



# Application of Gas Chromatography coupled with Mass Spectroscopy (GC/MS) to the analysis of archaeological ceramic amphorae belonging to the Carthaginian fleet that was defeated in the Egadi battle (241 B.C.)

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## ABSTRACT

The aim of this preliminary work was to identify characteristic compounds in 7 underwater marine ceramic amphorae sherds dating from the period of the battle of the Egadi Islands that decided the end of the First Punic War (241 B.C.) by Gas Chromatography coupled with Mass Spectroscopy (GC/MS).

**Section:** RESEARCH PAPER

**Keywords:** underwater ceramic amphorae; GS-MS; Egadi Battle; pine resin

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## 1. INTRODUCTION

Though remarkable underwater sites have been discovered in many parts of the world, the Mediterranean area is rightly recognized as one of the most fruitful locations for deep-water archaeology, due to the significant number of ancient shipwrecks discovered. Most of these are found intact, often with the contents in their original position, making it possible to understand the origin of the ship and to reconstruct the development of shipbuilding traditions [1].

The final battle of the First Punic War between Rome and Carthage, the battle of the Egadi Islands, took place in 241 B.C. and saw the victory of the Romans. Finds of multiple bronze warship rams, helmets, and amphorae, destined for a Carthaginian garrison on Sicily, confirm the naval battle general location and define its landscape.

In cargo ships, amphorae are known to have been used to carry wine, olive oil, spices and fish products, as well as several other liquid or semi-liquid goods [2]. Therefore, these objects are extremely precious for deducing agricultural and food-related practices, on the basis of direct archaeological evidence of residues found inside (relatively infrequent), or more frequently the traces found in these relics [3], [4].

Amphorae, in their many types and guises, were traditional package material throughout antiquity, producing a treasure of archaeological data about the rates of production, associated regional products, artisanal production organization, the different actors involved in production as well as exchange, amplitudes of distribution patterns and past networking, with a clear potential of sustaining historically inspired enquiries.

Compared to the archaeological prevalence of amphorae,

ancient sources on their use and content are rare and not always straightforward to interpret [5].

Archaeological data, together with chemical analysis of pottery remains, can provide information on the diet of a region's inhabitants during a chosen period of time. Organic residues recovered from pottery sherds can be used as fingerprints of the food materials once inside those containers.

The use of gas chromatography/mass spectrometry (GC/MS) in the analysis of organic residues and the use of biomarkers to identify the original source materials, which formed those residues, are well-established techniques in archaeological science.

The aim of this work was to identify the characteristic compounds in 7 underwater marine ceramic amphorae sherds, dating from the period of the battle of the Egadi Islands. Samples of underwater marine ceramic amphorae are shown in Figure 1.

Among 308 amphorae that were identified during the survey till 2013 (some of them were rescued to take samples for analysis), 278 are Greco-Italic (type MGS V-VI), while 33 are Punic (type: "ovoid maltese").

Since 5<sup>th</sup> century B.C. Maltese Islands produced amphorae having a typical shape, deriving from a Punic tradition. These

amphorae were ovoid in shape, without neck, with ear shaped handle placed on the upper part of the body and thin ribbon rim. These amphorae had a wide diffusion in Malta and their production would have continued until the Roman period. Clay fabric is orange-red with a cream coloured slip. Outside Malta these amphorae are present in central and western Mediterranean. Some of them are found in Camarina, Lilibeo, Ibiza, Cartage and Pantelleria in IV – II cent. BC layers.

## 2. METHODOLOGY

### Sample extraction

All samples were analysed at the Research Mass Spectrometry Laboratory of "Centro Grandi Apparecchiature - ATeN Center" of the University of Palermo.

Ceramic samples were analysed to identify the amphorae contents and organic coatings, and lipid extraction used to test for presence of lipids of either plant or animal origin that might suggest lipid-rich commodities (such as olive oil or garum), which may have been transported in the vessels.

The analysed samples belong to Greco-Italic amphorae (type MGS VI) dated to the 3<sup>rd</sup> century BC. These amphorae have a rim with triangular section, cylindrical neck, elongated body and pointed base.

The sherds were first surface-cleaned with a gentle stream of inert nitrogen gas before the ceramic sample for analysis was crushed in a mortar. No mechanical ablation procedure was used that could remove the compounds of interest. However, two samples were analysed in parallel, one before and one after the cleaning, in order to verify that the used cleaning procedure does not affect the result of analysis. Data (not showed in this paper) are the same for both samples.

All reagents and analytical standards were acquired from Sigma Aldrich.

The lipid extraction technique for ceramic samples was adapted from that used by Evershed et al [6]:

- Five grams of a sample was weighed;
- The lipids were extracted with 30 ml of chloroform and methanol (2:1 v/v) for 3 h, T=40 °C;
- The extraction solvent was then evaporated to dryness under a stream of nitrogen gas;
- The amount of dry extract obtained was around 100 mg/g sherd; samples were stored at -20 °C until analysis GC/MS.

Immediately prior to analysis, dry lipid extract was derivatized to obtain fatty acid methyl esters (FAMES):

Dry lipid extract was dissolved in 50 ml of toluene;

Subsequently 100 ml of methanolic potassium hydroxide (5 %) was added. After 5 min stirring the reaction was stopped by addition of 200 ml of bidistilled water;

FAMES were extracted with 1 ml of hexane, followed by solvent evaporation at reduced pressure at room temperature.

Dried lipid extract was dissolved in 500 µl of hexane for GC/MS analysis. For samples that appeared too concentrated to inject in splitless mode, analysis was first conducted in split mode (10:1) in order to access the quantity of organic constituents before being run in splitless mode.

### Analytical Conditions

Samples were thus analysed by GC/MS using a TRACE™ 1310 GC (Thermo Scientific) gas chromatograph equipped with a Phenomenex Zebtron ZB-5ms column (30 m length x 0.25 mm ID x 0.25 µm film thickness, 5 % diphenyl – 95 % dimethylpolysiloxane stationary phase). The mass spectrometer



Figure 1. Samples of underwater marine ceramic amphorae belonging to the Carthaginian fleet that was defeated in the Egadi battle (241 B.C.).

was a Thermo Scientific ISQ Single Quadrupole Mass Spectrometer, operated in electron ionization mode (70 eV). The scan range was  $m/z$  35–650.

The gas chromatograph conditions were as follows: inlet temperature 270 °C, flow rate 1.0 ml/min, transfer line temperature 265 °C. Helium was used as the carrier gas.

The temperature program for the GC oven was a 40–140 °C at 10 °C/min, 140–340 °C at 15 °C/min, with a 5 minutes isothermal hold at 340 °C.

Injections were made by a Thermo Scientific Autosampler AI/AS 1310, TriPlus™ RSH and sample injection volume was 1 µl in splitless mode.

### 3. RESULTS AND DISCUSSION

GC/MS analysis allowed the identification, among other compounds, of fatty acids (oleic, palmitic and stearic acids), steroids (stigmastanol) and resin sealants. These data suggest that Pinaceae resins were used as the amphorae sealants and that vegetable fat residues were present in all the analysed amphorae. Information regarding the making of the pitch sealants can be inferred from the chemical profile of the samples. Identification of marker compounds was achieved by comparison of retention times and mass spectra against authentic standards as well as comparison against the NIST11 mass spectral database (Table 1).

Typical chromatograms are shown in Figures 2 and 3.

The most abundant compound was the Oleic acid, which is one of the main constituents of olive oil, followed by the stearic and palmitic acids. The presence of these fatty acids, together with other acids such as lauric, palmitoleic, linoleic, behenic and lignoceric, shows the presence of fat, but does not allow us to attribute an animal or vegetable origin.

Stigmastanol, present in significant amounts in the analysed sherds, indicates presence of a vegetable fat. This compound originates from degradation processes occurred over the years, after reduction of the  $\beta$ -sitosterol that is the precursor and the typical marker of vegetable oils.

Dehydroabietic acid and its oxidation products are typical components of oxidised resin that exudes from plants of the Pinaceae family. Further confirmation derived from the identification of heating markers such as retene and 8-isopropyl-1,3-dimethyl phenantrene [7], [8].

The amount of Methyl dehydroabietate, observed in all samples, is significantly higher with respect to dehydroabietic acid. This compound is indicative of the pitch production method: it is formed by heating resin-bearing wood, where the wood is burned to simultaneously extract resin from the source wood and convert it to pitch [7], [8].

The lipid extracts analysed contain large amounts of organic substances, which allow us to hypothesize the presence of strongly degraded oil, presumably olive oil, beeswax and heat treated pine resin.

Beeswax is confirmed by the presence of free fatty acids from C22:0 to C30:0 with prevalence of the lignoceric acid and high levels of palmitic acid (Table 1). One of the most plausible hypothesis is the use of beeswax as waterproofing or sealant [9].

The presence of the stigmastanol together with oleic acid, in all samples, suggests that vegetable fat residue are present in the amphorae. Only the high level of stearic acid may suggest the presence of animal fats.

However, the absence of cholesterol and other animal sterols suggests that it may be a contamination, rather than an

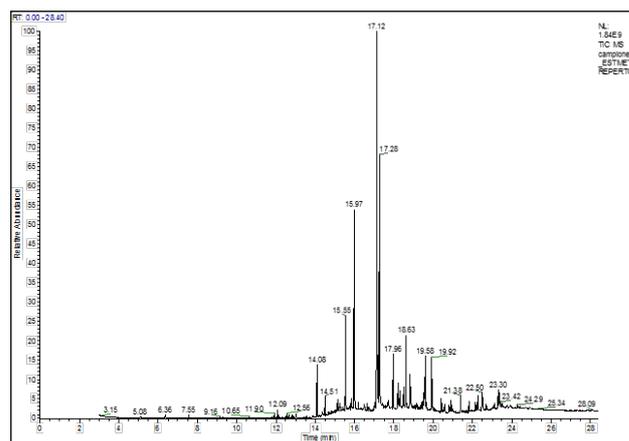


Figure 2. Total ion chromatogram of the lipid extraction of ceramic sample 1.

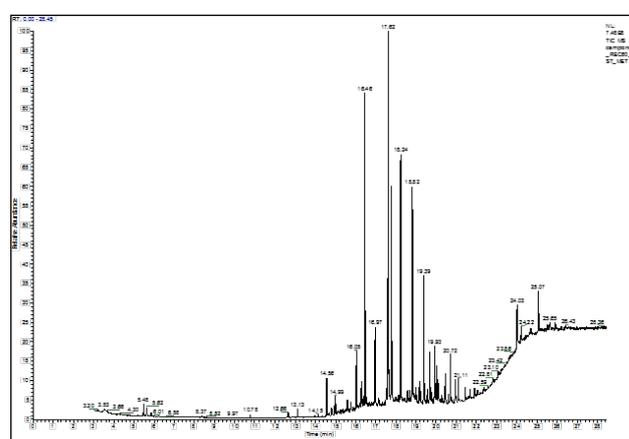


Figure 3. Total ion chromatogram of the lipid extraction of ceramic sample 5.

indication of the amphora content [9].

The chromatograms show several diterpenoid acids such as dehydroabietic acid and 7-oxodehydroabietic acid, together with polycyclic aromatic hydrocarbons, such as retene and dehydrogenated derivatives, that are markers for pine resin in aged materials [7], [10].

### 4. FINAL CONSIDERATIONS

The possibility to easily understand the contents of ancient container that, due to the long period of permanence in the sea, have disappeared, could be of great help for the archaeologists. Although we generally know that normally each amphorae type could be connected to a specified product such as oil, wine, grains, fish sauces (*garum* and other similar products), frequently amphorae were reused for different purposes and to contain different varieties of goods. It will be very important for archaeologists and historians to get the chance to understand the real contents of amphorae of an ancient wreck because it will be possible to frame the ancient sea routes and, consequently, understand the real economic dynamics either in a synchronic and in diachronic perspective. We need also to keep in mind that this information is very attractive for a wider set of people, such as the visitors of museums. Therefore, this methodology could have a wide application either in the field of scientific research and in the area of museography.

Table 1. Percentages relative areas of the marker compounds identified in 7 underwater marine ceramic amphorae samples belonging to the Carthaginian fleet that was defeated in the Egadi battle (241 B.C.).

Marker compounds	Sample 1	Sample 2	Sample 3	Sample 6	Sample 4 REC 58	Sample 5 REC 60	Sample 7 REC 57
	%Area	%Area	%Area	%Area	%Area	%Area	%Area
Lauric acid, methyl ester	0.27	0.02	0.03	0.20	-	0.14	0.11
Azelaic acid, dimethyl ester	0.20	-	0.01	-	-	-	-
Myristic acid, methyl ester	1.04	0.74	0.77	1.31	1.10	1.32	1.75
Palmitoleic acid, methyl ester	3.64	3.99	3.48	4.63	7.18	3.81	4.61
Palmitic acid, methyl ester	22.01	17.70	10.76	19.50	23.68	27.90	25.11
Linoleic acid, methyl ester	3.48	4.32	1.29	2.00	7.22	3.54	3.69
Oleic acid, methyl ester	26.64	34.30	23.52	24.70	34.34	34.02	34.25
Stearic acid, methyl ester	20.90	9.48	7.43	14.11	20.65	19.27	18.29
Retene (methyl isopropyl phenanthrene)	3.83	9.29	23.09	4.50	-	-	-
Methyl arachidate	1.05	1.32	1.17	1.05	2.86	0.75	1.16
8-Isopropyl-1,3-dimethyl phenanthrene	1.43	3.11	4.7	2.36	-	-	-
Dehydroabiatic acid, methyl ester	7.17	7.66	13.09	12.59	-	-	-
Behenic acid, methyl ester	-	-	-	1.36	-	2.62	2.01
7-OxoDehydroabiatic acid, methyl ester	4.56	6.01	7.13	6.27	-	-	-
Lignoceric acid, methyl ester	0.87	0.54	0.72	1.81	0.95	2.04	2.56
$\beta$ -Sitosterol	1.33	-	-	-	-	-	-
Stigmastanol	1.60	1.51	2.8	3.60	2.01	4.58	6.45

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