e-PS, 2006, **3**, 5-10
ISSN: 1581-9280 web edition
ISSN: 1854-3928 print edition

www.Morana-rtd.com

e-PRESERVATIONScience
published by M O R A N A RTD d.o.o.

IDENTIFICATION OF HISTORICAL INK INGREDIENTS USING PYROLYSIS-GC-MS. A MODEL STUDY.

FULL PAPER

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In the past centuries inks were prepared using many different recipes and contained a great variety of ingredients. This work presents analyses of some substances, which were used in addition to the common components of inks such as galls, vitriol and charcoal, in the course of ink preparation. Qualitative characterization of seeds and peel of pomegranate, gum arabic, apricot gum, saffron, henna and mustard are reported. The analyses were carried out using pyrolysis coupled to a gas chromatograph with mass-spectrometric detection (Py-GC/MS).

1. Introduction

The knowledge of material characteristics and the techniques used to create an object of art is fundamental in development of strategies to prevent their decay. In studies of degradation of works of art and documents on paper, it is thus important to know how inks were prepared and which substances were used in the process of their production. However, the problem is that during the past centuries a great variety of recipes were followed. In addition to the common components of inks, such as galls and vitriol in iron gall inks and charcoal in carbon-based ones, other ingredients were used to give inks specific properties like i.e., brightness, hue and intensity of colour. In Armenian manuscripts (a particularly beautiful example on Fig. 1) from different periods (12th-19th centuries), many ink recipes were recorded containing various ingredients such as seeds and peel of pomegranate, saffron, henna, mustard beside the more common gums.[1-3]

This study aims at providing a basic chemical characterization of these ingredients using pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). This powerful technique can often provide the qualitative information needed for identification of organic and some inorganic materials in artworks, while using up only a small sample.[4-14] Pyrolysis is thermal decomposition of macromolecules in the absence of oxygen into volatile and semivolatile molecules that can be easily analyzed using a GC/MS. The observed fragments provide a fingerprint which can be characteristic of a particular sample, in terms of both fragment nature and relative distribution. The pyrolysis profile is usually called pyrogram.

received: 22.02.2006
accepted: 14.04.2006

key words:
Pyrolysis-GC/MS, ink, saffron, henna, gum arabic, pomegranate, apricot gum, mustard

Fig. 1: Title page from the Gospel of Luke, 13-14th century, Matenadaran, Armenia.

2. Experimental

2.1. Materials

The materials used for chemical characterization and identification were all natural products and served as model samples. Each product was obtained from a single source, but each sample was independently analyzed three times. The use of these compounds as ingredients was indicated in various Armenian ink recipes.[3]

Saffron has been used in more than one recipe. In a manuscript from 1650, the following ingredients were used: gall nuts, water, iron vitriol, gum, lamp soot, saffron, sugar, and salt.

An example of a recipe containing pomegranate peel is: green gall nuts, rust, pomegranate peel, water, vinegar, gum, iron. It is dated from the 15th century and was used to write on parchment. Another recipe lists: pomegranate seeds (dried wild pomegranate), vinegar, vitriol, gall nuts, gum.

Henna (*Lawsonia inermis* L.) was also very often used, a recipe from a manuscript dated from the 17th century lists: green gall nuts, henna, ammonium chloride, salt solution, water.

The use of apricot gum is reported in a manuscript also from the 17th century, but in many recipes since 1400, gum arabic was more frequent.

2.2 Analysis

Pyrolysis was carried out at 500 °C using an SGE Pyrojector II microfurnace pyrolyzer. The samples were grinded to obtain powder and less than 1 mg was placed inside a disposable quartz tube 50 x 0.53 mm ID. The tube was attached to a solid sampling probe by means of a spring hook. The probe was inserted in the injection head, which was screwed onto the top of the Pyrojector furnace and carrier gas was connected to it. Once

No.	Compound	M (g mol^{-1})
1	pyrrole	67
2	toluene	92
3	1-hydroxy-2-butanone	88
4	phenol	94
5	3-methyl-1,2-cyclopentanedione	112
6	butyl-benzene	134
7	4-methyl- phenol	108
8	2-methoxy-phenol	124
9	2-methoxy-4-methyl-phenol	138
10	1,2-benzenediol	110
11	3-methoxy-1,2-benzenediol	140
12	2-methoxy-4-vinylphenol	150
13	2,6-dimethoxy-phenol	154
14	2-methoxy-4-(1-propenyl)-phenol	164
15	levoglucosan	162
16	acetyloxy-acetic acid	118
17	butanedial	86
18	propanoic acid,2-oxo-methylester	102
19	2-furaldehyde	96
20	3-furaldehyde	96
21	3-methyl-furan	82
22	cyclopentanone	84
23	2-hydroxy-cyclopenten-1-one	98
24	5-methyl-2-furancarboxyaldehyde	110
25	2,5- furandicarboxyaldehyde	124
26	dianhydromannitol	146
27	5-hydroxymethyl-2-furancarboxyaldehyde	26
28	propanal	58
29	2(5H)furanone	84
30	2-cyclopenten-1-one	82
31	1-acetyloxy-2-propanone	116
32	2-cyclopentene-1,4-dione	96
33	2-methyl-2-cyclopenten-1-one	96
34	1-(2-furanyl)-1-butanone	138
35	butyrolactone	86
36	2,3-dimethyl-2-cyclopenten-1-one	110
37	3,4-dimethylcyclohexanone	126
38	3-methyl-2-pentene	84
39	monosaccharide (generic)	150
40	1,2,3-benzenetriol	126
41	3,3-dimethyl-6-methylenecyclohexene	122
42	xylene	106
43	cyclohexanone	98
44	3,5,5-trimethyl-3-cyclohexen-1-one	138
45	3,5,5-trimethyl-2-cyclohexen-1-one	138
46	2,6,6-trimethyl-2-cyclohexene-1,4-dione	152
47	2-hydroxy-3,5,5-trimethyl-cyclohexen-2-one	154
48	unknown	154
49	3,5-dimethyl-benzaldehyde	134
50	4-hydroxy-3,5,5-trimethyl-cyclohex-2-enone	154
51	2,6,6-trimethylcyclohexa-1,3-dien-1-carboxaldehyde	150
52	1-methyl-1H-pyrrole	81
53	styrene	104
54	2,3-dihydro-benzofuran	120
55	indole	117
56	1-butyl-cyclopentene	124
57	propyl benzene	120
58	4-hydroxy-acetonitrile benzene	133
59	oleic acid	282
60	fatty acid (generic)	

Table 1. Principal pyrolysis products referred to in this paper.

the selected pyrolysis temperature was reached, the sample was expelled into a hot quartz furnace liner by depressing the plunger of the probe. The pyrolyzer was directly connected to the GC/MS system, which consisted of a CLARUS 500 gas chromatograph directly coupled to a mass spectrometer CLARUS 500 (Perkin Elmer).

The gas chromatograph was equipped with a 30m x 0.25 mm ID fused-silica column coated with 0.25 μm film of RTX® 5 (Cross bonded 5% diphenyl, 95% dimethyl polysiloxane).

The carrier gas was helium at a head pressure of 7.6 psi and 5 psi more in the pyrolyzer head.

The injector and transfer line temperatures were set at 250 °C and 220 °C, respectively. The samples were injected in the splitless mode and the oven temperature was programmed as follows: 45 °C during the starting 3 min, then 10 °C/min to 250 °C, which was held for 20 min. The operation conditions for electron impact mass spectrometer were: source temperature 220 °C, ionizing voltage 70 eV and scan range from m/z 25 to m/z 1200 with a scan time of 0.2 s. Structural assignment of the compounds (Table 1) was based on spectral matching with the library NIST 2002 (US National Institute of Standards and Technology).

3. Results and discussion

3.1 Pomegranate

Punica granatum L., popularly known as the pomegranate tree, is a densely branched bush with uneven oval leaves. Red flowers are followed by a hard-skinned fruit with a succulent pulp that contains numerous seeds covered in pink to purple-coloured pulp. The pharmacologically interesting parts of the plant are the roots, fruit rinds, seeds and flowers, as they contain different alkaloids (peretierin, isoperetierin, methylisoperetierin), tannins, pigments (e.g. anthocyanin, Fig. 2), organic acids (citric, malic, tartaric, ascorbic), thiamine, riboflavin and salts (containing elements such as phosphorus, potassium, sodium, calcium and iron).

Pomegranate was a very popular fruit all over the Middle East and might even have been the fruit of the “Tree of Knowledge” in the biblical story of creation.[15] The seeds of pomegranate contain lignin, cellulose and other polysaccharides, tannins (in lesser amounts than the peel), amino acids (bound to lignin and phenolic acids).[16] The pyrogram obtained from analysis of pomegranate seeds at 500 °C is shown in Fig. 3.

The composition of pomegranate seeds is reflected by the high amounts of phenolic derivatives among the fragmentation products. The compounds 2-methoxy-phenol (8), 2-methoxy-4-methyl-phenol (9), 2-methoxy-4-(1-propenyl)-phenol (14) are decomposition products of lignin. 2-Methoxy-4-vinylphenol (12) is probably a degradation compound of ferulic acid, which is itself formed as a decomposition product of lignin. Sinapyl alcohol, also a moiety of lignin, is probably the precursor of 2,6-dimethoxy-phenol (13). Pyrrole (1), toluene (2) and 1-hydroxy-2-butanone (3) are eluted at early retention times.

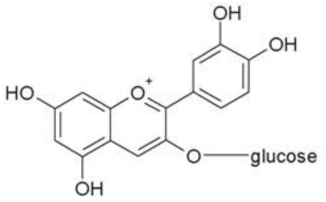

Fig. 2: A generic anthocyanin molecule linked to a molecule of glucose.

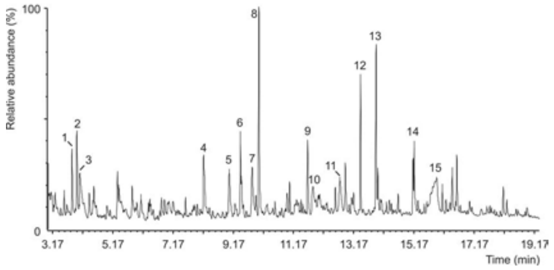

Fig. 3: Total ion chromatogram obtained from pyrolysis of pomegranate seeds at 500 °C. Peak designation according to Table 1.

The rind (peel) of pomegranate fruit, when dry, is brown on the outside, yellow within, smooth or finely tuberculate, hard, brittle, available in irregularly shaped fragments, non-odorous and of a very astringent, somewhat bitter taste. Its infusion gives an abundance of a dark-bluish precipitate with iron salts. Forbes writes: “In Mesopotamia the yellow dye was extracted as early as 2000 B.C. from pomegranate by grinding the rinds and extracting them with water. In Egypt it was in use from 1500 B.C. onwards as finds in tombs proved; in Palestine it was used in dyes and inks.”[17] The colouring agent in *Punica granatum* is granatonine, which is present in the peel in the alkaloid form *N*-methyl-granatonine, whose molecular structure is characterized by 2 condensed piperidine rings (Fig. 4).[18] Pseudopelletierine (*N*-methyl granatonine), $C_9H_{15}NO$, is a derivative of cyclooctane, and resembles tropine in that it contains a nitrogen bridge between two carbon atoms.[19]

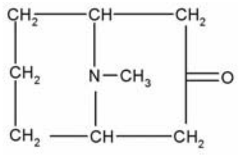

Fig. 4: Structural formula of granatonine.

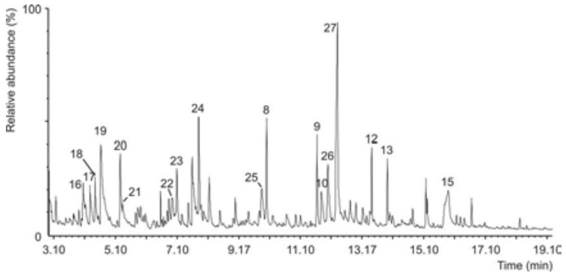

Fig. 5: Total ion chromatogram obtained from pyrolysis of peel of pomegranate at 500 °C. Peak designation according to Table 1.

A pyrogram obtained from the analysis of pomegranate peel at 500 °C is shown in Fig. 5.

The presence of phenolic derivatives such as 2-methoxy-phenol (8), 2-methoxy-4-methyl-phenol (9), benzenediol (10), 2-methoxy-4-vinylphenol (12) and 2,6-dimethoxy-phenol (13) reflects the composition of the peel of pomegranate, which is rich in polyphenolic compounds. Many derivatives of furan like 2-furaldehyde (19), 3-furaldehyde (20), 3-methyl-furan (21), 5-methyl-furancarboxyaldehyde (24), 2,5-furandicarboxaldehyde (25), 5-(hydroxymethyl)-furancarboxyaldehyde (27) are formed during pyrolysis. They are probably derived from polysaccharides present in the pomegranate peel. The formation of levoglucosan (15) can also be explained this way. The peel of pomegranate also contains some dianhydromannitol (26), which is present in the pyrogram.

3.2 Gum arabic

Gum arabic is a natural product of the tree *Acacia senegal* (L.) Willd. The gum is harvested as an exudate from trunks and branches. It is normally collected by hand and when dried, it resembles a hard, amber-like resin. Gum arabic is widely used in the food industry as an emulsifier, flavour encapsulator and thickening agent. It is mainly constituted of complex, highly branched polysaccharides composed of neutral monosaccharide and hexuronic acid monomers. Derrick and Stulik[20] used Py-GC technique to characterize natural gums used in works of art. Gum arabic, tragacanth, guar, ghatti and karaya all gave distinguishable and reproducible pyrograms, enabling their identification. Fig. 6 shows the pyrogram of gum arabic. It is characterized by the formation of cyclopentene and furan derivatives, as 2-furaldehyde (19), 2-cyclopenten-1-one (30), 2-hydroxy-cyclopenten-1-one (23), 3-methyl-1,2-cyclopentanedione (5), 2-methyl-2-cyclopenten-1-one (33) and 2,3-dimethyl-2-cyclopenten-1-one (36).

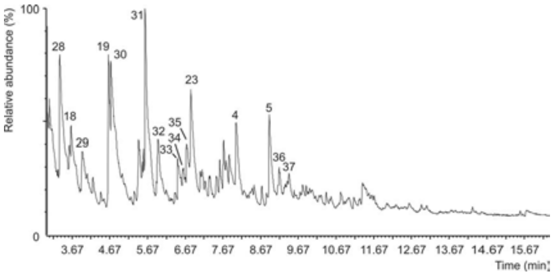

Fig. 6: Total ion chromatogram obtained from the pyrolysis of gum arabic at 500°C. Peak designation according to Table 1.

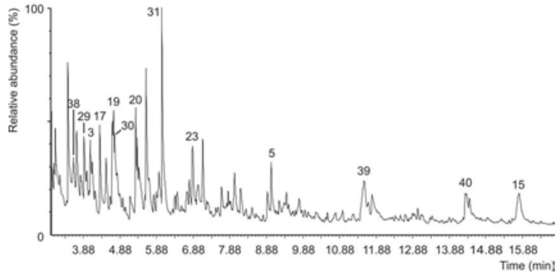

Fig. 7: Total ion chromatogram obtained from pyrolysis of apricot gum at 500 °C. Peak designation according to Table 1.

3.3 Apricot Gum

Apricot gum belongs to Prunus gums, exuded from different *Prunus* species like apricot, plum, peach and especially cherry tree. All these gums are very similar and not clearly distinguishable. The gums are partially soluble in water where they swell.[21]

In the apricot gum pyrogram (Fig. 7), as in the one of gum arabic, the thermal decomposition of monosaccharides leads to the formation of cyclopentene and furan derivatives. Some examples are 2-furaldehyde (19), 3-furaldehyde (20), 2-cyclopenten-1-one (30) and 2-hydroxy-2-cyclope-nten-1-one, (23). Levoglucosan (15) forms by dehydration of glucose.

3.4 Saffron

Saffron is considered to be one of the most expensive spices and consists of stigmas of flowers of *Crocus sativus* L., characterized by a distinct and unique colour, flavour and aroma.

The stigmas contain many chemical substances. There are carbohydrates, minerals, vitamins (especially riboflavin and thiamine) and pigments including crocin, anthocyanin, carotene, lycopene and zigzantin. There is also an aromatic terpenoid compound (safranal) and picrocrocin which gives saffron its distinctive flavour. Safflower contains 2 colouring compounds: the yellow one is soluble in water (‘safflower yellow’); the other one is of a beautiful red colour and appears greenish in reflected light, i.e. *carthamin*, or *carthamic acid* ($C_{14}H_{16}O_7$). It is insoluble in water, ether, and diluted acids, yet it is slightly soluble in alcohol, and readily soluble in alkaline solutions, in which, however, it readily decomposes.

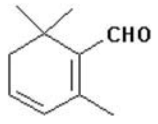

Fig. 8: Safranal.

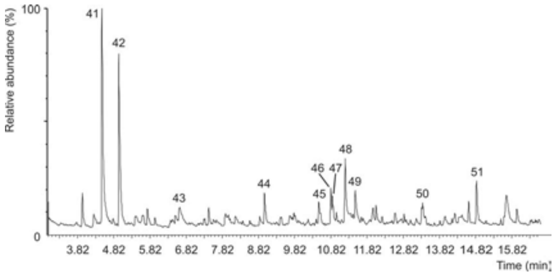

Fig. 9: Total ion chromatogram obtained from pyrolysis of saffron at 500 °C. Peak designation according to Table 1.

The compound with most pronounced organoleptic properties was found to be safranal (51), Fig. 8, (2,6,6-trimethylcyclohexa-1,3-dien-1-carboxyaldehyde), which is formed by de-glucosylation of picrocrocin.

In a series of analyses carried out by Tarantilis and Polissiou[22], they confirmed safranal to be a major volatile component. Cadwallader et.al. in 1997[23] reported on aroma dilution analysis of saffron volatiles and found that a minor component, 2-hydroxy-4,4,6-trimethyl-2,5-cyclohexadien-1-one (also isolated by Tarantilis and Polissiou by steam distillation of Greek red saffron), was the most intensive aroma constituent of saffron, followed by safranal. In Fig. 9 we show a pyrogram obtained by analysis of a saffron sample.

Safranal (51) is visible at 14.8 min. Some other compounds such as 3,5,5-trimethyl-3-cyclohexen-1-one (44), 3,5,5-trimethyl-2-cyclohexen-1-one (45), 2,6,6-trimethyl-2-cyclohexene-1,4-dione (46) 2-hydroxy-3,5,5-trimethyl-cyclohexen-2-one (47), 4-hydroxy-3,5,5-trimethyl-cyclohex-2-enone (50) are volatiles derived from the decomposition of carotenoids.[24]

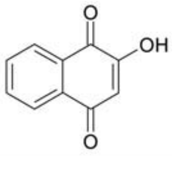

Fig. 10: Lawsone.

3.5 Henna

The red-orange dye henna derives from the plant *Lawsonia inermis* L. Its color is due to the compound lawsone (2-hydroxy-1,4-naphthoquinone), whose structure is given in Fig. 10.

The fractionation of methanolic extract of henna leaves by Botros et al.[25] resulted in isolation of seven compounds; three of them have been isolated for the first time and are p-coumaric acid, 2-methoxy-3-methyl-1,4-naphthoquinone and apiin, along with the previously isolated compounds: lawsone, apigenin, luteolin and cosmosiin. Fig.11 shows the pyrogram obtained by the analysis of a henna sample.

The most prominent peak in the pyrogram on Fig. 11 has been identified as 1-methyl-1H-pyrrole (52). Other nitrogen compounds are also formed, such as indole (55). Also, some aromatic compounds have been found, such as toluene (2), xylene (42) and styrene (53). The last part of the pyrogram is characterized by the presence of aliphatic compounds, marked by an asterisk. Most of them seem to be unsatured aliphatics.

3.6 White Mustard

White mustard (*Sinapis alba L.*) is a perennial plant probably originating in Asia and was exported to the West during the Roman age.[26] Its seeds are yellow to light brown in spite of the name "white mustard". They are mostly composed of proteins (28%) and fatty oils (35%). The typical taste of white mustard is given by sinalbin (Fig. 12), a compound of glucose and *p*-hydroxy-benzyl-isothiocyanate, which is hydrolyzed by the enzyme myrosinase to produce *p*-hydroxy-benzyl-isothiocyanate, a pungent and non-volatile substance.[15,27]

The pyrogram (Fig.13) is rich in non-identified unsatured aliphatic compounds, marked with an asterisk. One of the highest peaks has been identified as oleic acid (60), but also aromatic compounds have been found (2, 42, 53, 58, 59), along with phenol (4) and 4-methyl-phenol (7).

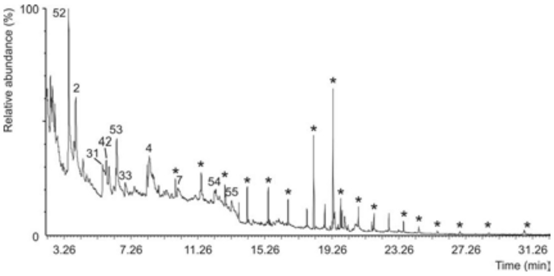

Fig. 11: Total ion chromatogram obtained from the pyrolysis of henna at 500 °C. Peak designation according to Table 1, asterisk denotes unclear assignation.

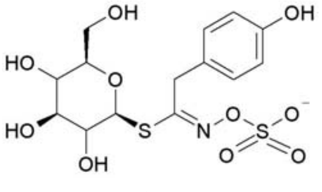

Fig.12: Molecule of sinalbin.

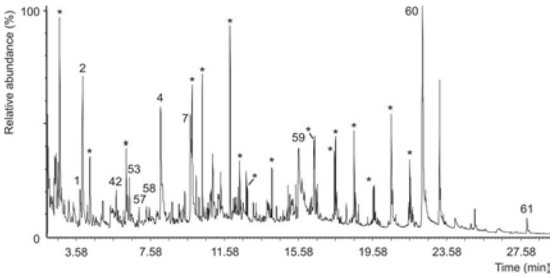

Fig. 13: Total ion chromatogram obtained from pyrolysis of white mustard seeds at 500 °C. Peak designation according to Table 1.

4. Conclusions

Py-GC/MS was shown to be a useful technique to discern between ingredients of inks of organic origin, such as gums, extracts of seeds and peel of pomegranate, henna, mustard, etc, which were used in medieval to 19th-century ink recipes. A variety of compounds have been identified in the pyrograms, some of them characteristic of the particular ingredient. However, more work is needed to see how the aged ingredients can be analyzed and how other constituents of the inks influence the pyrolysis process, before the technique can be applied to originals.

5. Acknowledgement

We gratefully thank Gayane Eliazyan from Institute of Ancient Manuscripts (Matenadaran) in Armenia and Barbara Rittmeier from Hildesheim University of Applied Sciences and Arts for kindly providing all the natural products used as samples.

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