Universitatea „Babeş-Bolyai” Cluj-Napoca

Facultatea de Chimie şi Inginerie Chimică

PhD Thesis Summary

CONTRIBUTIONS TO THE STUDY OF ORGANIC MATERIALS IN PAINTED SURFACES

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Table of contents (as in the full text)

Keywords

Organic binding media of painted surfaces, GC-MS analyses, analytical procedure, proteinaceous, saccharide, lipid and resinous materials, microsample, glass icons, painted coffered ceilings, wall paintings, Transylvanian heritage.

Abstract

Organic materials of painted surfaces have a major influence on the painting technique, aspect and condition of the paint. The thesis presents the first research related to organic materials in paint layers of Romanian heritage objects. Analyses were performed by gas chromatography coupled with mass spectrometry, GC-MS, applying a methodology which enabled the identification of proteinaceous, polysaccharide, lipid and resinous compounds of the paint layer from the same microsample, avoiding interferences due to the inorganic compounds of the paint. Analyses were performed on 81 samples coming from 38 glass icons belonging to different icon painting centers, the painted ceiling and woodwork of five Transylvanian churches and two wall paintings. Conclusions were drawn with respect to the painting technique of the objects and characteristic features of the studied centers.

Introduction and general presentation of the thesis

In order to better understand, appreciate and protect a value it is necessary to know it as well as possible.

Painted surfaces of Romanian heritage were less studied scientifically. Analytical data on their material composition could bring a significant contribution to the knowledge of the painted heritage items, can reinforce the actual knowledge about them or can put the objects in a new light, promoting their real knowledge.

The representative cultural heritage of the Western countries is fairly well characterized from scientific point of view. In Romania this was not a priority, consequently there is no overall knowledge not even regarding the outstanding cultural heritage of the country. More analytical data is available on the inorganic components of exterior wall paintings of the word heritage churches in Bucovina, but even here – according to our present knowledge - analyses concerning the organic components of the paint layer were not performed yet.

The present thesis would like to contribute to the study of organic materials in painted surfaces of Romanian heritage objects using an analytical method that provides most information

based on the analysis of one single microsample. The methodology is elaborated and used by the research group „Chemical Science for the Safeguard of Cultural Heritage", led by Prof. Maria Perla Colombini, active within University of Pisa, Faculty of Chemistry and Industrial Chemistry, where the practical part of the thesis was performed. Since the necessary instrumentation is available also in Cluj, in the research group led by Prof. Dr. Luminița Silaghi-Dumitrescu, the method could be implemented in the future also here for further studies of the Romanian cultural heritage.

Improved knowledge on the material composition of paint layers on heritage items will allow a better understanding of the painting technique used by the authors, will contribute to the characterization of national authors and workshops, assignment and authentication of painted heritage objects, sustainable conservation of the heritage and detection of counterfeits.

Chemical composition of an art object is influenced by several factors: the painting technique used by the artist in order to create the object, the decay processes affecting the painting materials over time, due to environmental factors and the different materials eventually used for the conservation of the object. The present thesis focuses mainly on the study of natural organic material used in our geographic area.

Organic materials can be used in painted surfaces for different purposes: as adhesives, when joining together two surfaces; as binding media, if applied in order to assure cohesion in a paint layer; as consolidant if used to return the lost coherence a paint layer; as varnish or protective layer of the painted surface. Frequently, the same material originally used as binding medium can be also used as conservation material that makes the identification of the initial binder more complicated. The kind of binding media used influences significantly the aspect of the painted surface and consequently will decide its painting technique.

The **first chapter** of the thesis presents based on the available technical literature the main organic materials used as binding media of the painted surfaces. Chemically these materials belong to five major classes: proteins, sugars, lipid materials, terpens/ terpenoids or resinous materials and bituminous materials or tars and pitches. The biological sources of these materials are presented, together with their major chemical components, their properties and use in paintings and their main decay processes.

The **second chapter** presents first the ethical and technical aspects of sampling from heritage objects. Then it gives an overview of the major methods used for binding media analysis based in the cited technical literature. It presents techniques from simple micro- and histochemical tests up to different advanced analytical methods, revealing advantages and limits for each of them. Based on this data arguments sustaining the choice of the analytical method applied for the practical part of the thesis are given. GC-MS analysis of paint samples proved to

be the most suitable tool for identifying their binding media. It enables the separation of the complex mixtures and the identification of each organic component from tiny microsamples, even if they are present in low amount and suffered decay processes. A chemical preteatment of the samples is necessary. The method allows quantitative measurements and it has a good reproductibility.

Beginning form **third chapter** the thesis presents the binding media analysis of Romanian heritage objects. First, the principles and steps of the applied analytical methodology are presented as described in technical literature and experienced during the practical work performed at the laboratory in Pisa. The methodology was applied to 71 samples coming from different Transylvanian heritage objects and three wall painting samples from other regions. Another series of 10 samples belonging to glass icons were analyzed with a more simple methodology applied at the scientific laboratories of the Kunsthistorisches Museum in Vienna. The samples were taken from 38 glass icons made in the 19th century in several icon creating centers of Transylvania, five coffered painted ceilings and a pew parapet from the 18th century, belonging to churches of the region and two wall paintings. Data interpretation methodology is illustrated with chromatograms resulting from their analyses.

The next subchapters present, mainly synthesized in tables, the detailed sample description, overall and detailed results provided by the analyses. Data interpretation is sustained with arguments and conclusions are drawn regarding the objects and the centers they belong to.

The **fourth chapter** presents in detail the working conditions and the results for each sample.

The thesis ends with general conclusions and the list of consulted bibliography. Annexes contain the chromatograms acquired for each sample.

I. Organic materials in painted surfaces

Chemical composition of a paint layer depends on the applied painting technique, the effect of the environmental factors the object was exposed to since its manufacture and the materials used for an eventual conservation. The aim of the thesis is the analysis of the organic compounds in paint layers, of those characteristic for our geographic region. These organic materials are film-forming and can occur in each layer of a painted surface. They are called differently according to their role in the layer: adhesives if applied for joining two surfaces, binders if used as a matrix of the paint, varnishes if applied as a protective layer or consolidants if used for the conservation of a fragile paint. If consolidants are natural organic materials, this can complicate the understanding of the original painting technique.

The binder has a major contribution to the aspect of the painting and consequently will decide the painting technique of the object. Encaustic painting has wax as binding medium. The binder of oil paint is siccative oil, mainly linseed oil. Tempera painting is based on various emulsions, having a proteinaceous component (egg, gelatin, animal glue, casein, or mixture of these) lipid additives, as siccative oils (if present in higher amounts the technique is called tempera grassa), sometimes also saccharide materials (arabic gum, fruit tree gums), resins or other additives as honey (plasticizer) or ox gall (emulsifier). The binding medium of aquarelle painting is mainly arabic gum. Modern painting is realized using different synthetic resins[1].

Chemically the organic materials of the paint layer can belong to five major chemical classes presented below, together with main representatives. They can occur in the paint layer alone, but mainly in mixtures.

Lipid materials contain esters of saturated or unsaturated fatty acids or hidroxiacids. Drying oils are glycerolipids with high content of unsaturated fatty acids. Most used is linseed oil, but poppy and walnut oil may also occur. Waxes are mainly represented by beeswax, paraffin and microcrystalline waxes. The latter are made up of hydrocarbons; bee wax contains beside hydrocarbons, different esters, free fatty acids, and free alcohols. Cholesterol, the sterol present in egg yolk together with egg glycerolipids, acts as emulsifier of the binder emulsions, as also the sterols from ox gall.[2].

[1] Laurie 1967; Havel 1980; Lăzărescu 2009; Gettens 1966.
[2] Masschelain 1996; Colombini, Modugno 2009a; Andreotti el al. 2008; Theophilus 1986; Mills&White 1987; Istudor 2006; Welthe 2004; Gettens 1966; Cennini 1977.

From naturally occurring polysaccharides starches and dextrins, arabic gum and fruit tree gums (cherry, plum, peach) were mainly used in art objects. These are natural polymers made up of .different simple sugars and uronic acids[3]. Honey was used as plasticizer.

Proteinaceous materials, like egg (yolk and/ or white), gelatin, animal glue or casein, are most frequently used in art as binders. They contain polypeptide chains made up of amino acids. Each protein has a specific amino acid composition From vegetal proteins garlic was used as adhesive of gold foils[4].

Terpens and terpenoids, basically natural resins are mainly used as protective layers. They are complex natural mixtures with components build up of isoprene units. Mono- and sesquiterpenoids occur in volatile oils like turpentine or lavender oil. Diterpenoids are extruded by *Pinaceae* and *Caesalpiniaceae* species and are represented by colofonium, sandarac or copal resins. Di- and triterpenoids never occur together. The later are produced by *Angiosperm* plants. Most used are dammar and mastic resins. Myrrh, Peru or Copaiba balms, amber, shellac or urushi lacquer contain beside tepenoid compounds also different other substances[5].

Bituminous materials are complex mixtures obtained directly from natural sources (bitumen, asphalt) or resulting from the pyrolysis of resinous wood or coal (tars and pitches).

Organic materials in paint layers undergo different deterioration processes in time resulting from both free radical reactions (mainly auto oxidations) and ionic processes. The later are predominantly of hydrolytic nature[6]. Biodeterioration by enzymatic processes is also a significant decay factor. Understanding the mechanism of decay processes and identifying the resulting degradation products is essential for the identification of organic compounds in paint layers, but also for finding methods to prevent, stop or slow down these processes in order to assure the long term preservation of cultural heritage.

[3] Colombini, Modugno 2009a; Bonaduce et al. 2007
[4] Colombini, Modugno 2009a; Mills&White 1987; Balázsy 1993; Spyros, Anglos 2006; Welthe 2004; Bodaduce et al. 2006; Yarosh 1990; Istudor 2011
[5] Mills&White 1977; Masschelain 1996; Andreotti el al. 2008; Colombini, Modugno 2009a.
[6] Mills&White 1987; Doménech-Carbó 2008; Andreotti el al. 2008

II. Analytical methods for the study of natural organic binders

Since the first reported analytical studies (end of 18th century), the number of analytical methods and techniques applied to the study of cultural heritage constantly grew, trying to improve the detection limit, sensitivity, resolution, reproducibility and accuracy of the analytical results. Measurements are fairly difficult due to the complex character of the aged organic mixtures and the small amount of available samples. Inorganic components of the layer might interfere with the analysis of organic material. Recently the multi-analytical approach of organic material identification gain ground.

The first investigation were based on physical, micro- and histochemical tests, leading to low specificity results, but localized to the different layers of the painted surface[7]. Methods are low cost and accessible.

An improvement of histochemical identifications of proteins was the use of immunological techniques. The high specificity of the antigen-antibody reaction enables discrimination of similar proteins originating from different species. Three such techniques are applied, the immunofluorescence microscopy, (IMF)[8], enzyme-linked immunosorbent assays (ELISA)[9] and a combined IFM – ELISA method[10]. These techniques are suitable only for identification of proteinaceous material.

Spectroscopic techniques were widely used in the last decades.

Nuclear magnetic resonance (NMR) was first applied for the analysis of organic residues in archaeological objects, but also for identification of organic materials in pictorial layers. Analysis is performed on solvent extracts[11]. Sample preparation is fairly simple, but the method has restrained applicability and data interpretation is difficult.

Fourier transform infrared spectroscopy (FTIR) and different developments of the technique like diffuse reflection FTIR (DRIFT), attenuated total reflection (ATR), photoacustic spectroscopy (FTIR -PAS), FTIR microscopy in transmission or reflected mode, synchrotron radiation FTIR (ST-FTIR)[12] are among the most used in art. Sample preparation is simple and analyses are not time consuming. Sample size goes down to nanograms and the spatial resolution is 20-100 μm^2. Portable instrumentation was developed and rich spectral libraries were

[7] Plesters 1956; Schramm&Hering 1978; Gay 1970; Martin 1977
[8] Ramírez-Barat 2001; Dolci et al. 2008; Sciutto et al. 2011
[9] Doménech-Carbó 2008
[10] Mazurek et al. 2008
[11] Spyros, Anglos 2006
[12] David et al. 2004; Doménech-Carbó et al. 1996; Doménech-Carbó 2008; Nevin et al. 2009.

established[13]. The disadvantage of the technique is a relatively low specificity, identifying only the main groups of organic materials encountered in paint layers. Recently chemometric approach was applied to spectra interpretation. Using as PCA variables the absorption bands in two characteristic spectral windows, one corresponding to C-H vibrations and the other to carbonyl bands, organic materials were better distinguished[14].

FTIR was applied also to the study of Romanian heritage, for identification of inorganic materials and classes of organic binders[15].

Raman spectroscopy gradually gains ground in art analyses[16]. Preparation of samples is simple. Coupling the technique with confocal microscopy increased considerably its spatial resolution and enabled the selective determination of organic binders. Due to optic fibers the development of portable instrumentation became possible[17]. Still, the method is more successful in the analysis of inorganic compounds and proves more difficult for the identification of organic materials. Articles reporting Raman identification of pigments would use GS-MS for the analysis of binding media[18].

Mass spectrometry (MS) and related methods (direct infusion DIMS, direct pyrolisis DPMS, direct temperature resolved DTMS) imply a relative simple sample preparation. Their major disadvantage is the limited applicability for complex organic mixtures, which can be solved by coupling the method with a separation technique[19]. Still, using DIMS for the analysis of chemically pretreated samples, with positive ion electrospray ionization (ESI) and ion trap detection system, applying chemometrics (DLA – Liniar Discriminant Analysis) for data interpretation some proteinaceous and lipid binders were satisfactory differentiated[20].

Using MADLI (Matrix Assisted Laser Desorption Ionization) and TOF (time of flight) analyzer different proteins could be distinguished. The molecules were previously fractioned by enzymatic cleavage[21].

Separation techniques, mainly chromatographic ones, are widely applied in the analysis of binding media given their ability of separating organic mixtures. Applications closely followed the development of the technique, evolving from paper chromatography (PC) and thin layer chromatography (TLC) to liquid- and gas chromatography (LC, GC) coupled with MS

[13] Derrick et al. 1999; Meilunas et al. 1990; http://www.irug.org/ed2k/search.asp
[14] Sarmiento et al. 2011
[15] Maruțoiu et al. 2011; Merticaru, Petroviciu 2005; Merticaru, Istudor 2005; Baciu et al. 2010.
[16] Smith, Clark 2001.
[17] Doménech-Carbó 2008.
[18] Bersani et al. 2008; Abdel-Ghani et al 2008.
[19] Doménech-Carbó 2008; Colombini, Modugno 2009.
[20] Peris-Vincente et al. 2005; Peris-Vincente et al. 2007.
[21] Kuckova et al. 2005

detection. Thus, organic components in paint layer can be better distinguished and identified. Compared the other techniques, sample preparation is more complex and time consuming. Illustrating the importance of the method an international Users' Group for Mass Spectrometry and Chromatography (MaSC) in art was established [22].

Different LC techniques, also high performance LC (HPLC) were applied for the identification of proteins and siccative oils[23].

As alternative to chromatographic separations, capillary electrophoresis (CE) was used, showing a higher efficiency and speed. Derivatization is not needed prior to the analysis, but the method has a low sensitivity[24].

For high molecular weight polymers pyrolysis (Py)-GC-MS is successfully applied. Sample preparation is simple; the method is highly sensitive and shows a low limit of detection (LOD). The resulting pyrograms are rather complicated and more difficult to interpret as the GC chromatograms of the same materials[25].

At the moment GC-MS is considered the most suitable and proves the most widespread technique for identification of organic materials in paint layers[26]. Sample preparation involves complex chemical pretreatments and the method provides bulk results for multilayered structures if not previously separated. But specificity, sensitivity and reproducibility of the method is high. Sample size can be reduced to 0.1 mg; limit of detection is at microgram level. The practical part if the thesis is based on this method.

[22] http://www.mascgroup.org/
[23] Colombini et al. 2002a; Colombini, Modugno 2004
[24] Mazanek et al. 2006; Harrison et al. 2005a; Größl et al. 2005; Harrison et al. 2005b.
[25] Chiavari et al. 1998; Bonaduce, Andreotti 2009
[26] Andreotti el al. 2006; Andreotti el al. 2008; Bonaduce et al. 2009; Casoli et al. 1996; Colombini et al. 2010; Colombini, Modugno 2004; Gautier, Colombini 2007; Gimeno-Adelentado et al. 2002; Kenndler et al. 1992; Kouloumpi et al. 2007a; Lluveras et al. 2010; Marinach et al. 2004; Schilling 1996;

III. Analysis of organic materials in the paint layers of Transylvanian heritage objects

Analysis of Romanian cultural heritage was performed mainly connected to restorations. According to present knowledge no extensive study was performed in order to fully characterize the materials used by a national author or workshop. No comprehensive instrumental analyses of organic binding media was reported.

The GC-MS analytical protocol adopted for the experimental part of the thesis provided qualitative and quantitative information on the organic binders of the studied paint layers. The method was elaborated and validated by the research group „Chemical Science for the Safeguard of Cultural Heritage" within Pisa University, lead by Prof. Dr. Maria Perla Colombini, an internationally recognized expert group with decades of experience in the analyses of organic materials in art objects. The experimental part of the thesis was performed in this laboratory. Premises of applying the method in the future also within Cluj University exist.

III.1 Brief description of the working methodology

The samples were analyzed according to a complex analytical procedure that characterizes all usual classes of organic binding medium from the same microsample, avoiding interferences from inorganic media[27]. The method is based on a multi-step chemical pretreatment of the sample. First proteins and polysaccharide materials were subjected to ammonia extraction in order to separate them from lipid and resinous materials. Proteins and sugars were separated afterwards by monolithic sorbent tip technology with a C4 stationary phase and purified before hydrolysis. Lipids and resins were subjected to saponification. Three fractions were generated and analyzed separately by GC/MS, enabling a quantitative analysis of the components in each fraction. Proteinaceous materials were identified based on the percentage composition of 11 determined amino acids applying principal component analysis (PCA) for data interpretation. The percentage of aldoses and uronic acids in the saccharide fraction enabled the identification of the saccharide materials of the samples. Glycerolipid identification used the mono- and dicarboxylic aliphatic acid content resulting from the lipid fraction. Waxes and natural resins were recognized from the same fraction based on molecular patterns and specific degradation products.

The procedure consists of 16 steps. A simplified scheme is presented in Fig.1.

[27] Lluveras et al. 2010

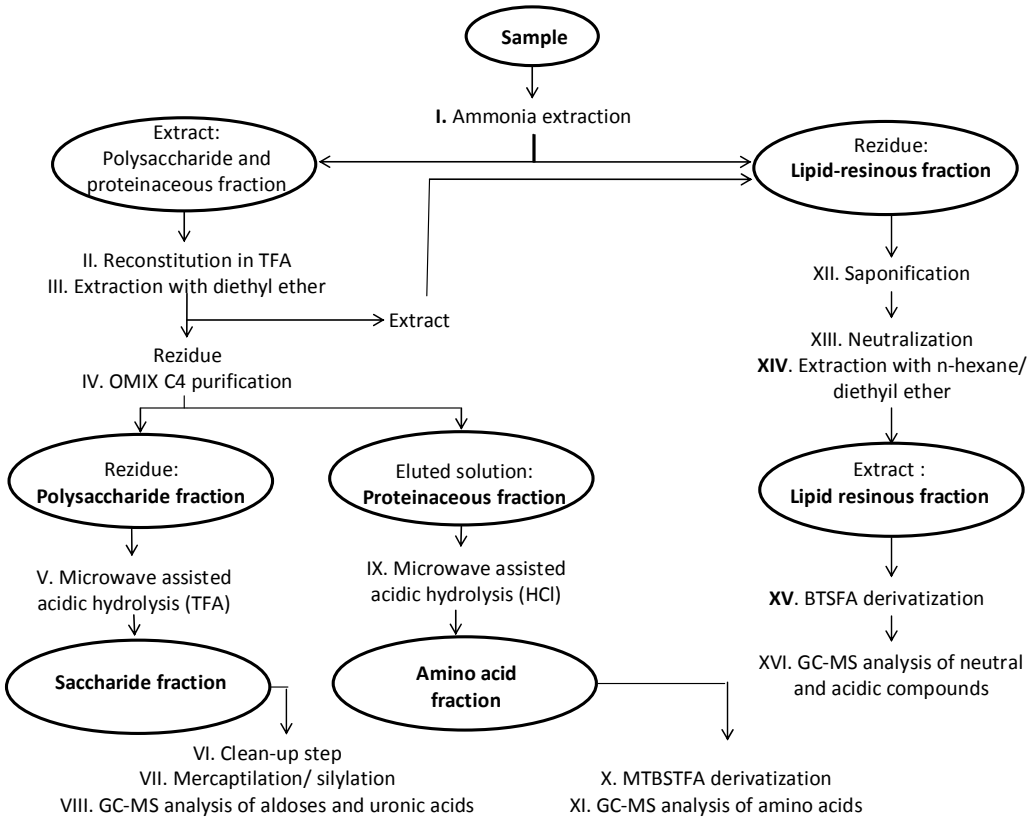


Fig. 1. Simplified scheme of the procedure[27].

The quantitative analysis of the three fractions is performed by using building calibration curves based on standard solutions of amino acids, aldoses and uronic acids or aliphatic mono- and dicarboxylic acids respectively. The individual response of each analyt is evaluated by daily recoveries. Individual derivatizations and injections are controlled by internal standards (norleucine, manitol, tridecanoic acid and hexadecane). Running blanks of the procedure revealed low levels of contamination. Limit of detection (LOD) and quantification (LOQ) were evaluated periodically for each analyte. In order to assure reproducibility all vials were subjects of a rigorous cleaning procedure.

III.2. Data interpretation

In order to understand properly the analytical results of the above procedure and avoid misinterpretations due to unilateral thinking, it is necessary to apply an interdisciplinary approach, to have a good knowledge of the traditional painting techniques and of the related technological sources, and to develop close, effective collaboration with restorers, museologists and art historians.

Chromatograms were evaluated in SIM mode, based on the retention time and mass spectrum of each peak. Mass spectrums were attributed by direct comparison with Wiley 275 spectral database or with spectra of reference materials recorded in the same condition.

III.2.1. Identification of proteins

Kind and relative percentage composition of amino acids resulting from the hydrolysis of a protein depends on the nature of the protein. Table 4 presents the main proteins encountered in paint layers with the corresponding relative percentage amino acid composition of each of them. Values are average of tens of measurements performed on reference materials[28].

	Ala	**Gly**	**Val**	**Leu**	**Ile**	**Ser**	**Pro**	**Phe**	**Asp**	**Glu**	**Hyp**
casein	5.0	3.0	7.6	11.9	6.6	5.8	11.5	5.9	8.5	22.2	0.0
egg	7.7	4.8	7.7	11.0	6.7	10.3	5.7	6.4	12.6	15.0	0.0
animal glue	12.3	29.4	3.9	4.7	2.5	3.8	12.4	2.8	6.6	9.9	7.7

Table 1. Average relative percentage composition of amino acids fractions resulting from reference samples of the main proteinaceous binders

The relative amino acid percentage content resulting from the analysis of the samples was subjected to a multivariate statistical analysis using principal components analysis (PCA). Identification of the source proteins in the resulting score plot was made compared to a data set of 121 reference samples of animal glue, egg, and casein[28]. Fig. 2. shows the chromatograms related to one of the egg containing samples and Fig. 3. the PCA biplot of the 121 data set of reference samples used for the identification of the protein content of the real samples.

[28] Colombini et al. 1999, Andreotti el al. 2006

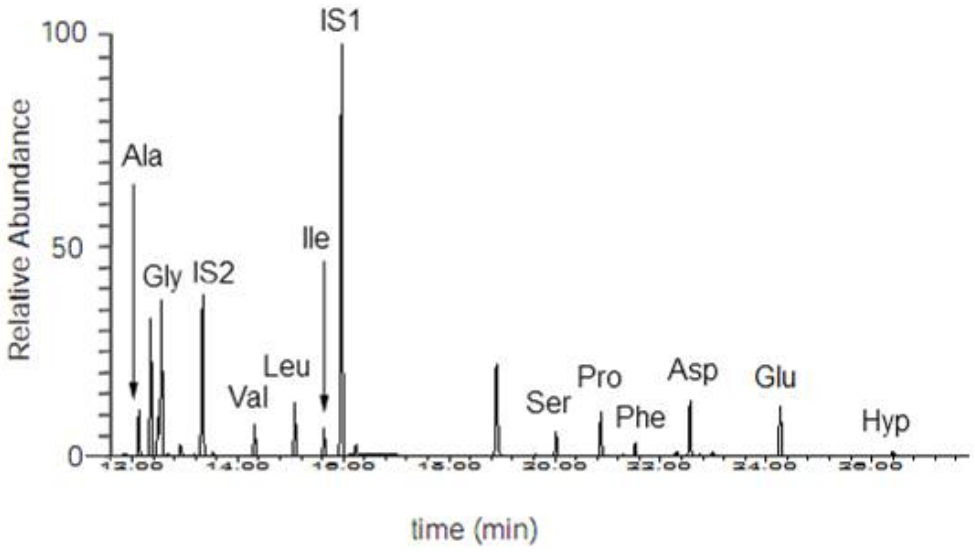


Fig. 2. Selected ion monitoring chromatogram (SIM) of amino acid fraction in sample PIV-3 (Baptism of the Lord, Braşov area) showing the profile of egg; IS – internal standard, IS_1 norleucine, IS_2 hexadecane

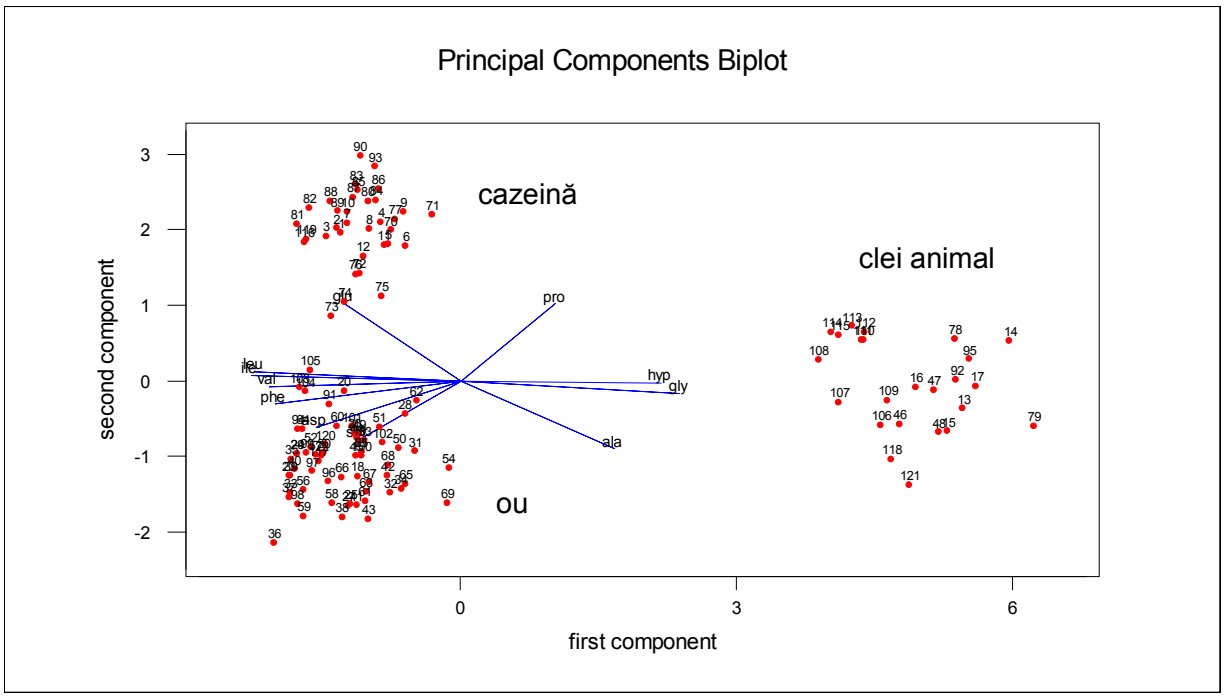


Fig. 3 PCA biplot of the 121 data set of reference samples used for the identification of the protein content of real samples[29].

[29] Colombini et al. 1999, Andreotti el al. 2006

III.2.2. Identification of lipids

Chromatograms of lipid resinous fraction can contain peaks of aliphatic mono- and dicarboxylic acids, hydroxiacids, sterols, alcohols, alkanes, acidic and neutral terpenoid compounds. The percentage relative fatty and dicarboxylic acid content of the sample results from the corresponding peak areas in the SIM. The source of lipid materials can be evaluated based on the ratio between the relative content of palmitic acid and stearic acid (P/S), the ratio between the relative content of azelaic acid and palmitic acid (A/P), the sum of the percentage content of dicarboxylic acids (ΣD) and the presence or absence of cholesterol. Values in Table 2 show the characteristic parameters of main siccative oils, egg lipids and tempera grassa, and were determined applying the procedure to reference samples[30]. Cholesterol can not always be identified in the chromatogram, even if the lipid profile of the sample is corresponding to egg; this is due to the difficulty of detecting decayed cholesterol, present in very low concentrations.

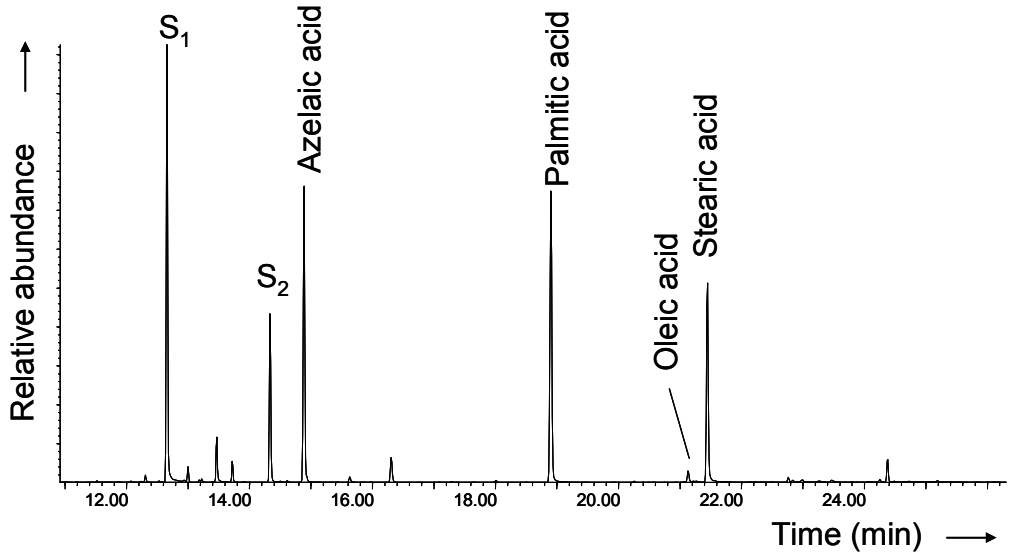


Fig. 4 SIM chromatogram of sample III-3 (icon Jesus on the Throne, Olt County) illustrates the profile of aged linseed oil

[30] Colombini et al. 2002b, Andreotti el al. 2006

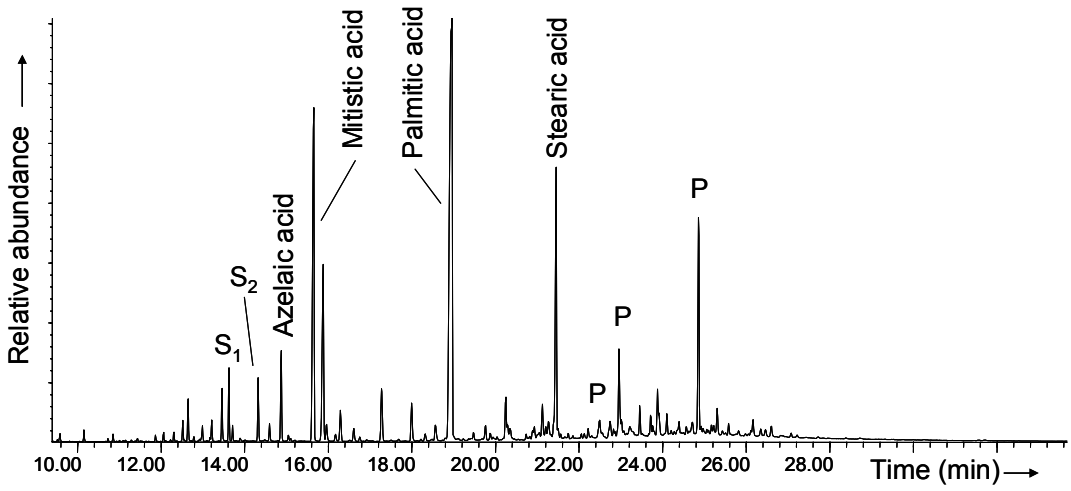


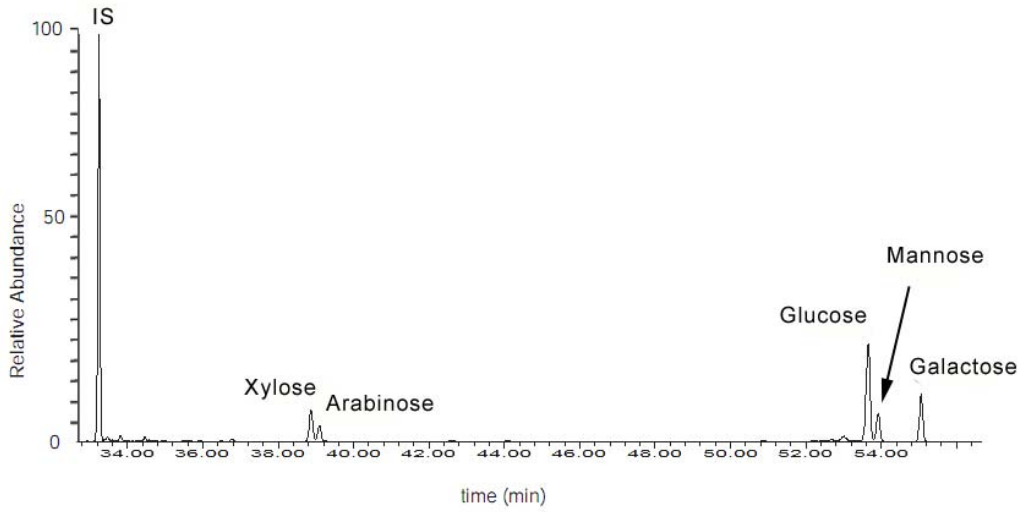
Fig. 5; TIC chromatogram recorded for sample IV-3 (icon Baptism of the Lord, Brasov area) showing the characteristic profile of egg lipids; this contains also the characteristic peaks for pine resin (P); S – internal standard

	Linseed oil	Walnut oil	Poppy seed oil	Egg lipids	Tempera grassa
P/S	<2	2.2-3.0	>3	2.7-3.2	1.8-2.3
A/P	>1	>1	>1	<0.3	0.5-1
ΣD	>40	>40	>40	<10%	10-20%
cholesterol	-	-	-	present	present

Table 2 Characteristic parameters of different siccative oils, egg and egg oil mixture (tempera grassa); P –palmitic acid, S –stearic acid, A –azelaic acid, ΣD – sum of percentage dicarboxylic acid content

III.2.3. Identification of sugars

Saccharide materials were identified after quantification of aldoses and uronic acids from SIM. of saccharide fraction, based on the relative percentage content in sample of each saccharide above detection limit. Since sugars are frequent contaminants of the environment quantitation of each component of the saccharide fraction is necessary. The chromatogram below illustrates the SIM of the saccharide fraction of sample XII-2, a glass icon from Nicula, containing arabic gum. Table 3 presents the relative percentage saccharide content of polysaccharides encountered in paint layers.



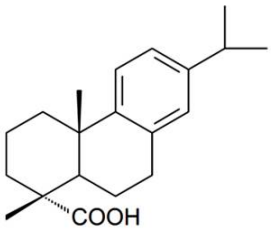
	xil	ara	ram	fuc	a.gal	a.glu	man	gal
Arabic gum	0	36.1	10.8	0	0	7.3	0	45.8
Tragacant gum	17.6	39.6	2.9	9.3	16.6	3.6	0	10.4
Cherry gum	6.2	35.8	2.4	0	0	13.1	6.2	36.3
Peach gum	6.7	32.4	3.2	0	0	14.2	5.4	38.1
Locust beam	0	1.5	0	0	0	0	81	17.5

Table 3 Relative percentage saccharide content of the main polysaccharides encountered in paint layers, average values measured for reference materials[31].

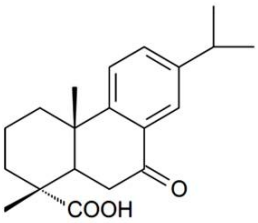
[31] Bonaduce et al. 2007

III.2.4.Identification of resins

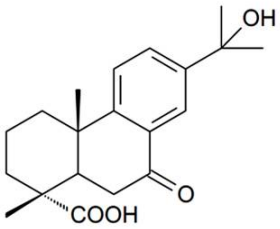
Resins were identified from TIC chromatograms of lipid resinous fractions from their charachteristic degradation products (molecular markers). For pine resin these are listed below:



dehidroabietic acid



7-oxo-dehidroabietic acid



7-oxo-15-hidroxidehidroabietic acid

Marker molecules are usually detected in the extract ion chromatogram (EIC) according to their characteristic mass fragments. For pine resin the extracted ions are m/Z 237 (didehidroabietic acid), 239 (dehidroabietic acid) , 253 and 268 (acid 7-oxo-dehidriabietic) in the time range of 22-25 minutes. Resulting characteristic profile is shown below.

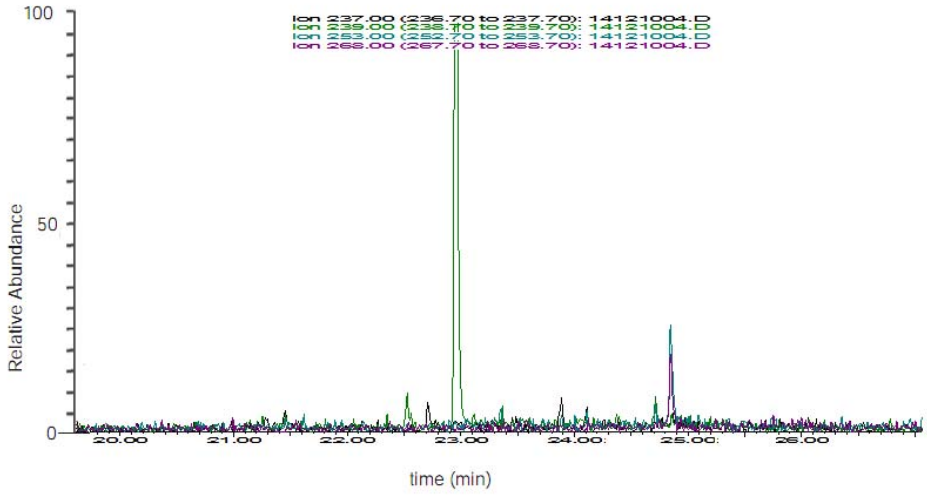


Fig. 6 Extract ion chromatogram (EIC) for m/Z 237, 239, 255 and 268 and the characteristic profile for pine resin recorded for sample III-3 (glass icon Jesus on the Throne from Olt county)

III.4. Binding media analyses of Transylvanian glass icons

Analyses were performed on available samples, mainly coming form the glass icon collection of ASTRA Museum in Sibiu, and from church and private collections. Samples were kindly provided by senior restorers dr. Olimpia Coman-Sipeanu, dr. Geanina Ionescu-Curcă and Mirel Bucur, together with the images of the source objects and connected information.

A number of 56 samples coming from 38 glass icons were analyzed, from which:

- 10 from 7 glass icons belonging to Nicula center
- 20 from 15 glass icons made in Olt county (Ţara Oltului, zona Făgăraşului), also by two famous icons painters of the region (Matei Ţimforea, 3, Savu Moga, 5)
- 22 from 12 glass icons made in Brasov area (Şcheii Braşovului)
- 4 from 4 glass icons belonging to other centers

The aim of the research was gain analytical information on the organic materials used for glass icon painting in order to better understand and characterize their painting technique. It was important to find out how complex the binding media mixtures used for glass icons were, if they show any characteristic features related to a certain glass icon center or historic period. On the other hand the obtained data was important to better plan the sustainable conservation.

III.4.2. Glass icons from Nicula

According to the analytical results binding media used in Nicula was more diverse and complex as supposed based on the scarce documentary sources.

Protein content was close to detection limit in four of the icons; it could be identified as egg only in two of them. Higher protein amounts of the other three samples permitted their more confident identification. One proved to be egg, the other egg mixed with animal glue, the third one casein with small amounts of animal glue. Finding casein was unexpected, but the analytical results – score plot position of sample XI-1 together with the high glutamic acid content of the protein - sustain the identification. Even if not mentioned as a glass icon binder, the use of casein as a gluing agent was recorded in the region[32]. Further studies would be necessary in order to decide if the presence of casein in this icon was an exception or a specific proteinaceous binder used in Nicula at the beginning of 19th century.

Analyses of the saccharide fraction led in case of sample XIII-2 to the conclusion that arabic gum and sugars of the egg are present in the paint layer, the same is supposed for sample XIII-1. In the other samples in which saccharide fraction was analyzed sugar content was either below detection limit or the saccharide profile of the sample suggested a contamination.

Linseed oil was present in every sample, in higher amounts, showing the adding oil to the paint layer was usual in Nicula in the 19th century.

Pine resin was identified in one single sample and present in traces in two other ones.

Detailed results are presented in the tables below.

[32] Mihalcu 2009, p.15

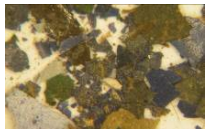
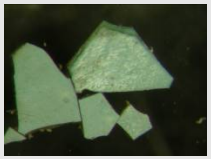
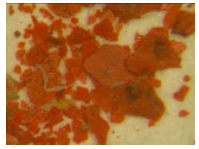
Sample	Source object description	Inventory number.	Sample weight	Sample description	Micro photography
XI-1	**Archangel Michael** (Archangelul Mihail), first part of the 19th c.	T78-OC	1.1 µg	Red paint layer fragments from the edge of the glass	
XI-2	**Holy Mother with Child** (MD cu Pruncul) first part of the 19th century	T90-OC	0.6 µg	Some blue fragments chosen from available dislocated paint fragments	
I-7 **V-1**	**Crucifixion** (Rastignirea) second part of the 19th century	Private collection (MP)	0.9ug 1.5 µg	Blue fragments chosen from available dislocated paint fragments	
XIII-1	**Grieving Holy Mother** (MD Indurerata, middle of the 19th century	T93-OC	0.6 µg	White and blue fragments chosen from available dislocated paint fragments	
XIII-2 **KHM2**	**Saint George** (Sf Gheorghe) first part of the 19th century	T96-OC	1.2 µg qualitative analysis	Some blue fragments chosen from available dislocated paint fragments	
XIV-2 **XVI-1**	**Grieving Holy Mother** (MD Indurerata), Nicula, middle of the 19th century	1491-OC	<0.1ug? 0.1 ug	Red fragments with less dirt chosen from available dislocated paint fragments	
KHM1	**Crucifixion** (Răstignirea), Northern Transylvania, second part of 19th century	Private collection (HC)	qualitative analysis	Some fragments chosen from available dislocated paint fragments	-

Table 4 . Sample and source object descriptions for the Nicula (Northern Transylvania) glass icons analyzed

Name and manufacturing period if the icons	Sample (weight)	Proteinaceous fraction (weight and percentage in sample, remarks)	Saccaride fraction (weight and percentage in sample, remarks)	Lipid-resinous fraction (weight and percentage in sample, remarks)
Archangel Michael (Archangelul Mihail), first part of the 19th c.	**XI-1** (1.1 µg)	**Casein and traces of animal glue** (8.4 µg , 0.8%, Hyp present)	Not analyzed	**Aged linseed oil** (14.4 ug, 1.3%)
Holy Mother with Child (MD cu Pruncul) first part of the 19th century	**XI-2** (0.6 µg)	**Egg and animal glue** (4.7 ug, 0.8%, Hyp present)	Not analyzed	**Aged linseed oil** (19.3 ug, 3.2%)
Crucifixion (Rastignirea) second part of the 19th century	**I-7** (0.9ug) **V-1** (1.5 µg)	**Egg** (0.3 µg , 0.03%)	Below detection limit	**Aged linseed oil** **Pine resin** (32.6ug, 2.7%, traces of cholesterol)
Grieving Holy Mother (MD Indurerata, middle of the 19th century	**XIII-1** (0.6 µg)	**Egg** (0.2 µg, 0.03%)	**Arabic gum and sugars of the egg?** (0.4ug/ 0.07%)	**Aged linseed oil** (16.0ug, 1.3%)
Saint George (Sf Gheorghe) first part of the 19th century	**XIII-2** (1.2 µg)	**Egg** (cu Omix, 1.2 µg, 0.1%)	**Arabic gum and sugars of the egg** (1.1ug/ 0.1%)	**Aged linseed oil** (45.0ug, 3.8%)
	KHM 2	**Traces of egg (?)**	Not analyzed	**Linseed oil** **Traces of pine resin**
Grieving Holy Mother (MD Indurerata), Nicula, middle of the 19th century	**XIV-2** (<0.1 µg ?) **XVI-1** (0.1 ug)	**Egg** (0.4 µg, 0.4%)	**Saccaride material** (probably wood contamination) (1ug)	**Aged linseed oil** (11.5ug)
Crucifixion (Răstignirea), Northern Transylvania, second part of 19th century	**KHM1**	**Traces of a protein**	Not analyzed	**Linseed oil** Traces of pine resin (?)

Table 5. Overview of the organic materials identified in the samples from Nicula glass icons

Sample	ala	gly	val	leu	ile	ser	pro	phe	asp	glu	hyp	Protein content	PC1	PC2
XI-1	5.9	8.1	6.4	11.1	4.2	4.5	7.3	5.9	15.8	28.8	1.9	8.4 µg 0.8%	-0.0171	0.86866
XI-2	11.4	14.1	11.2	13.5	8.8	3.5	8.5	2.8	4.3	11.9	10.0	4.7 ug 0.8%	1.6978	0.0126
I-7	8.5	12.4	9.0	15.0	9.2	13.5	5.4	7.8	8.8	10.3	0.0	0.3 µg 0.03%	-1.1258	-1.5355
XIII-1	5.8	15.0	6.5	12.0	6.1	6.3	5.5	8.2	8.4	23.4	2.8	0.2 µg, 0.03%	0.0733	0.4965
XIII-2	8.2	11.8	8.4	11.9	6.5	9.1	5.2	6.8	12.1	19.8	0.3	1.2 µg 0.1%	-0.4545	-0.6395
XVI-1	6.3	18.1	7.1	13.5	7.1	12.8	3.2	7.1	9.2	15.7	0.0	0.2 µg 0.2%	-0.4342	-1.2018

Table 6 Relative amino acid percentage content of the proteins in the samples from Nicula glass icons and the two principal components resulting from PCA

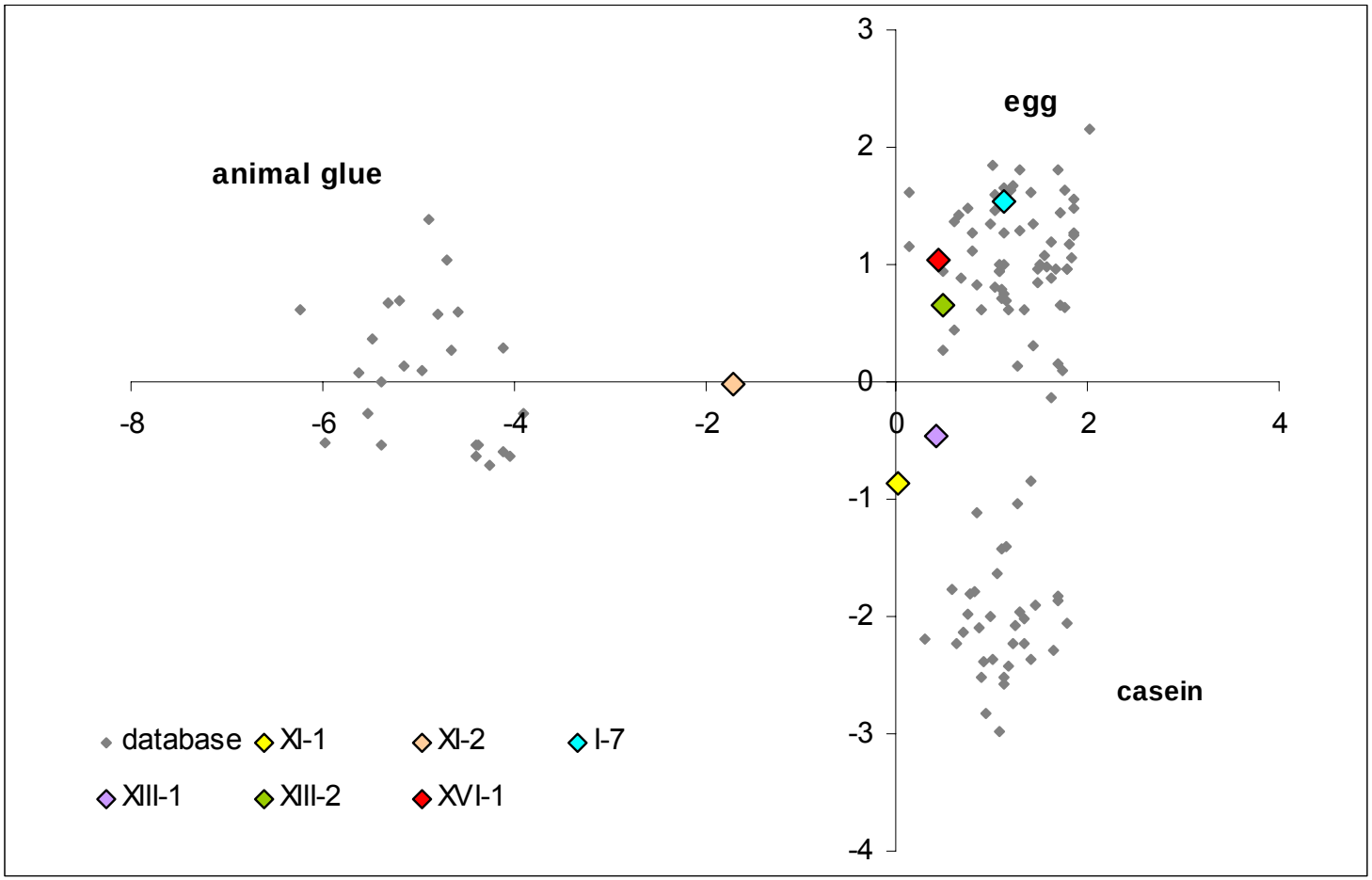


Fig. 7 PCA score plot of the relative amino acid percentage content of the samples from Nicula glass icons

Sample	Characteristics of lipid-resinous fraction of the samples						Saccharide profile of the samples									Content µg/%
	P/S	A/P	Σ DC	Content µg/%	Chole sterol	Pine resin	Xyl	Ara	Ram	Fuc	A.gal	A.glu	Glu	Man	Gal	
XI-1	1.7	2.7	66.0	14.1/ 1.3	ND	ND	-	-	-	-	-	-	-	-	-	-
XI-2	1.0	6.0	77.9	13.9/ 3.2	ND	ND	-	-	-	-	-	-	-	-	-	-
V-1/ I-7	1.1	1.8	55.9	32.6/ 2.7	traces	present	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	< DL
XIII-1	1.1	1.6	52.1	31.2/ 5.2	ND	ND	NC	31.7	0.0	0.0	0.0	0.0	< DL	39.6	28.7	0.41/ 0.07
XIII-2	1.5	1.5	51.9	45.1/ 3.8	ND	ND	< DL	7.1	0.0	0.0	0.0	0.0	< DL	47.5	45.5	1.13/ 0.1
XIV-2	0.8	2.7	58.5	11.5/ ?	ND	ND	NC	41.5	< DL	< DL	< DL	< DL	NC	36.4	22.1	1.02/ ?

Table 7 Analytical results for the lipid-resinous and saccharide fraction of the samples from Nicula glass icons
(P/S- palmitic/ stearic acid percentage ratio; A/P- azelaic/ palmitic acid percentage ratio; Σ DC- sum of dicarboxylic acids percentage amounts;
ND – not detected; NC – not considered; < DL – below detection limit; - no data available)

III.4.3. Glass icons from Olt county (Ţara Oltului also called Făgăraşului)

Icons analyzed from this center were mainly from the first part of the 19th century and showed a relatively consistent binding media usage, mainly in mixtures. In the protein fraction of the icons higher or lower amounts of egg was detected. The lipid fraction contained relatively high amounts of linseed oil, but in one case, when the lipid profile was characteristic for egg lipids. Pine resin was present in traces in three of the samples. The saccharide profile of two of the samples suggests the presence of arabic gum in the paint layer.

The painting technique of Savu Moga - an icon painter of the region who mainly signed his icons and worked in the second part of 19th century – seams to follow the art of the anonymous painters in Olt county. His binders contain egg as proteinaceous component (animal glue identified in one single case was probably a restoration material). Lipid fractions showed higher amounts of linseed oil in most of the icons. In one icon the characteristic profile of egg lipids was found; this icon is not signed by the author, just assigned to him. Compared to results above, the analytical data is not sustaining the assignment. Saccaride fraction of his icons was analyzed just in one instance and it proved below detection limit.

The three glass icons by Matei Ţimforea (another famous painter of the region, active in the second part of 19th century) were analyzed. His paint layer fragments were of higher quality, more thin, hard and homogeneous as any other samples analyzed. Based in these few analyses the painting technique of his icons seams different from the one of the other icons of the region analyzed. Mixtures of egg, animal glue, arabic gum and linseed oil were found in his binders, but the pine resin, considered to be a specific additive of paint layers, was present in traces just in one of the icons. Of course, further research on other icons by Matei Ţimforea is needed in order to consolidate these results and draw strong conclusions.

Detailed results are presented in the tables below.

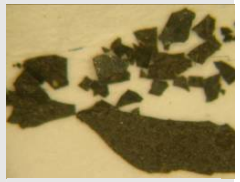
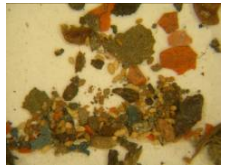
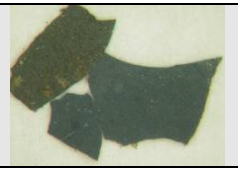
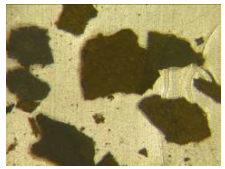
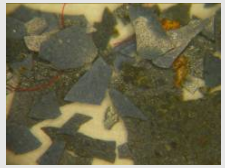
Sample	Source object description	Inventory number.	Sample weight	Sample description	Micro photography
XI-3	**Saint Nicolas** (Sf. Nicolae), first part of the 19th c.	T45-OC	0.2 mg	Black paint layer fragments	
XI-4	**Saint Nicolas** (Sf. Nicolae), first part of the 19th c.	T50-OC	0.1 mg	Blue fragments chosen from available dislocated paint fragments	
III-3	**Jesus on the throne** (Iisus pe tron), 1837	134-OC	< 0.1 mg	Blue paint layer fragments	
XIII-3	**Saint Demetrios** (Sf. Dumitru) first part of the 19th century,	14-OC	0.1 mg	Fragile blue paint layer fragments with dirt deposits	
XIII-4	**Saint Nicolas** (Sf. Nicolae), middle of the 19th century	101	0.2 mg	Cleaner blue fragments chosen from available dislocated paint fragments	
KHM 2	**Beheading of St John the Baptist**, first part of the 19th century	2779-OC	-	Dislocated fragments	-
KHM1	**Icon from Olt county,** dated 1883 (Tămas?)	Private collection (P)	-	Dislocated fragments	-

Table 8. Sample and source object descriptions for the Olt county glass icons

Sample	Source object description	Inventory number.	Sample weight	Sample description	Micro photography
XIV-6	**Annunciation** (Bunavestire), second part of the 19th century	2616-OC	<0.1mg	Red paint layer fragments	-
XIV-7 **KHM2**	**Crowning of the Virgin** (Încoronarea Fecioarei) second part of the 19th century	1142	0.1 mg	Light blue paint layer fragments	-
XIV-8 **KHM2**	**Candlemas** (Stretenia) Savu Moga?, second part of the 19th century	1115	0.1 mg	White paint layer fragments	-
XIV-5 **XVII-1**	**Jesus on the throne** (Iisus pe tron), Savu Moga?, second part of the 19th century	T79-OC	0.1 mg 0.75 mg	Dislocated paint layer fragments, some cleaner blue and white fragments chosen	
KHM1	**Praznicar icon** (placed before the altar), middle of the 19th century	-	-	Blue and red paint layer fragments	-

Table 9. Sample and source object descriptions for the glass icons by Savu Moga

Sample	Source object description	Inventory number.	Sample weight	Sample description	Micro photography
XIV-3 **XVI(16)-2**	**Saint Elijah** (Sf Ilie), second part of the 19th century	T83-OC (white-black)	1.1mg 0.2 mg	Thin, hard, homogenous dislocated fragments, two white fragments were chosen and cleaned from dirt deposits	
XIV-4 **XVI(16)-3**	**Saint Haralambos** (Sf Haralambie) second part of the 19th century	2751	<0.1mg? 0.2 mg	Cleaner red dislocated paint fragments	
KHM1	**Grieving Holy Mother** (MD Indurerata)**,** dated 1884	187-OC	-	White paint layer from the top right margin	-

Table 10. Sample and source object descriptions for the glass icons by Matei Țimforea analyzed

Name and manufacturing period if the icons	Sample (weight)	Proteinaceous fraction (weight and percentage in sample, remarks)	Saccaride fraction (weight and percentage in sample, remarks)	Lipid-resinous fraction (weight and percentage in sample, remarks)
Saint Nicolas (Sf. Nicolae), first part of the 19th c.	**XI-3** (0.2 mg)	**Egg** (5.0 ug, 2.6%)	Not analyzed	**Aged linseed oil** (9.6 ug, 4.8%)
Saint Nicolas (Sf. Nicolae), first part of the 19th c.	**XI-4** (0.1 mg)	**Egg** (0.5 ug, 0.5%)	Not analyzed	**Aged linseed oil** (1.9 ug, 1.9%)
Jesus on the throne (Iisus pe tron), 1837	**III-3** (<0.1mg)	**Egg** (0.44ug)	Below detection limit	**Aged linseed oil** traces of pine resin (3.9ug)
Saint Demetrios (Sf. Dumitru) first part of the 19th century,	**XIII-3** (0.1 mg)	**Egg** (cu Omix, 1.0ug, 1.0%)	**Arabic gum and sugars of the egg** + traces of a contamination (xyl, gluc > DL) (1.5ug/ 1.5%)	Almost **Egg profile** (2,4 µg/ 2.4%)
Saint Nicolas (Sf. Nicolae), middle of the 19th century	**XIII-4** (0.2 mg)	**Egg** (cu Omix, 0.31ug, 0.2%)	**Arabic gum and sugars of the egg** (1.0ug/ 0.5%)	**Aged linseed oil** (22.6ug, 11.3%)
Beheading of St John the Baptist, first part of the 19th century	**KHM 2**	**Traces of egg** (?)	Not analyzed	**Linseed oil** traces of pine resin
Icon from Olt county, dated 1883 (Tămas?)	**KHM1**	**Egg, traces of animal glue**	Not analyzed	**Linseed oil** traces of pine resin

Table 11. Overview of the organic materials identified in the samples from Olt county glass icons

Name and manufacturing period if the icons	Sample (weight)	Proteinaceous fraction (weight and percentage in sample, remarks)	Saccaride fraction (weight and percentage in sample, remarks)	Lipid-resinous fraction (weight and percentage in sample, remarks)
Annunciation (Bunavestire), second part of the 19th century	**XIV-6** (<0.1mg)	**Egg** (0.35ug, ?%)	Not analyzed	**Aged linseed oil** (2.3ug)
Crowning of the Virgin (Încoronarea Fecioarei) second part of the 19th century	**XIV-7** (0.1mg)	**Egg** no Omix, (0.36ug/ 0.36 %)	Not analyzed	**Aged linseed oil** (2.6ug, 2.6%)
	KHM2	**traces of animal glue**	Not analyzed	**Linseed oil**
Candlemas (Stretenia) Savu Moga?, second part of the 19th century	**XIV-8** (<0.1mg)	**Animal glue** no Omix (2.8ug)	Not analyzed	**Aged linseed oil** (8.5ug)
	KHM2	**Animal glue**	Not analyzed	**No oil detected** (sample too small?)
Jesus on the throne (Iisus pe tron), Savu Moga?, second part of the 19th century	**XIV-5** (0.1mg) **XVII-1** (0.75mg)	**Egg** with Omix (3.5ug, 0,5%)	Below detection limit	Almost **Egg profile** (8.1 ug, 1%)
Praznicar icon (placed before the altar), middle of the 19th century	KHM1	**Egg,** traces of animal glue (?)	Not analyzed	**Linseed oil**, traces of pine resin, traces of cholesterol

Table 12. Overview of the organic materials identified in the samples from glass icons by Savu Moga

Name and manufacturing period if the icons	Sample (weight)	Proteinaceous fraction (weight and percentage in sample, remarks)	Saccaride fraction (weight and percentage in sample, remarks)	Lipid-resinous fraction (weight and percentage in sample, remarks)
Saint Elijah (Sf Ilie), second part of the 19th century	**XIV-3** (1.1mg?) **XVI(16)-2** (0.2 mg)	**Egg and animal glue** (no Omix, 0.47ug, 0.2%)	Sugars present, complex saccharide profile**,** **Arabic gum?** and wood contamination (1.2ug/ ??0.1%)	**Aged linseed oil** (90.6ug, 8.2%)
Saint Haralambos (Sf Haralambie) second part of the 19th century	**XIV-4** (<0.1mg?) **XVI(16)-3** (0.2 mg)	**Egg and animal glue?** (traces de Hyp) (no Omix, 0.8ug, 0.4%)	**Arabic gum and sugars of the egg** + possible contamination (xyl, gluc > DL) (1.4ug, ?%)	**Aged linseed oil** (11.5ug, ?%)
Grieving Holy Mother (MD Indurerata)**,** dated 1884	**KHM1**	**Animal glue, traces of egg**	**Not analyzed**	**Linseed oil,** traces of cholesterol, traces of pine resin

Table 13. Overview of the organic materials identified in the samples from glass icons by Matei Țimforea

Sample	ala	gly	val	leu	ile	ser	pro	phe	asp	glu	hyp	Protein content	PC1	PC2
XI-3	8.1	7.7	8.0	12.1	7.1	6.3	4.1	7.8	19.6	19.1	0.0	5.0 ug, 2.5%	-1.3212	-1.2524
XI-4	8.8	15.1	8.6	16.0	8.4	6.6	5.6	6.2	8.2	16.6	0.0	0.5 ug, 0.5%	-0.5149	-0.2819
III-3	10.2	8.1	11.6	17.1	8.5	9.3	2.3	0.0	16.3	16.7	0.0	0.44ug,0.44%	-1.1361	-1.4902
XIII-3	9.9	15.2	9.5	12.8	6.9	3.7	8.0	5.6	11.5	16.9	0.0	1.0ug, 1.0%	0.2360	-0.1933
XIII-4	7.6	13.8	6.6	10.0	5.4	11.9	5.6	6.9	12.6	19.6	0.0	0.31ug, 0.2%	0.0602	-0.8512
XIV-6	5.6	16.9	6.3	10.9	6.1	15.3	3.4	6.0	14.8	14.6	0.0	0.35ug	-0.2525	-1.6361
XIV-7	8.3	18.0	8.7	16.8	8.2	8.9	4.0	5.8	11.0	10.3	0.0	0.36ug/ 0.36 %	-0.6301	-1.3789
XIV-8	11.9	32.5	5.4	6.7	3.4	3.6	11.1	3.2	8.3	10.2	3.7	2.8ug	4.1828	-0.4026
XVII-1	8.0	8.3	9.9	12.6	6.8	11.4	6.8	5.5	12.2	18.4	0.0	3.5ug/ 0,5%	-0.7623	-0.5991
XVI(16)-2	8.5	21.4	5.7	8.2	4.0	6.3	6.4	4.3	13.2	18.5	3.4	0.46 µg/ 0.2%	1.9527	-0.4064
XVI(16)-3	8.9	16.7	7.4	10.6	5.5	15.1	5.5	4.6	9.7	13.9	1.9	0.84 µg/ 0.4%	0.9262	-1.3851

Table 14. Relative amino acid percentage content of the proteins in the samples from Olt County glass icons, icons by Savu Moga and icons by Matei Țimforea and the two principal components resulting from PCA

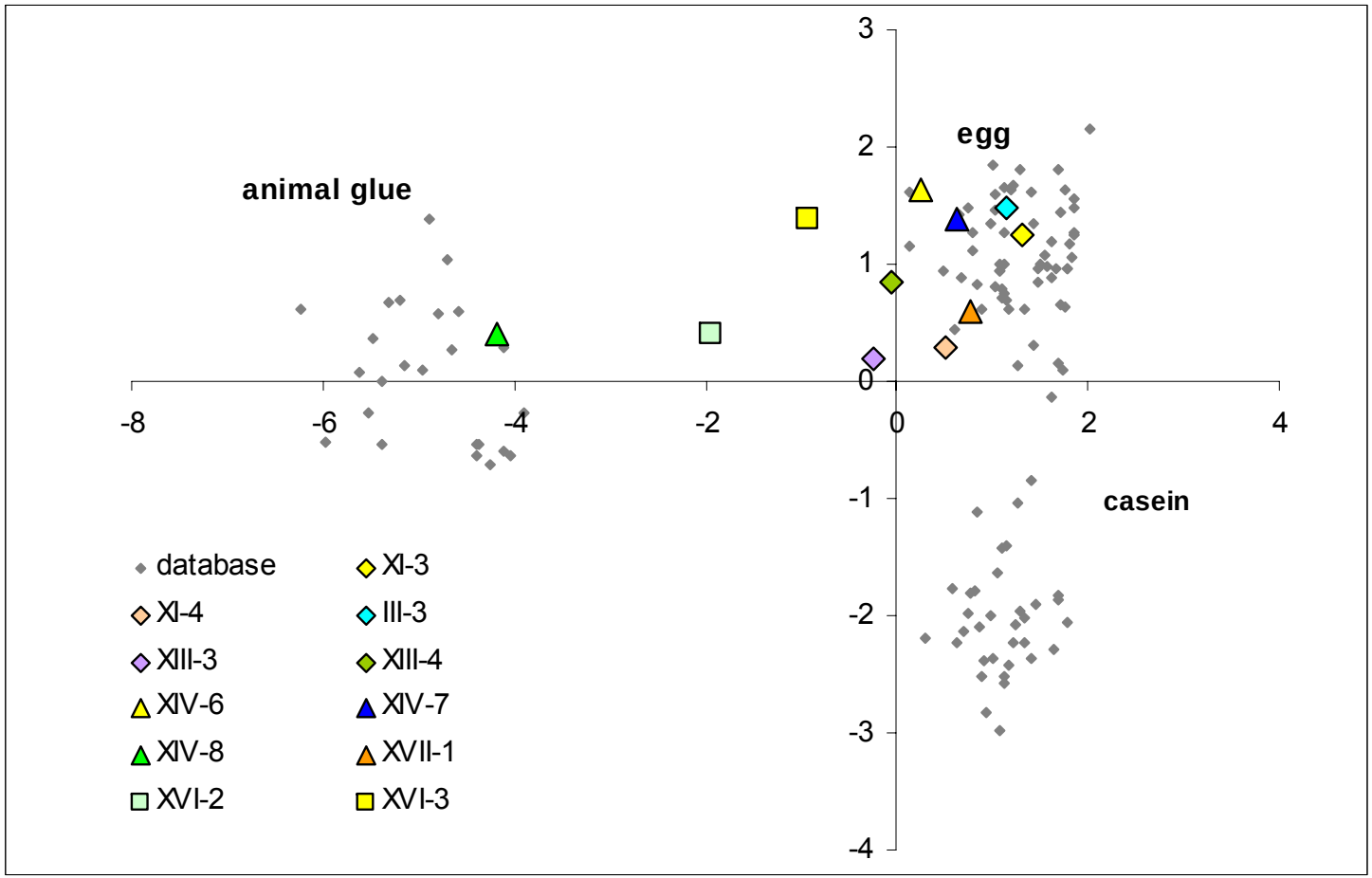


Fig. 8 PCA score plot of samples from Olt County icons and icons by Savu Moga and Matei Țimforea

Sample	Characteristics of lipid-resinous fraction of the samples						Saccharide profile of the samples									Content µg/%
	P/S	A/P	Σ DC	Content µg/%	Chole sterol	Pine resin	Xyl	Ara	Ram	Fuc	A.gal	A.glu	Glu	Man	Gal	
XI-3	1.1	3.1	66.9	9.6 / 4.8	ND	ND	-	-	-	-	-	-	-	-	-	-
XI-4	0.9	1.7	47.4	1.9/1.9	ND	ND	-	-	-	-	-	-	-	-	-	-
III-3	1.2	1.7	51.8	3.9	ND	traces	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	< DL
XIII-3	1.7	0.02	1.5	2.4/ 2.4	ND	ND	NC	38.1	0.0	0.0	0.0	0.0	NC	30.5	31.5	1.5/ 1.5
XIII-4	1.1	1.9	53.2	22.6/ 11.3	ND	ND	NC	16.4	4.6	0.0	0.0	0.0	NC	44.2	34.8	1.03/ 0.5
XIV-6	0.7	1.3	39.5	2.3	ND	ND	-	-	-	-	-	-	-	-	-	-
XIV-7	0.8	1.4	43.0	2. 6/ 2.6	ND	ND	-	-	-	-	-	-	-	-	-	-
XIV-8	0.8	3.7	67.1	8.5	ND	ND	-	-	-	-	-	-	-	-	-	-
XVII-1	1.4	0.1	6.7	8.1/ 1.0	ND	ND	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	< DL
XIV-3	1.4	4.2	74.3	90.6/ 8.2	ND	ND	< DL	7.1	0.0	0.0	0.0	0.0	< DL	47.5	45.5	1.15
XIV-4	0.9	3.2	63.3	11.5	ND	ND	NC	16.4	4.6	0.0	0.0	0.0	NC	44.2	34.8	1.45

Table 15. Analytical results for the lipid-resinous and saccharide fraction of the samples from Olt County glass icons, icons by Savu Moga and by Matei Ţimforea
(P/S- palmitic/ stearic acid percentage ratio; A/P- azelaic/ palmitic acid percentage ratio; Σ DC- sum of dicarboxylic acids percentage amounts;
ND – not detected; NC – not considered; < DL – below detection limit; - no data available)

Glass icons from Brasov area (Şcheii Braşovului)

The objects sampled from this region were from the second part of the 19th century.

The results of the analyses showed that mixtures were used as binders of the paint layer samples. The proteinacous compound of the binding media was egg in about 60% of the samples and animal glue in 30% of the samples; for the remaining two icons egg with animal glue were identified. Based on the results from the lipid resinous fraction of the samples, linseed oil was added to most binders: just one of the samples revealed the lipid profile of an egg. Pine resin was found in 35% of the icons. Traces of cholesterol were detected just in three of the egg containing samples .

Detailed results are presented in the tables below.

Name and manufacturing period if the icons	**Sample** (weight)	**Proteinaceous fraction** (weight and percentage in sample, remarks)	**Saccaride fraction** (weight and percentage in sample, remarks)	**Lipid-resinous fraction** (weight and percentage in sample, remarks)
0	**1**	**2**	**3**	**4**
Burial of Jesus (Inmormantarea lui Iisus), second part of the 19th century	**II-4** (<0.1mg) **V-2** (0.9mg)	**Animal glue** (7.2ug, 0.8%)	**Sugars present** Contamination?	(6.3ug) **Aged linseed oil** Traces of pine resin and cholesterol (V-2)
Baptism of the Lord (Botezul Domnului), second part of the 19th century	**IV-3** (1,4mg) **V-3** (0.9mg)	**Egg** (Omix, 0.39ug, 0.03%)	**Sugars present** Contamination?	(17.4ug, 1.2%) **Egg profile** **Pine resin**
Heavenly feast (Masa Raiului) end of the 19th century	**IV-4** (0,6mg) **VI-3** (<0.1mg) **VII-2** (0.7 mg)	**Inconclusive results**	**Sugars present** Contamination?	**Aged linseed oil** **Pine resin** (9.3ug, 1.3%)
Sf Paraschiva, second part of the 19th century	**VI-2** (<0.1mg) **VII-1** (<0.1mg)	(no Omix, 0.6ug) **Egg and animal glue**	Not analyzed	(1.7ug) **Aged linseed oil**
Heavenly feast (Masa Raiului) second part of the 19th century	**VI-1** (<0.1mg)	**Egg** (no Omix, 7.2ug)	Not analyzed	**Aged linseed oil** **Pine resin** (3.0ug)

0	1	2	3	4
Resurrection (Învierea lui Iisus) second part of the 19th century	**VI-4** (<0.1mg) **VII-3** (0,2mg) **X-3** (<0.1mg)	**Egg** (1.9ug, no Omix)	Not analyzed	(3.9ug, 1.9%) **Aged linseed oil** (5.1ug)
Crowning of the Virgin (Încoronarea Maicii Domnului) end of the 19th century	**VII-4** (0,6mg) **X-4** (<0.1mg)	**Egg** (no Omix, 7.0ug)	Not analyzed	(9.7ug. 1.6%) **Aged linseed oil** (4ug)
Sf Paraschiva, end of the 19th century	**VIII-1** (0.3mg) **X-1** (<0.1mg)	**Egg** (no Omix, 4.6ug)	Below detection limit	**Aged linseed oil** (7.6 ug)
Holy Mother (Maica Domnului alăptând) end of the 19th century	**VIII-2** (0.1mg) **X-2** (<0.1mg)	**Egg** (2.3ug, ?%)	Below detection limit	**Aged linseed oil** (5.9 ug)
Last supper (Cina cea de taina), end of the 19th century	**VIII-3** (<0.1mg)	**Animal glue** (cu Omix, 0.23ug, la DL)	Below detection limit	**Aged linseed oil** (5.7 ug)
Three Saints (Cei Trei Ierarhi), end of the 19th century	**VIII-4** (<0.1mg)	**Animal glue** (cu Omix, 3.3ug,)	Below detection limit	**AG below detection limit**

Table 16 Overview of the organic materials identified in the samples from Brasov area glass icons

Sample	ala	gly	val	leu	ile	ser	pro	phe	asp	glu	hyp	Protein content	PC1	PC2
II-4	11.4	30.6	4.6	5.8	2.8	6.8	12.7	1.5	9.8	9.9	4.1	7.2µg, 0.8%	4.4726	-0.4035
IV-3	6.8	17.7	5.5	10.4	5.0	13.9	7.1	4.0	12.6	17.1	0.0	0.4µg, 0.03%	0.8709	-0.6867
VI-2	6.4	20.2	4.0	7.4	3.6	6.3	9.6	5.2	19.1	13.2	4.1	0.6µg	2.0171	-0.6871
VI-1	8.5	9.2	10.0	16.8	6.5	6.6	4.4	6.3	15.2	16.5	0.0	7.1µg	-1.3040	-1.01038
X-3	9.3	17.0	9.2	13.8	7.0	4.0	9.7	3.3	13.6	13.0	0.0	1.9µg	0.4769	-0.21809
X-4	7.5	19.0	6.7	12.9	5.2	9.1	4.9	4.2	15.6	14.6	0.0	7.0µg	0.4084	-1.15919
X-1	5.4	9.2	7.3	11.5	5.2	9.2	10.9	6.6	14.6	20.1	0.0	4.6µg	-0.3334	0.36342
X-2	10.4	24.1	7.8	15.6	6.1	4.1	7.9	4.7	9.0	10.3	0.0	2.3µg	1.1349	-0.58167
VIII-3	5.1	10.9	4.1	6.1	3.5	3.7	8.8	3.3	5.7	20.6	28.2	0.22µg	4.5749	-1.65977
VIII-4	9.9	24.0	3.2	4.2	1.9	3.6	14.5	4.5	10.9	16.0	7.4	3.3µg	4.4394	0.48536

Table 17. Relative amino acid percentage content of the proteins in the samples from Braşov area and the resulting two principal components

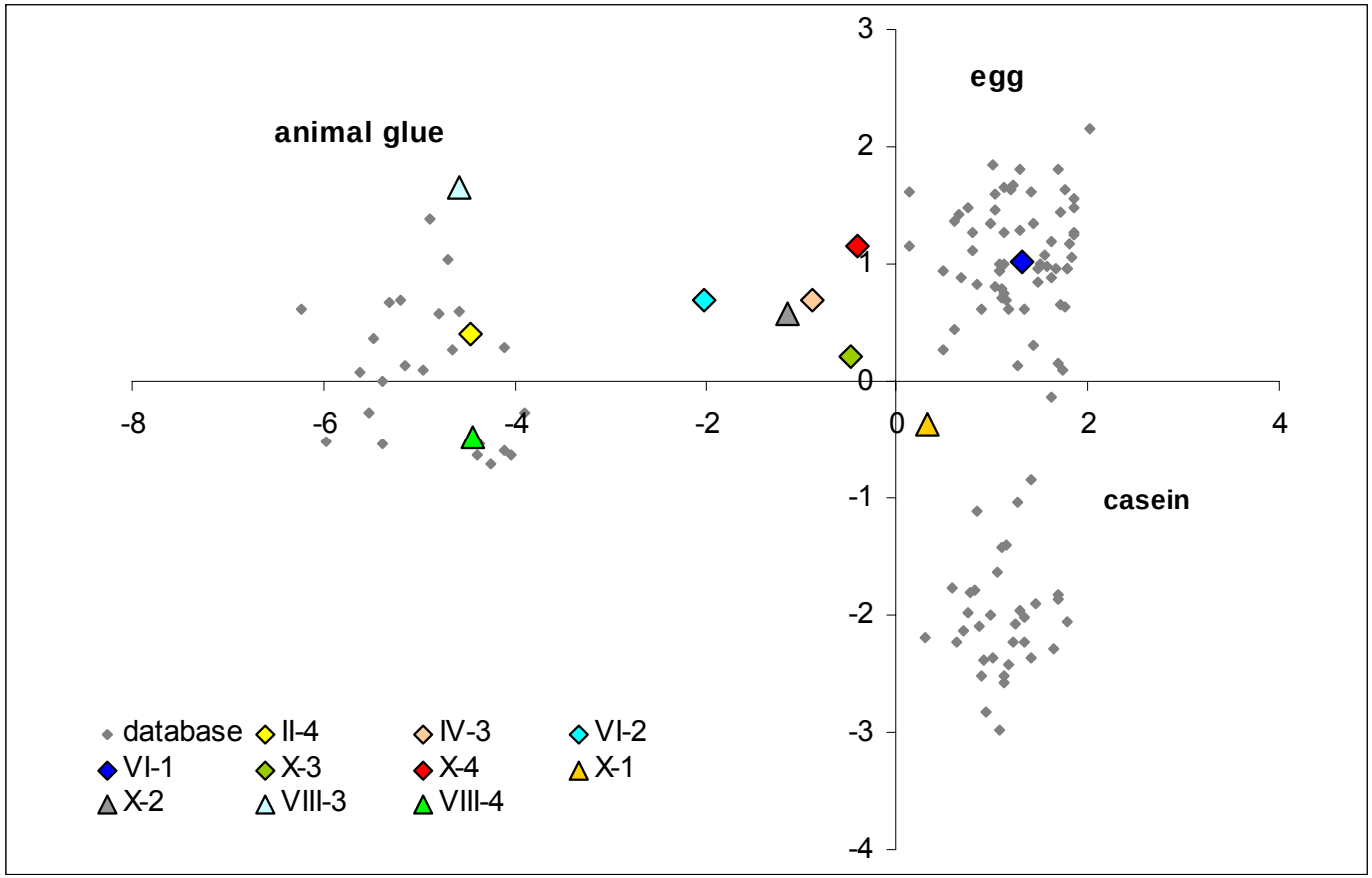


Fig. 9. PCA score plot of samples from Braşov area

Sample	Characteristics of lipid-resinous fraction of the samples					
	P/S	A/P	Σ DC	Content µg/%	Cholesterol	Pine resin
II-4	0.6	1.7	45.9	6.3/ 1.3	ND	traces
IV-3	3.2	0.1	7.3	17.4/ 1.2	traces	present
VII-2	1.1	1.8	55.9	9.3/ 1.3	traces	present
VI-2	0.7	2.0	46.8	1.7	ND	ND
VI-1	0.7	1.8	40.3	3.0	ND	present
VII-3	0.9	1.3	43.1	3.9/ 1.9	ND	ND
VII-4	1.2	1.4	48.5	9.7/ 1.6	ND	ND
X-1	0.8	3.8	65.0	7.6	ND	ND
X-2	0.9	2.4	56.3	5.9	ND	ND
VIII-3	1.1	1.9	56.1	5.7	ND	ND
VIII-4	-	-	-	0.56	ND	ND

Table 18 Analytical results for the lipid-resinous of the samples from Brasov area glass icons
(P/S- palmitic/ stearic acid percentage ratio; A/P- azelaic/ palmitic acid percentage ratio; Σ DC- sum of dicarboxylic acids percentage amounts; ND – not detected; NC – not considered; < DL – below detection limit; - no data available)

III.5. Organic materials in the paint layer of Transylvanian painted ceilings

Many of the ecclesiastical and secular monuments of Transylvania are decorated with painted coffered ceilings and other kind of painted woodwork. No research concerning the organic materials of these painted surfaces was carried out up to now. Samples analyzed within the thesis were collected by restorer Mihály Ferenc form five medieval churches in different regions of Transylvania. Their pained woodwork dated from 17th and 18th century.

The aim of the research was to characterize the painting technique of the ceilings and woodwork, to see if different colors have similar binder and to compare the techniques used by different workshops. It was also important to better understand the observed decay processes of the paint layers based on scientific data and to improve their conservation strategies.

All together 22 samples were analyzed.

The proteinaceous fraction of the samples was mainly animal glue. Three samples contained egg mixed with animal glue, they all were from paintings of Umling Lőrinc the elder, from white or white and blue surfaces. Interestingly, the other 4 colors (black, green, red and ochre) analyzed from paint layers by this painter (even on the same object where white with egg and animal glue was identified) had only animal glue as binder.

Saccharide content was analyzed just in some of the samples. Five of these contained some sugars, but judging from the saccharide profile of the samples this was more probably due to wood contamination then to an intentionally added polysaccharide binder. Two wood samples were analyzed in order to understand the problem, but this issue needs further studies.

Lipid content of all samples was below detection limit, it contained no resinous component or waxes. The results are in good agreement with the aspect mat aspect of the paint.

So, basically the painter-carpenters used simple binding media applying colors mainly with animal glue in all five studied churches. The only noticed exceptions are connected to white and blue colors of Umling Lőrinc the elder, who applied also egg mixed with animal glue, probably because his known background in panel painting.

The overall results of the analyses are presented below.

Name and manufacturing period if the painted woodwork	Sample (weight)	Proteinaceous fraction (weight and percentage in sample, remarks)	Saccaride fraction (weight and percentage in sample, remarks)	Lipid-resinous fraction (weight and percentage in sample, remarks)
0	2	3	4	5
Painted ceiling of reformed church in Alunişu (Magyarókereke, CJ), Umling, the elder, 1746; sample XII-1 comes from a paint layer (white), the other two samples from the wood of the ceiling	**XII-1** (<0.1 mg)	**Egg and animal glue** (cu Omix, 0.38 ug, traces de Hyp)	Sugars present Probably due to wood contamination	**Below detection limit** (0.7 ug)
	XIV-1 (<0.1mg)	-	Sugars of the wood 1,4 ug (DL<Ram, Glu<QL, Xyl, Ara, Man, Galct>QL)	-
	XVII-6 (1.4mg)	-	Sugars of the wood 0,96ug, 0.1%	-
Painted ceiling of reformed church in Alunişu (Magyarókereke, CJ), , Umling, the younger, 1786	**XII-2** (0.6 mg)	**Animal glue** (1.4 ug, 0.2%)	Sugars present Probably due to wood contamination	**Below detection limit**
Painted ceiling of reformed church in Luna de Sus (Magyarlóna, CJ), Umling, the elder, 1752, coffer G13, (white)	**XV-1** (1.1 mg)	I **Egg and animal glue** (0.3ug, 0.3%)	Not analyzed	**Below detection limit**
	XVI(16)-4 (0.1 mg)			
Idem, (black)	**XV-2** (2.2 mg)	**Animal glue** (3.8ug, 0.4%)	Not analyzed	**Below detection limit**
	XVI(16)-5 (0.9 mg)			
Idem, (green)	**XV-3** (1.0 mg)	**Animal glue** (3.9ug, 0.8%)	Not analyzed	**Below detection limit**
	XVI(16)-6 (0.5 mg)			

0	2	3	4	5
Painted ceiling of the reformed church in Luna de Sus (Magyarlóna, CJ), Umling, the elder, 1752, coffer G13, (red),	**XV-4** (2.8 mg) **XVI(16)-7** (0.8 mg)	**Animal glue** (13.8ug, 1.7%)	Not analyzed	**Below detection limit**
Idem, (ochre)	**XV-5** (0.2 mg)	**Animal glue** (aprox. 46,0ug, 23,0%)	Not analyzed	**Below detection limit**
Idem, an other coffer (blue)	**XV-6** (1.3 mg) **XVI(16)-8** (0.4 mg)	**Egg and animal glue** (1.3ug, 0.3%)	Not analyzed	**Below detection limit**
Reformed church in Luna de Sus (Magyarlóna, CJ), pew parapet, Umling the younger (blue and black), 1768	**XV-7** (0.5 mg) **XVII-5** (0.4mg)	**Animal glue** (12,9 ug, 3,2%)	Not analyzed	**Below detection limit**
Painted ceiling of the catholic church in Ghelința (Gelence, CV), 1628, (green)	**IX(9)-3** (0.7 mg)	**Animal glue** (6,8ug, 1.0%)	Sugars present Probably due to wood contamination	**At detection limit** (1,3 ug) Typical blank profile
Painted ceiling of the reformed church in Petrindu (Nagypetri, SJ), painter Zilahi Asztalos János, 1713 (red)	**XII-3** (0.3 mg)	**Animal glue** (1.8ug, 0.6%)	Sugars present Probably due to wood contamination	**Below detection limit**
Painted ceiling of the reformed church in Crasna (Kraszna, SJ), Pataki Asztalos János, 1736. (green)	**XII-4** (<0.1 mg)	**Animal glue** (0,6ug)	Sugars present Probably due to wood contamination	**Below detection limit**
Idem, (flaking red)	**XV-8** (1.4 mg) **XVII-2** (1.1mg)	**Animal glue** (14,9 ug, 1,4%)	**Below detection limit**	**Below detection limit** (0,7ug, 0.06%)

Table 19. Overview of the organic materials identified in the samples from painted coffered ceilings

Sample	ala	gly	val	leu	ile	ser	pro	phe	asp	glu	hyp	Protein content	PC1	PC2
XII-1	8.2	22.8	4.6	7.4	4.0	9.7	11.0	3.1	12.4	15.9	0.9	0.4 ug	2.3933	0.0994
XII-2	9.3	23.3	4.0	5.7	3.1	5.1	15.7	3.3	9.0	12.5	8.9	1.4ug/ 0.2%	4.2862	0.5748
XVI-4	10.8	19.3	6.8	8.5	4.7	2.9	10.9	4.1	13.3	16.7	2.0	0.3ug,/ 0.3%	1.9073	0.0062
XVI-5	11.9	33.0	4.2	5.1	2.7	2.6	14.6	2.9	10.2	10.9	1.9	3.8ug/ 0.4%	4.3799	0.0719
XVI-6	11.3	22.5	5.5	5.5	2.9	6.2	10.9	3.1	9.0	10.0	13.2	3.9ug/ 0.8%	4.5593	-0.5454
XVI-7	10.1	24.2	3.1	4.5	2.0	3.5	15.4	2.7	8.5	15.6	10.3	13.8ug/ 1.7%	5.1520	-0.9742
XV-5	14.3	35.7	3.7	3.3	1.8	3.9	6.6	1.9	12.2	14.8	1.9	46,0ug/ 23,0%	4.7389	-1.4094
XVI-8	11.2	21.4	6.8	8.5	4.4	3.5	14.2	4.4	13.3	11.5	0.9	1.3ug/ 0.3%	2.3532	0.1118
XVII-5	10.3	25.4	3.4	4.1	1.9	3.2	13.5	2.6	10.1	16.2	9.4	12,9 ug/ 3,2%	5.0519	0.5314
IX-3	9.8	29.5	3.9	4.6	2.2	2.9	18.5	2.5	6.7	14.9	4.5	6,8ug/ 1.0%	4.8952	0.5917
XII-3	9.8	26.1	2.9	4.1	2.0	3.4	14.5	2.6	8.4	14.8	11.5	1.8ug/ 0.6%	5.4117	0.7396
XII-4	9.2	26.3	4.2	7.4	3.6	6.1	11.6	2.7	10.1	12.3	6.5	0.6ug	3.8532	-0.0173
XVII-2	10.6	27.1	4.0	5.5	2.4	3.7	16.5	3.2	8.0	12.1	6.9	14,9 ug/ 1,4%	4.8847	0.6510

Table 20 Relative amino acid percentage content of the proteins in the samples from painted ceilings and the two principal components resulting from PCA

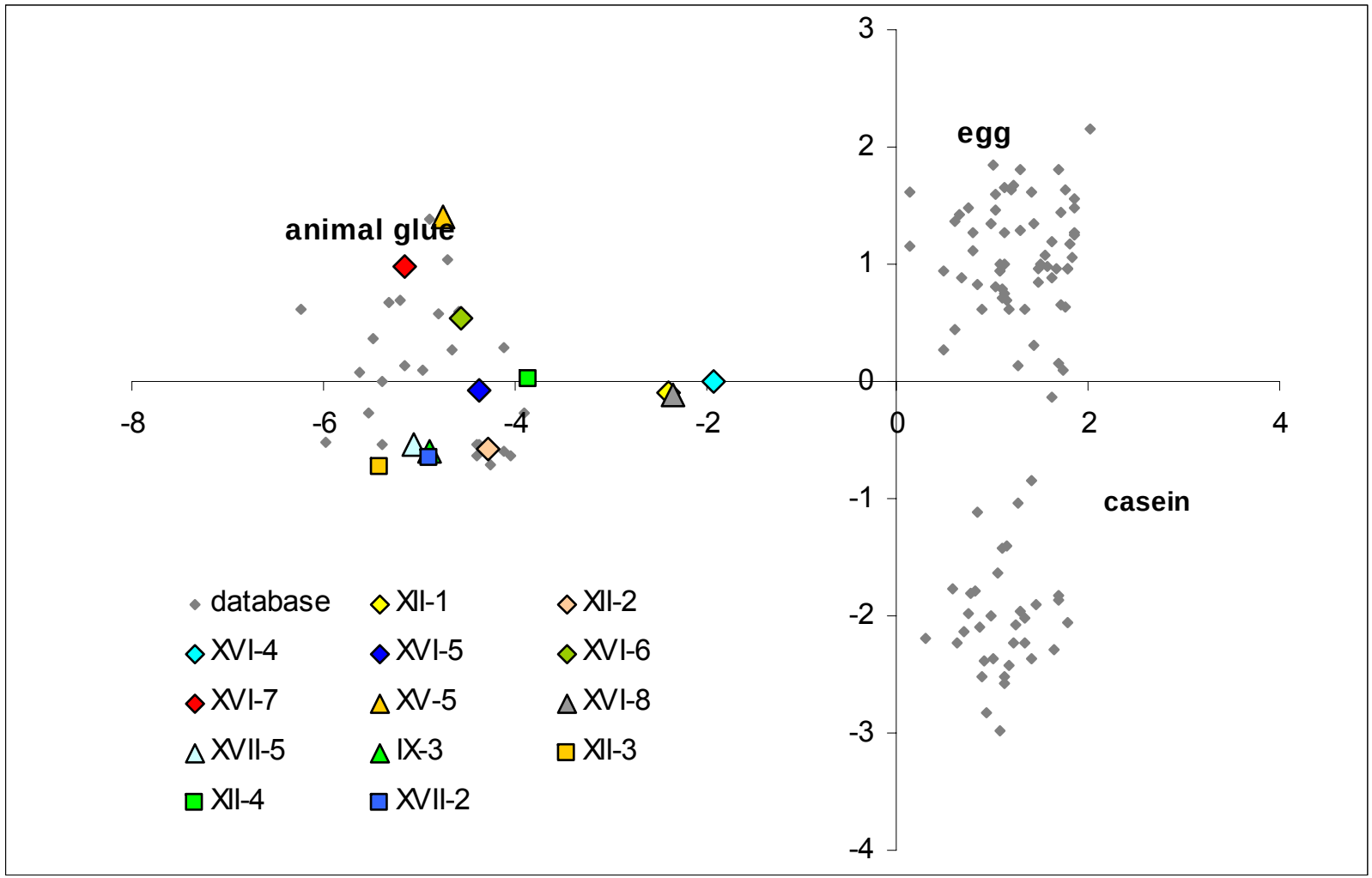


Fig. 10. PCA score plot of the proteinaceous fractions belonging to painted ceiling samples

III.6. Organic materials of two wall paintings

The painting of the renaissance prayer niche in castle Siklós

The analyzed paint sample originates from the wall painting of a renaissance prayer niche recently discovered at Siklós castle, Hungary. From the aspect of the painting it was obvious that it is painted with secco technique, using an organic binder. The analysis of the binder was necessary in order to characterize the painting and to plan its consolidation.

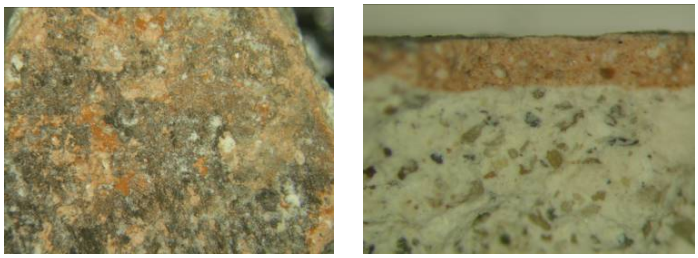


Fig. 11. View and cross section of the paint layer; sample IV-1was scarped from the surface

The content of the lipid and proteinaceous fractions of the sample was below detection limit. The saccharide fraction (SIM chromatogram shown in Fig 11.) contained arabinose, ramnose, glucose and galactose above detection limit. The profile was characteristic to arabic gum.

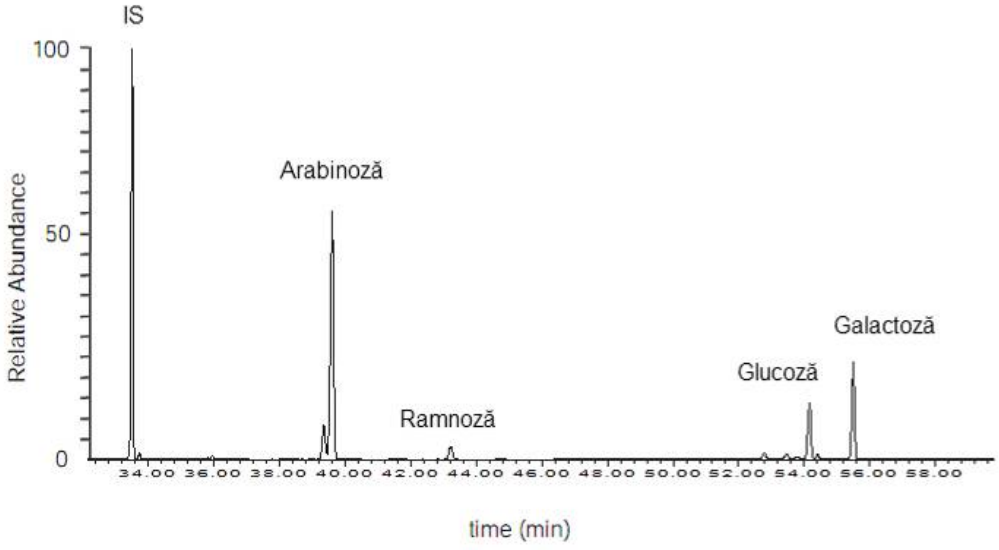


Fig. 12. SIM chromatogram of sample IV-1

Sample description	Saccharide profile									Content µg/%
	Xyl	Ara	Ram	Fuc	A.gal	A.glu	Glu	Man	Gal	
Siklós, red paint layer sample (IV-1, weight 2.3mg)	< DL	47.9	6.9	< DL	< DL	< DL	< DL	< DL	45.1	1.24/ 0.05
Average saccharide content of reference arabic gum samples[33]	0	36.1	10.8	0	0	7.3	NC	0	45.8	

Table 21. Saccharide profile of sample IV-1 compared to the average saccharide content of reference arabic gum samples (< DL – below detection limit; NC – not considered)

The binder of the blue of Voroneț

The 15th century churches in Bucovina (Northen Romania) with exterior and interior wall paintings were declared world heritage sites in 1993. The blue on the exterior of Voronet monastery church is particularly famous, because it preserved its vivid, bright aspect even in places where other colors were lost (Fig. 12). Studies up to now showed that the pigment is azurite $2CuCO_3 \cdot Cu(OH)_2$.[34], applied on a carbon black layer with an organic binder (the use of azurite in fresco technique is not possible because its sensitivity to alkaline solutions). The same layer structure was observed for the green surfaces painted with malachite $CuCO_3 \cdot Cu(OH)_2$. In order to analyze the organic binder of the blue and green layers, two samples were kindly provided by ing. Ioan Istudor (Fig. 13).

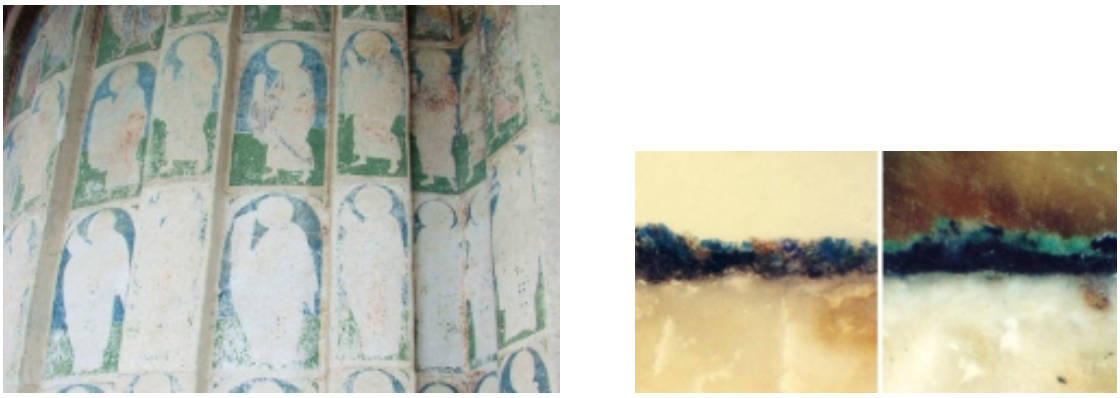


Fig. 13. Detail from the outside painting of Voronet church showing the surprising stability of blue and green surfaces (photo CERECS ART, 2007) and the cross section of two samples taken from blue (left) and green (right) prepared by ing Ioan Istudor

[33] Bonaduce et al. 2007
[34] Istudor 2009

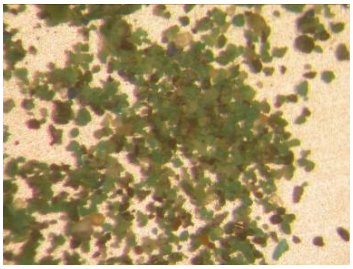
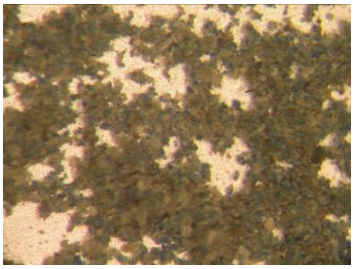



Fig. 14. Microscopic aspect of the samples: green, left, XVII-3, weight 0.9mg, blue, right, XVII-4, weight 0.6mg

Organic material content of sample XVII-3 (green, weight 0.9 mg) was below detection limit. In the proteinaceous fraction of sample XVII-4 (blue, weight 0.6 mg) a considerable amount of protein was detected, identified by PCA as egg. As shown in Table 22 the relative amino acid percentage content was close the one of egg measured in reference samples. The saccharide and lipid-resinous fraction of the same sample were below detection limit.

Even though the result for one of the samples was conclusive, analyses of further samples would required to draw final conclusions regarding the way of applying the blue and the green, and in generally, regarding the whole exterior painting technique.

	Relative amino acid content (%) of the protein										
	ala	**gly**	**val**	**leu**	**ile**	**ser**	**pro**	**phe**	**asp**	**glu**	**hyp**
Sample XVII-4	7.1	11.7	7.5	13.8	7.0	10.9	4.7	6.4	13.0	17.9	0.0
Average values for egg	7.7	4.8	7.7	11.0	6.7	10.3	5.7	6.4	12.6	15.0	0.0

Table 22. Relative amino acid percentage content of the sample with blue and the one of egg measured in reference samples

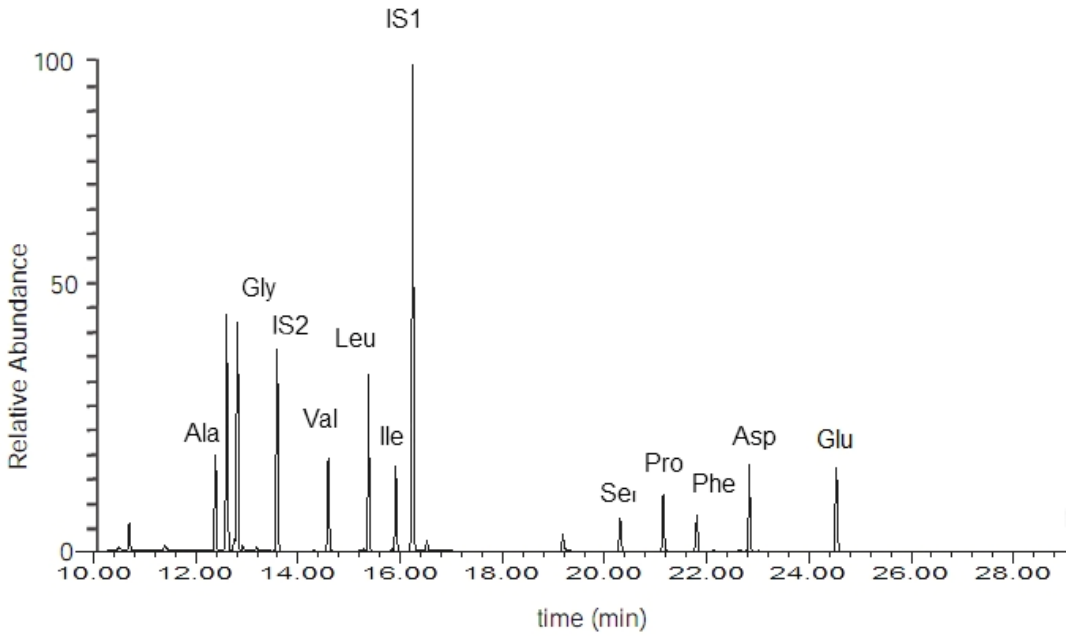


Fig. 15. SIM chromatogram of the proteinaceous fraction of sample XVII-4

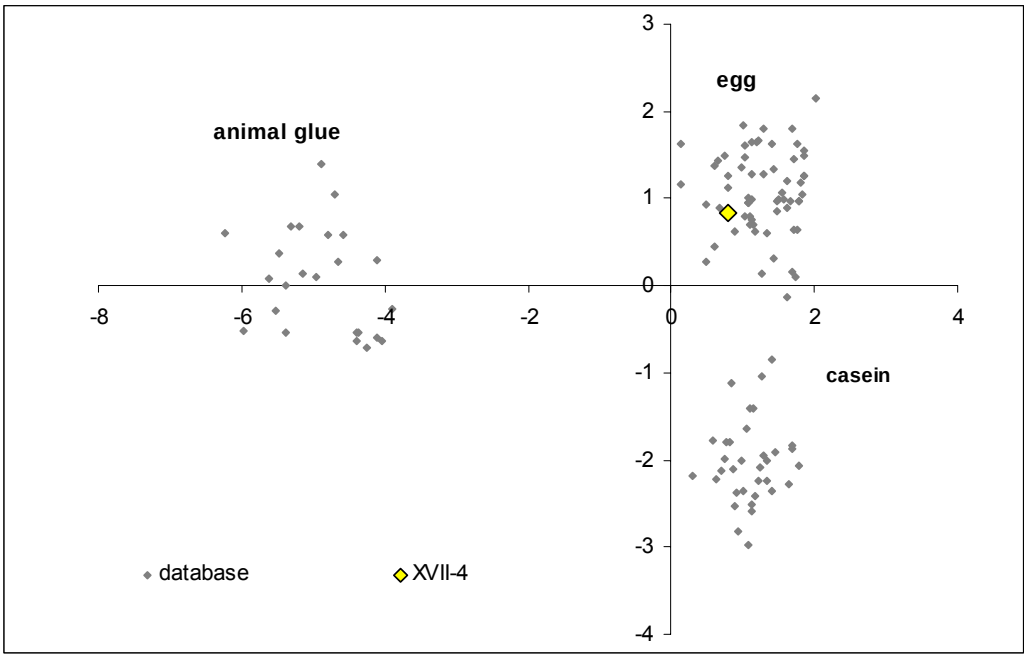


Fig. 16. PCA score plot of sample XVII-4

V. Conclusions

Comprehensive analysis of organic materials in paint layers belonging to Transylvanian heritage objects bring an awaited contribution to the characterization of these items.

Analyses were performed by GC-MS, adopting a working methodology which enables the specific and quantitative identification of all main organic compounds encountered in paint layers. The procedure was applied to the analysis of 71 samples, 10 more were analyzed with a simplified qualitative method. All together 38 Transylvanian glass icons, five painted ceilings, a pew parapet and two wall paintings were characterized.

Evaluating the result for the studied glass icons one can conclude that the binding media used for the paint layer of the icons consisted of complex mixtures based on larger diversity of materials as supposed according to the few inherited documentary sources. The binder of most studied icons had two or three components.

Egg was the proteinaceous component in 54% of the studied objects, in the others animal glue, or a mixture of egg and glue were found. Finding casein with animal glue in one of the glass icons from Nicula was an unexpected result. Animal glue as only protein component of the binder was encountered just in Brasov area.

The lipid fraction of almost each sample contained linseed oil, detected also in high amounts, proving that adding oil to the binding media was more common as supposed, in every center, already from the beginning of the 19th century. Pine resin was present in each center, but in less than 20% of the studied icons, partly in traces. Sugars were found in all three centers, proving to be arabic gum and/ or sugars of the egg and/ or remains of a contamination.

Results could not reveal characteristic binding media usage for any of the studied glass icon centers, but showed some possible particularities. These conclusions are not unexpected, as icon painters were self taught artists, unrestrained by prescription, working in modest conditions and using cheap and handy materials.

Analyses performed on the samples from painted ceilings and a pew parapet, belonging to Transylvanian churches from 17^{th} and 18^{th} century, showed a more simple binding media usage. In most of the samples only animal glue was detected. One single painter, Umling Lőrinc the elder, used egg with animal glue for two of his colors, white and blue, using just glue for four other colors analyzed. Lipid content of all samples was below detection limit and no pine resin was found, which is in good agreement with the matte aspect of the paintings. In some of the samples sugar content was detected, but the saccharide profile suggested wood contamination.

The procedure was applied to the analyses of two valuable wall painting samples. In the renaissance painting from Siklós arabic gum was detected. The famous blue from Voronet proved to have egg as binder.

The above results report the first conclusions regarding the binding media of different Transylvanian painted heritage objects. They provide a useful contribution to the understanding of their painting technique and to the characterization of the different centers. The results were conveyed to the specialists providing the samples, helping them to better understand the objects and to adopt suitable conservation strategies.

The above results, together with the widespread interest at international level in the study of organic binders in paint layers, are arguments that sustain further research on our cultural heritage.

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