to increase proteome coverage and sample recovery for quantitative proteomic experiments. J Proteome Res. 2014;13(4):1885–95.
5. Hayes J. Gainsborough drawings: a supplement to the catalogue raisonné. Master drawings. 1983;21(4):367–91.
6. Fiddyment S, Holsinger B, Ruzzier C, Devine A, Binois A, Albarella U, Fischer R, Nichols E, Curtis A, Cheese E, Teasdale MD. Animal origin of 13th-century uterine vellum revealed using noninvasive peptide fingerprinting. PNAS. 2015;112(49):15066–71.
7. Roepstorff P, Fohlman J. Proposal for a common nomenclature for sequence ions in mass spectra of peptides. Biomed Mass Spectrom. 1984;11(11):601.
8. Bär C, Mathis D, Neuhaus P, Dürr D, Bisig W, Egger L, Portmann R. Protein profile of dairy products: simultaneous quantification of twenty bovine milk proteins. Int Dairy J. 2019;97:167–75.
9. Dewettinck K, Rombaut R, Thienpont N, Le TT, Messens K, Van Camp J. Nutritional and technological aspects of milk fat globule membrane material. Int Dairy J. 2008;18(5):436–57.
10. Boland M, Singh H, Thompson A, editors. Milk proteins: from expression to food. 2nd ed. Cambridge, MA: Academic Press; 2014.
11. Farrell JH. Milk proteins | Casein nomenclature, structure, and association. In: Fuquay JW, Fox PF, McSweeney PLH, editors. Encyclopedia of dairy sciences. 2nd ed. Cambridge, MA: Academic Press; 2011. p. 765–71.
12. Deeth HC, Lewis MJ. High temperature processing of milk and milk products. Hoboken, NJ: John Wiley & Sons; 2017.
13. Gasparini A, Buhler S, Faccini A, Sforza S, Tedeschi T. Thermally-induced lactosylation of whey proteins: identification and synthesis of lactosylated β-lactoglobulin epitope. Molecules. 2020;25(6):1294.
14. Czerwenka C, Maier I, Pittner F, Lindner W. Investigation of the lactosylation of whey proteins by liquid chromatography — mass spectrometry. J Agric Food Chem. 2006;54(23):8874–82.
15. Barbana C, Pérez MD. Interaction of α-lactalbumin with lipids and possible implications for its emulsifying properties–A review. Int Dairy J. 2011;21(10):727–41.
16. Vegarud GE, Langsrud T, Svenning C. Mineral-binding milk proteins and peptides; occurrence, biochemical and technological characteristics. Br J Nutr. 2000;84(S1):91–8.
17. Singh H. Milk protein products | Functional properties of milk proteins. In: Fuquay JW, Fox PF, McSweeney PLH, editors. Encyclopedia of dairy sciences. 2nd ed. Cambridge, MA: Academic Press; 2011. p. 887–93.
18. Lordan R, Tsoupras A, Zabetakis I. Phospholipids of animal and marine origin: Structure, function, and anti-inflammatory properties. Molecules. 2017;22(11):1964.
19. Mather IH. Milk lipids: milk fat globule membrane. In: Fuquay JW, Fox PF, McSweeney PLH, editors. Encyclopedia of dairy sciences. 2nd ed. Cambridge, MA: Academic Press; 2011. p. 680–90.
20. Blundel R, Tregear A. From artisans to "factories": the interpenetration of craft and industry in English cheese-making, 1650–1950. Enterp Soc. 2006;7(4):705–39.
21. Atkins PJ. The pasteurization of England: the science, culture and health implications of milk processing, 1900–1950. In: Smith DF, Phillips J, editors. Food, science, policy and regulation in the twentieth century: international and comparative perspectives. London: Routledge; 2000. p. 37–51.
22. Park YW, Haenlein GF, editors. Milk and dairy products in human nutrition: production, composition and health. Hoboken, NJ: John Wiley & Sons; 2013.
23. Fox PF. The major constituents of milk. In: Smit G, editor. Dairy processing: improving quality. Boca Raton, FL: CRC Press and Woodhead Publishing Limited; 2003. p. 5–41.
24. Zheng H, Jiménez-Flores R, Everett DW. Bovine milk fat globule membrane proteins are affected by centrifugal washing processes. J Agric Food Chem. 2013;61(35):8403–11.
25. Vincent D, Ezernieks V, Elkins A, Nguyen N, Moate PJ, Cocks BG, Rochfort S. Milk bottom-up proteomics: method optimization. Front Genet. 2016;6:360.
26. Haddadian Z, Eyres GT, Carne A, Everett DW, Bremer P. Impact of different milk fat globule membrane preparations on protein composition, xanthine oxidase activity, and redox potential. Int Dairy J. 2017;64:14–21.
27. Shelley M. Painting in the dry manner the flourishing of pastel in 18th-century Europe. Bull Metropolitan Museum Art. 2011;68(4):5–6.
28. Baetjer K, Shelley M. Pastel portraits: images of 18th-century Europe. New York: The Metropolitan Museum of Art; 2011. p. 4–56.
29. Ellis MH. The shifting function of artists' fixatives. J Am Inst Conserv. 1996;35(3):239–54.
30. Norgate E, Muller JM, Murrell J. Miniatura, or, The art of limning. Edited from the Manuscript in the Bodleian Library and collated by Martin Hardie. Oxford, UK: Oxford at the Clarendon Press; 1929. p. 8-9.
31. Coltelli MB, Wild F, Bugnicourt E, Cinelli P, Lindner M, Schmid M, Weckel V, Müller K, Rodriguez P, Staebler A, Rodríguez-Turienzo L. State of the art in the development and properties of protein-based films and coatings and their applicability to cellulose based products: an extensive review. Coatings. 2016;6(1):1.
32. Chen H, Wang J, Cheng Y, Wang C, Liu H, Bian H, Pan Y, Sun J, Han W. Application of protein-based films and coatings for food packaging: a review. Polymers. 2019;11(12):2039–71.
33. Imbrogno J, Nayak A, Belfort G. Egg white varnishes on ancient paintings: a molecular connection to amyloid proteins. Angew Chem Int Ed. 2014;53(27):7014–7.
34. Derow JP. Gainsborough's varnished watercolor technique. Master drawings. 1988;26(3):259–308.

Publisher's Note