

Identification of lipid- and protein-based binders in paintings by direct on-plate wet chemistry and matrix-assisted laser desorption ionization mass spectrometry

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Abstract Direct on-target plate processing of small (ca. 100 µg) fragments of paint samples for MALDI-MS identification of lipid- and protein-based binders is described. Fragments were fixed on a conventional stainless steel target plate by colloidal graphite followed by in situ fast tryptic digestion and matrix addition. The new protocol was first developed on paint replicas composed of chicken egg, collagen, and cow milk mixed with inorganic pigments and then successfully applied on historical paint samples taken from a fifteenth century Italian panel painting. The present work contributes a step forward in the simplification of binder identification in very small paint samples since no conventional solvent extraction is required, speeding up the whole sample preparation to 10 min and reducing lipid/protein loss.

Keywords Painting · Colloidal graphite · MALDI · Lipid · Protein

Introduction

Paint layers of polychrome works of art are typically made up of colored pigments and a medium or binder such as drying

oil, egg, animal glue, casein, and vegetal gum. Identification of the original binder components through their constitutive molecules (e.g., lipids, proteins, polysaccharides, etc.) and investigation of their modifications induced by ageing processes are of primary importance for the conservation and preservation of paintings and other polychrome artefacts. For instance, the characterization of organic materials in artistic paintings is typically performed by gas and liquid chromatography (GC and LC) or pyrolysis-GC [1] that, however, require several pre-analytical steps including laborious methods of hydrolysis and derivatization.

The introduction of soft ionization (I) methods like matrix-assisted laser desorption (MALD) and electrospray (ES) has greatly contributed to the explosion of mass spectrometry (MS)-based techniques in the field of the omics sciences. As an obvious consequence, MS-based proteomics studies started to be increasingly appreciated also in the field of cultural heritage where non-destructive techniques are typically preferred and the sample amount for classical analysis is drastically limited. Such a limitation can be easily faced by the inherently high sensitivity of MALDI or nano-LC-ESI-MS as demonstrated by several papers reporting protein identification by the classical bottom-up approach [2–4]. Sample pretreatment is generally quite laborious: proteins are extracted, making use of appropriate alkaline or acidic media, and then submitted to processes such as denaturation/reduction/alkylation and finally enzymatic (usually tryptic) digestion. Otherwise, digestion with trypsin may be directly performed on paint fragments or cross sections, followed by purification of the peptide solution before MALDI or nano-LC-ESI-MS analysis [5, 6].

Although MALDI-MS has been widely used in the field of cultural heritage for protein analysis, its application to non-proteinaceous materials is much more limited and has been reported in only a few studies. For instance, van den Berg et al. [7, 8] have investigated the oxidative ageing of drying oil,

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while van den Brink et al. reported on cholesterol oxidation products [9] and on the effects of ageing on egg-based paint specimens [10]. Moreover, some MALDI-MS studies have been dedicated to colorants [11], terpenoid resins [12], and polyethylene glycols [13].

A new protocol, based on different extraction solvents, for MALDI analysis of lipids, e.g., phospholipids (PLs) and triacylglycerols (TAGs), and proteins contained in a single small specimen of paint has been recently reported [14–16]. The present work contributes a step forward in the simplification of such a protocol as no conventional solvent extraction is required. The paint fragment (ca. 100 μg) was fixed on the MALDI target plate, and the wet chemistry steps required for protein digestion were performed directly on the plate that, after matrix addition, was analyzed by MALDI-MS for the simultaneous identification of proteinaceous and lipid markers of the most commonly used binders. Colloidal graphite was used to assure the adhesion of the painting fragment (as such or after grinding) on a conventional 96-well stainless steel target plate; graphite is known to enhance thermal and electrical conduction, possibly favoring laser desorption ionization (LDI) [17–19]. Indeed, graphite-assisted (GA)LDI has been used, in the field of cultural heritage, for the identification of triterpenoid resins [20, 21] and other organic materials (e.g., natural resins, oils, waxes, and ketone resins) after solvent extraction [22].

The protocol here proposed was developed on specimens from paint replicas made of egg yolk, milk, and different types of animal glue mixed with various pigments; its potential was finally demonstrated analyzing a fifteenth century Italian panel painting.

Experimental

Materials

α -Cyano-4-hydroxycinnamic acid (CHCA), ACTH 18-39 fragment, angiotensin I, renin, trypsin proteomic grade, ammonium bicarbonate, trilaurin (LLL), trimyristin (MMM), tripalmitin (PPP), and tristearin (SSS) were obtained from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA). HPLC-grade water, acetonitrile (ACN), trifluoroacetic acid (TFA), and methanol (MeOH) (Sigma-Aldrich) were used without further purification. α -Cyano-4-chlorocinnamic acid (CCICA) was synthesized according to a previously reported procedure [23]. Fresh chicken eggs and whole cow milk were purchased at local supermarkets. Prussian blue, ultramarine, red ochre, vermilion, red lead, San Giovanni white, Naples yellow, and burnt Siena pigments were purchased from Carezza (Bari, Italy). Azurite, indigo, and lead white pigments and the collagen standards (bovine bone glue (63000), bovine skin glue (63010), rabbit skin glue (63025), and Saliansky

isinglass glue (63110)) were obtained from Kremer Pigmente GmbH & Co (Aichstetten, Germany). Conducting graphite paint (code n. AGG3418), containing colloidal graphite and a thermoplastic resin as binder (in isopropanol), was obtained from Agar Scientific (Stansted, UK).

Paint replicas were prepared on 76×26 mm glass slides by applying mixtures of chicken egg yolk, cow milk, and various types of collagen with different, abovementioned, pigments. Some egg yolk/pigment samples were naturally aged indoors, in ambient conditions for 1.5 years. Micro-samples (ca. 100 μg) were collected from various zones of the polyptych “Christ in Pity, St. Louis of Toulouse, St. Francis of Assisi, St. John Baptist, St. Anthony of Padua” (1467) by Antonio Vivarini (Pinacoteca Provinciale “Corrado Giaquinto”, Bari, Italy).

Analytical protocol

Few fragments (ca. 100 μg) were taken from each paint replica or paint sample and were applied, as such or after finely grinding, on the target spot previously covered by a small drop of conducting graphite paint. For on-spot protein digestion, 1 μL of trypsin solution (0.04 $\mu\text{g}/\mu\text{L}$ in 50 mM NH_4HCO_3) was deposited, followed by 1 μL of 50 mM NH_4HCO_3 . The target plate was placed in a Petri dish containing a wet piece of paper (to ensure a humid environment), and kept in the oven at 40 °C for 10 min (this step can be, obviously, omitted when the analysis is limited to lipids). Then, 1 μL of CCICA (10 mg/mL in ACN:H₂O 2:1 with 0.1 % TFA) was added, the spots were allowed to air-dry, and the MALDI-MS analysis (positive ion mode) was run simultaneously for both peptide and lipid fractions. For some samples, on-target washing with 1 μL of LC-MS water was carried out.

Before introducing the target plate into the instrument, it was blown with a flux of nitrogen in order to eliminate any graphite or solid sample fragments that did not adhere to the surface.

Database search

The raw MS files relevant to tryptic digests were searched against the non-redundant database Swiss-Prot utilizing the Mascot (or MS-Fit) server. The search was performed against the Chordata (or *Gallus gallus*) database for egg samples and Mammalia (or *Bos taurus*) for milk and collagen samples, setting the oxidation of methionines as a variable modification. Up to three missed cleavages were accepted, and a mass tolerance of 0.15 Da was set. Alternatively, the search was performed using the FindPept database. In this case, a list of all possible proteins as contained in egg, milk, and collagen was created and raw MS files relevant to

Table 1 List of main *m/z* ions observed in the spectra derived from on-spot tryptic digestion of paint replicas composed of different collagen standards mixed with vermilion and their possible attributions as found (a) by the FindPept tool or in (b) Tripkovic et al. [25], (c) Kirby et al. [24], and (d) Fremout et al. [2]

Bovine bone glue	Bovine skin glue	Rabbit skin glue	Isinglass glue	Protein
1088.51 ^b	836.18 ^a	836.22 ^a	1427.52 ^{a,b,c,d}	Collagen alpha-1(I) chain precursor (alpha-1 type I collagen) <i>Bos taurus</i> (bovine)
1105.56 ^{b,c,d}	886.24 ^a	1088.56 ^b		
1121.56 ^b	898.40 ^{b,d}	1095.53 ^b		
1264.56 ^a	1088.48 ^b	1105.52 ^{b,c,d}		
1427.67 ^{a,b,c,d}	1095.49 ^b	1427.78 ^{a,b,c,d}		
1568.77 ^a	1105.52 ^{b,c,d}	1435.66 ^b		
1931.84 ^a	1121.52 ^b	1459.68 ^b		
	1177.61 ^b	1586.64 ^{b,d}		
	1201.51 ^{b,d}	1975.90 ^{a,b}		
	1332.61 ^b			
	1427.62 ^{a,b,c,d}			
	1459.59 ^b			
	1568.65 ^a			
	1586.66 ^{b,d}			
	1975.85 ^{a,b}			
	2056.87 ^{a,b}			
	2130.92 ^b			
	2884.13 ^a			
821.19 ^a	852.17 ^a	1095.53 ^b	1427.52 ^{a,b,c}	Collagen alpha-2(I) chain precursor (alpha-2 type I collagen) <i>Bos taurus</i> (bovine)
1427.67 ^{b,d}	868.20 ^a	1267.58 ^b		
1568.77 ^a	898.40 ^{a,b}	1427.78 ^{a,b,c}		
2011.75 ^a	1095.49 ^b	1975.90 ^b		
2027.77 ^a	1240.58 ^a			
	1267.59 ^b			
	1427.62 ^{a,b,d}			
	1562.73 ^b			
	1568.65 ^a			
	1975.85 ^b			
	2130.92 ^{b,c}			
837.16 ^a	836.18 ^a	836.22 ^a	1427.52 ^{a,b,c,d}	Collagen alpha-1(II) chain precursor (alpha-1 type II collagen) <i>Bos taurus</i> (bovine)
1096.53 ^a	852.17 ^a	1427.78 ^{a,c}		
1333.65 ^a	868.20 ^a	1975.90 ^a		
1425.70 ^a	1177.61 ^a			
1447.67 ^a	1427.62 ^{a,c}			
1931.84 ^a	1568.65 ^a			
	1975.85 ^a			
887.27 ^a				
1096.53 ^a				
1241.58 ^a				
837.16 ^a				
1202.51 ^a				
		1088.56 ^a		Collagen alpha-1(III) chain <i>Bos taurus</i> (bovine)
		1976.91 ^a		Collagen alpha-4(IV) chain (fragment) <i>Bos taurus</i> (bovine)
			1504.52 ^a	Collagen alpha-1(XII) chain <i>Oryctolagus cuniculus</i> (rabbit)
			1818.60 ^a	Collagen alpha-2(I) chain precursor (alpha-2 type I collagen)
			2139.64 ^a	<i>Oncorhynchus mykiss</i> (rainbow trout) (<i>Salmo gairdneri</i>)

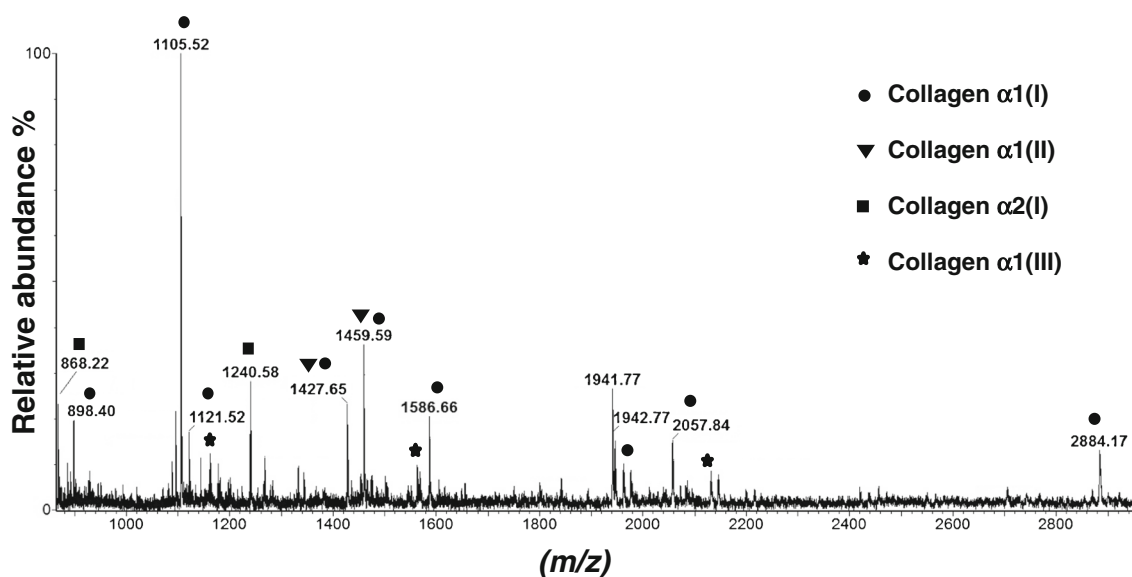


Fig. 1 MALDI-TOF mass spectrum of the tryptic digest obtained from direct on-spot digestion of the model paint composed of bovine skin collagen mixed with vermilion

tryptic digests were searched against one protein at a time. Other modifications, such as oxidation of methionine, proline, cysteine, and tryptophan, and deamidation of asparagine and glutamine, likely occurring during ageing processes, were included for aged samples. For lipid analysis, the main ions in the mass spectra were assigned by using the LIPID MAPS database [<http://www.lipidmaps.org/>].

Instrumentation

MS experiments were performed using a Micromass M@LDITM-LR time-of-flight mass spectrometer (Waters MS Technologies, Manchester, UK) equipped with a nitrogen UV laser (337 nm wavelength), a precision flat target plate sample introduction system bearing a micro-titer target plate, reflectron optics, and a fast dual micro-channel plate (MCP) detector.

Positive ion mass spectra were acquired in the mass range 400–4000 m/z in reflectron mode; the following voltages were applied: pulse, 2610 V; source, 15,000 V; reflectron, 2000 V; and MCP, 1900 V. The resulting spectra were averaged, background subtracted, and smoothed by a Savitzky-Golay algorithm. A time lag focusing (TLF) delay of 500 ns was used between the laser pulse and the application of the accelerating voltage. Mass calibration was performed using a peptide mixture composed of fragment 18–39 of the adrenocorticotrophic hormone (ACTH), renin and angiotensin for peptide analysis, and a triacylglycerol mixture composed of trilaurin, trimyristin, tripalmitin, and tristearin for lipid analysis. For the sake of clarity, spectra are shown in the ranges 400–900 m/z for lipids and 900–4000 m/z for peptides.

Results and discussion

Paint replicas

To prove the feasibility of the proposed protocol for analysis of organic binders, paint replicas composed of chicken egg yolk, different types of animal glue (collagen), and cow milk mixed with various pigments were used. At first, paint replicas composed of collagen, i.e., bovine bone glue, bovine skin glue, rabbit skin glue, and Saliensky isinglass glue, mixed with vermilion were analyzed. From the database search and comparison with literature, it was possible to assign the main detected peptides (Table 1). In particular, the peptide masses were compared with those previously reported by Kirby et al. in a study on collagen-based materials used in cultural heritage artefacts [24], by Tripković et al. in a MALDI-MS/MS study on the identification of protein binders [25], and by Fremout et al. [2]. Figure 1 reports the MALDI mass spectrum obtained from on-spot tryptic digestion of the model sample made of bovine skin collagen mixed with vermilion. The main detected peptides (marked in Fig. 1) were assigned to different collagen chains: alpha-1(I) (COL1A1), alpha-2(I) (COL1A2), alpha-1(II) (COL2A1), and alpha-1(III) (COL3A1) (mass accuracy on m/z value is typically ± 0.1 Da). The highest number of attributed peptides was found for bovine glues and for the rabbit skin glue, while for the isinglass glue only few masses could be recognized. Almost all attributed peptides of the rabbit glue coincide with those of the bovine glues, whereas the isinglass glue peptides are different. In fact, distinction between mammalian and non-mammalian (e.g., sturgeon) origin of animal glue is rather feasible, but discrimination between different mammalian species is much more difficult

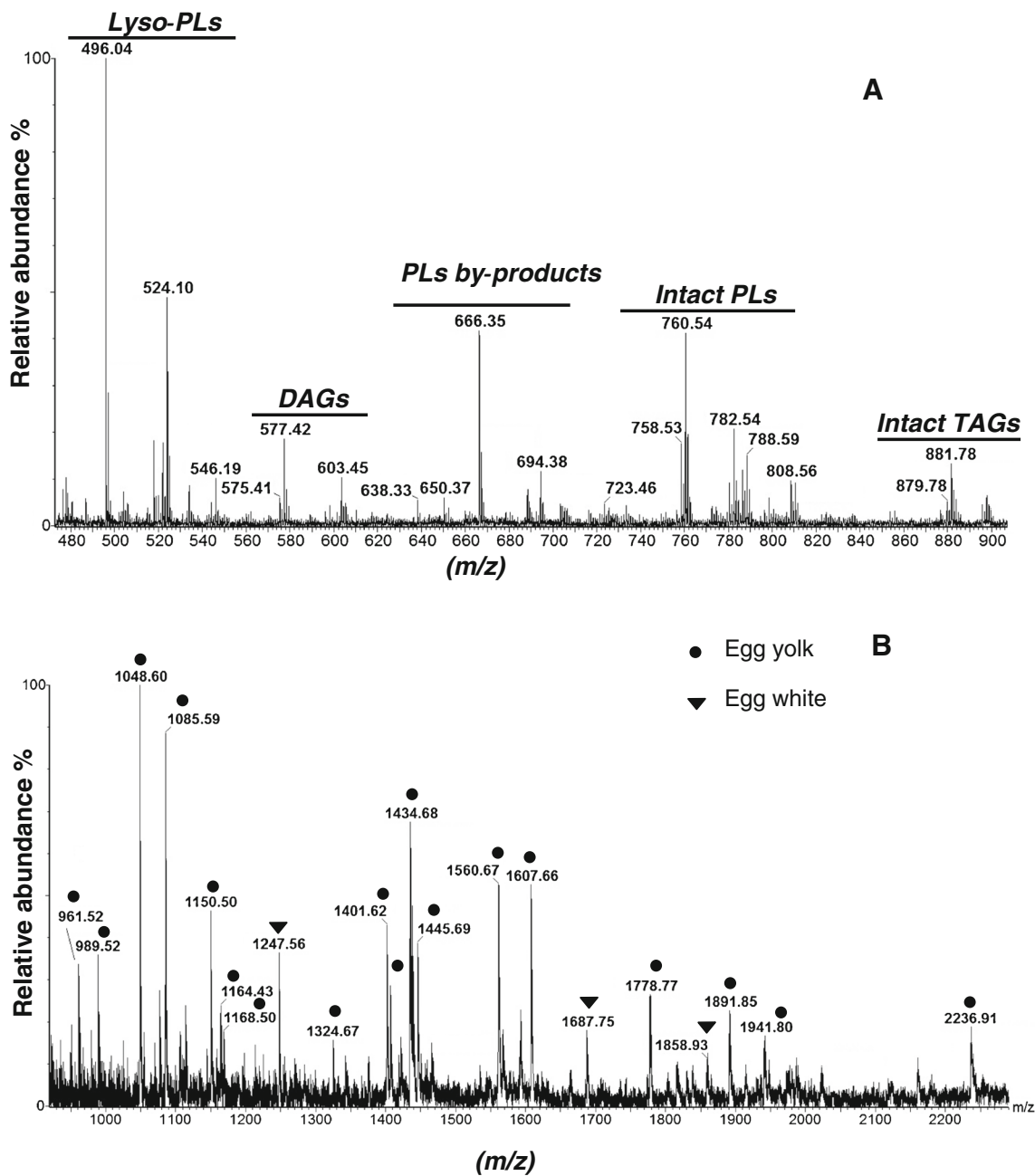


Fig. 2 MALDI-TOF mass spectra of the lipids (A) and on-spot tryptic digest (B) obtained from the aged model paint composed of egg yolk mixed with ultramarine

[2]. This is due to a strong homology (in some cases up to 92 %) and to a lack of data on collagen sequences; it is worth noting that, very recently, new peptide markers have been proposed for discrimination of some mammalian species [24, 26, 27].

In conclusion, our data show that all the information necessary to discriminate between collagen binders of mammalian (i.e., bovine and rabbit) or fish (i.e., sturgeon) origin was acquired in about 15 min without any particular sample pre-treatment.

To further assess the potential of the procedure in characterizing organic materials in aged paintings, different egg yolk/pigment paint replicas aged for 1.5 year were analyzed. Figure 2 shows the mass spectra of the lipids (A) and the tryptic digest (B) obtained from an aged paint replica composed of egg yolk and ultramarine pigment. Previous studies on the lipid composition of egg yolk [15] showed that most of them were phosphatidylcholines (PCs) with small amounts of TAGs. However, as expected, after the ageing process, the occurrence of additional signals due to oxidation by-products

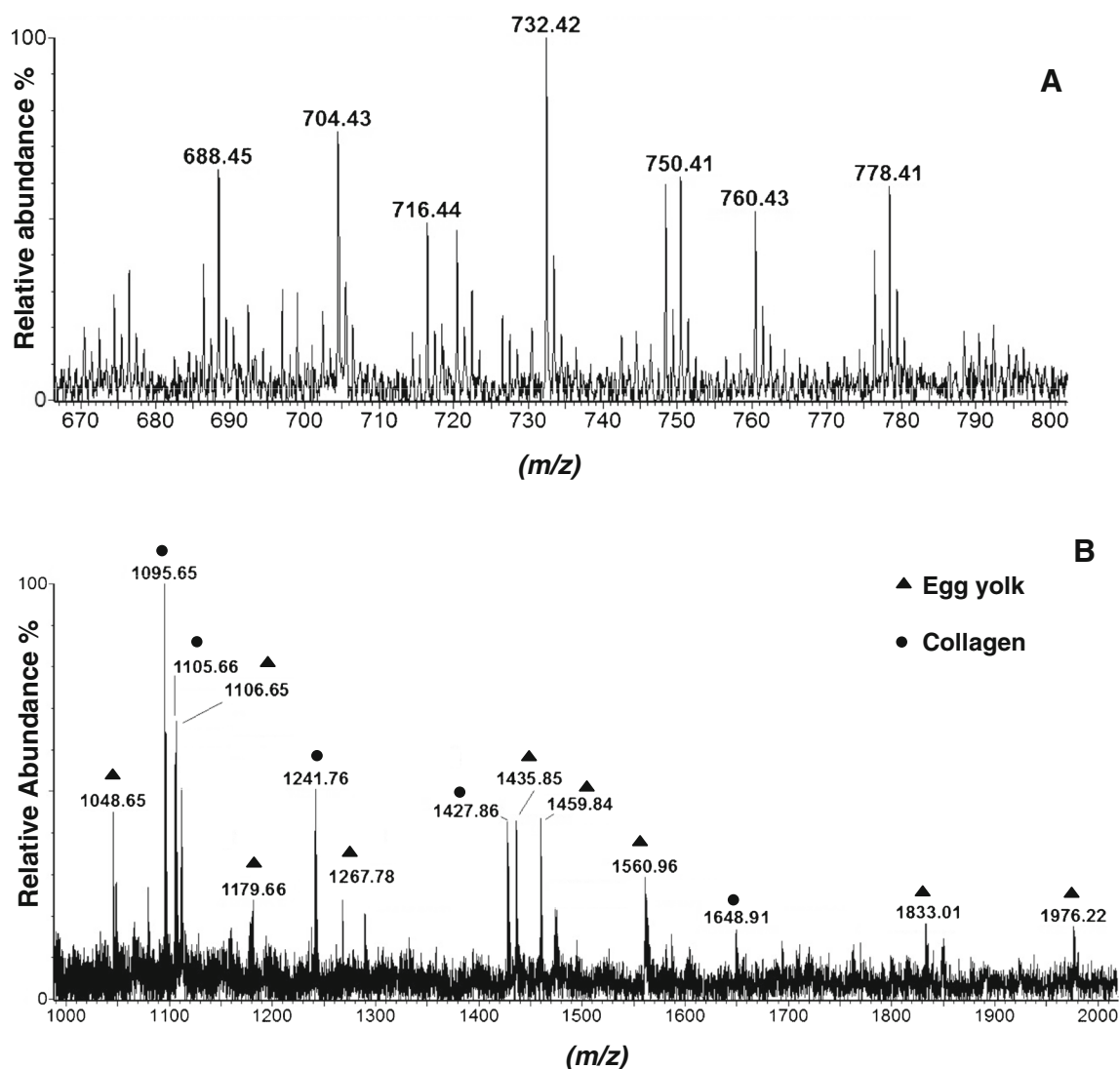


Fig. 3 MALDI-TOF mass spectra of the lipids (A) and on-spot tryptic digest (B) obtained from a sample of the polyptych by Antonio Vivarini (1467)

was observed. All the main ions in the mass spectrum were assigned by using the LIPID MAPS database [<http://www.lipidmaps.org/>] and the existing literature on this topic [15, 28]. In detail, the m/z range from 470 to 610 (see Fig. 2A) includes signals consistent with their assignment to lysophospholipids (Lyso-PLs) and diacylglycerols (DAGs) formed by hydrolysis of native phospholipids and TAGs, respectively. The m/z range between 620 and 700 is characterized by ions that can be attributed to by-products, likely formed through a β -cleavage mechanism of PLs. Phospholipids are also present as intact or mono-oxidized species in the m/z range of 750–820. Finally, TAGs are observed in the mass window of 850–900 m/z , mainly as sodiated ion adducts. These results clearly show that the developed protocol allowed a rapid identification of all the marker lipids of the egg-based binder.

As for the protein fraction (Fig. 2B), the major proteins of egg yolk and some peptides from egg white could be identified. In particular, many peptides could be attributed to vitellogenin-1 (m/z 1077.53, 1085.57, 1105.50, 1144.62, 1406.58, 1418.70, 1439.70, 1663.88), vitellogenin-2 (m/z 961.52, 989.52, 1048.60, 1114.57, 1164.48, 1324.67, 1401.62, 1434.68, 1435.67, 1445.69, 1560.67, 1607.66, 1777.78, 1890.84, 2235.93), vitellogenin-3 (m/z 1150.50, 1437.68, 1815.88, 1940.74), avidin (m/z 1247.56), and ovalbumin (m/z 1687.75, 1858.93). The presence of few peptides of egg white proteins in the egg yolk/pigment samples is probably due to contamination during the membrane cutting of the egg yolk or by natural migration of egg white proteins through the membrane known to occur in old eggs [29].

In order to validate our protocol, the peptide list of the egg yolk samples was compared with literature data

obtained by using various mass spectrometric techniques (MALDI-MS, LC-MS/MS) and different sample pre-treatments [2–5]. This comparison clearly proved the soundness of our procedure since it allowed the identification of almost all the peptides reported in the literature studies.

It should be pointed out that analogous results were obtained for the different tested pigments both for proteins and lipids, suggesting that their presence does not affect the efficiency of the proposed protocol.

In order to further evaluate the effectiveness of the novel strategy, replicas composed of cow milk and vermilion were investigated. On-spot tryptic digestion yielded a mass spectrum in which all the main peptides attributed to caseins and whey proteins were clearly observed, providing a coverage of 45 % for α -s1-casein, 53 % for α -s2-casein, and 30 % for β -casein. Moreover, the mass spectrum showed intense peaks attributed to sodiated TAG species containing short- and medium-chain saturated fatty acids according to the milk fatty acid composition [30]. So, the proposed protocol seems also to be a valuable tool for identification of casein/milk binders.

Historical paint samples

Finally, the protocol was applied to historical paint sample analysis. In particular, some micro-samples (ca. 100 μ g) taken from the polyptych by Antonio Vivarini were examined. The fragments were crushed and deposited on the colloidal graphite as described for the paint replicas. Figure 3B reports the MALDI mass spectrum relevant to the tryptic digest of a sample taken from the white signature painted on a red-orange background. The pigments were identified, by micro-Raman spectroscopy, as lead white and red ochre.

It should be highlighted that most proteins in real samples show some degree of modification [31], due to ageing processes that contribute as variable modifications during database search (see “Database search”). Through this approach, it was, indeed, possible to identify many peptides (m/z 1095.65, 1105.65, 1241.76, 1427.86, 1459.84, 1473.82, 1648.91—mass accuracy of typically 0.05 Da) attributed to collagen [2, 24, 25], indicating that animal glue had been used in the gypsum ground and/or paint layer. Moreover, the presence of some peptides (m/z 1048.59, 1106.66, 1179.66, 1267.78, 1435.85, 1459.84, 1560.96, 1833.01, 1976.22) from vitellogenin-1 and vitellogenin-2, already observed in the egg paint replicas, suggests the use of an egg-based paint binder.

Analysis of the lipid fraction (Fig. 3A) led to the detection of ions at m/z 688.45, 704.48, 716.47, 732.42, 750.41, 760.43, and 778.41, likely related to phospholipids and their degradation products [14–16], thereby confirming, once again, the

presence of egg. For comparison, some paint fragments were analyzed with the protocol based on different extraction solvents [14, 16], providing very similar results.

Conclusions

A novel strategy for on-spot analysis of lipids and proteins in paint samples is proposed. On-target lipid analysis and protein digestion using colloidal graphite as a support were shown to be very efficient in speeding up the sample preparation. Graphite seems to improve the digestion efficiency simplifying, at the same time, the purification step since desalting can be efficiently accomplished by on-target washing. The subsequent MALDI-MS analysis provides important and exhaustive information for the recognition of organic binders through the detection of specific peptide and lipid markers. The main point of strength of our approach is that it would allow to easily manage very small (<100 μ g) paint fragments, with minimum sample processing, thus avoiding the risk of either material loss or contamination.

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References

1. Colombini MP, Modugno F (2004) *J Sep Sci* 27:147–160
2. Fremout W, Kuckova S, Crhova M, Sanyova J, Saverwyns S, Hynek R, Kodicek M, Vandenabeele P, Moens L (2011) *Rapid Commun Mass Spectrom* 25:1631–1640
3. Tokarski C, Martin E, Rolando C, Cren-Olivé C (2006) *Anal Chem* 78:1494–1502
4. Leo G, Cartechini L, Pucci P, Sgamellotti A, Marino G, Birolo L (2009) *Anal Bioanal Chem* 395:2269–2280
5. Kuckova S, Hynek R, Kodicek M (2007) *Anal Bioanal Chem* 388: 201–206
6. Kuckova S, Sandu ICA, Crhova M, Hynek R, Fogas I, Muralha VS, Sandu AV (2013) *Microchem J* 110:538–544
7. van den Berg JDJ, Vermist ND, Boon JJ (2011) *Adv Mass Spectrom* 15:823–824
8. van den Berg JDJ, Vermist ND, Carlyle L, Holcapek M, Boon JJ (2004) *J Sep Sci* 27:181–199
9. van den Brink O, Ferreira ESB, van der Horst J, Boon JJ (2009) *Int J Mass Spectrom* 284:12–21
10. van den Brink OF, Boon JJ, O’Connor PB, Duursma MC, Heeren RMA (2001) *J Mass Spectrom* 36:479–492
11. Maier MS, Parera SD, Seldes AM (2004) *Int J Mass Spectrom* 232: 225–229
12. Scalpone D, Duursma MC, Boon JJ, Chiantore OJ (2005) *J Mass Spectrom* 40:1527–1535
13. Hoogland FG, Boon JJ (2009) *Int J Mass Spectrom* 284:66–71
14. van der Werf ID, Calvano CD, Palmisano F, Sabbatini L (2012) *Anal Chim Acta* 718:1–10
15. Calvano CD, van der Werf ID, Palmisano F, Sabbatini L (2011) *Anal Bioanal Chem* 400:2229–2240

16. van der Werf ID, Calvano CD, Laviano R, Simonetti A, Sabbatini L (2013) *Microchem J* 106:87–94
17. Sunner J, Dratz E, Chen YC (1995) *Anal Chem* 67:4335–4342
18. Li X, Wilm M, Franz T (2005) *Proteomics* 5:1460–1471
19. Larsen MR, Cordwell SJ, Roepstorff P (2002) *Proteomics* 2:1277–1287
20. Zumbuhl S, Knochenmuss R, Wulfert S, Dubois F, Dale MJ, Zenobi R (1998) *Anal Chem* 70:707–715
21. Dietemann P, Kälin M, Zumbühl S, Knochenmuss R, Wulfert S, Zenobi R (2001) *Anal Chem* 73:2087–2096
22. Dietemann P, Herm C (2009) In: Colombini MP, Modugno F (eds) *Organic mass spectrometry in art and archaeology*. John Wiley & Sons, pp 131–163
23. Carulli S, Calvano CD, Palmisano F, Pischetsrieder M (2011) *J Agric Food Chem* 59:1793–1803
24. Kirby DP, Buckley M, Promise E, Trauger SA, Holdcraft TR (2013) *Analyst* 138:4849–4858
25. Tripković T, Charvy C, Alves S, Lolić AD, Baošić RM, Nikolić-Mandić SD, Tabet JC (2013) *Talanta* 113:49–61
26. Buckley M, Collins M, Thomas-Oates J, Wilson JC (2009) *Rapid Commun Mass Spectrom* 23:3843–3854
27. Buckley M, Kansa SW, Howard S, Campbell S, Thomas-Oates J, Collins M (2010) *J Archaeol Sci* 37:13–20
28. Fuchs B, Schiller J, Süß R, Schürenberg M, Suckau D (2007) *Anal Bioanal Chem* 389:827–834
29. Sugimoto Y, Sanuki S, Ibrahim HR, Aoki T, Kusakabe T, Koga K (2001) *Biochim Biophys Acta* 1526:1–4
30. Zou X, Huang J, Jin Q, Guo Z, Liu Y, Cheong L, Xu X, Wang X (2013) *J Agric Food Chem* 61:7070–7080
31. Leo G, Bonaduce I, Andreotti A, Marino G, Pucci P, Colombini MP, Birolo L (2011) *Anal Chem* 83:2056–2064