Talanta 118 (2014) 195–200

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Identification of oil residues in Roman amphorae (Monte Testaccio, Rome): A comparative FTIR spectroscopic study of archeological and artificially aged samples

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ARTICLE INFO

Article history:
Received 21 February 2013
Received in revised form
5 September 2013
Accepted 8 September 2013
Available online 19 October 2013

Keywords:
Archeological vessel
Organic residues
FTIR spectroscopy
Monte Testaccio
Residues extraction

ABSTRACT

The application of Fourier Transform InfraRed (FTIR) spectroscopy to the analysis of oil residues in fragments of archeological amphorae (3rd century A.D.) from Monte Testaccio (Rome, Italy) is reported. In order to check the possibility to reveal the presence of oil residues in archeological pottery using microinvasive and\or not invasive techniques, different approaches have been followed: firstly, FTIR spectroscopy was used to study oil residues extracted from roman amphorae. Secondly, the presence of oil residues was ascertained analyzing microamounts of archeological fragments with the Diffuse Reflectance Infrared Spectroscopy (DRIFT). Finally, the external reflection analysis of the ancient shards was performed without preliminary treatments evidencing the possibility to detect oil traces through the observation of the most intense features of its spectrum. Incidentally, the existence of carboxylate salts of fatty acids was also observed in DRIFT and Reflectance spectra of archeological samples supporting the roman habit of spreading lime over the spoil heaps. The data collected in all steps were always compared with results obtained on purposely made replicas.

1. Introduction

Organic residues can survive in different forms in association with archeological pottery. In few cases, in fact, they are trapped in the clay porous matrix of the ceramic and possibly preserved as vessel fills. In this case it is important to avoid confusion between original residues and post-burial fills. More often, surface residues appear as visible residues on the exterior or interior of vessels or, on the contrary, they are invisible to the naked eye since absorbed by the vessel wall [1].

As organic materials contained in archeological ceramics represent an essential evidence of human activities through years shedding some light on several aspects of daily life concerning diet, medical practice and ritual habits, they have been objects of many scientific investigations aiming to their detection and identification. Particular attention has been devoted to the chemical characterization of animal and vegetable fats as they are important constituents of food whose procurement was and is a fundamental block of the human experience both on individual and social level [2].

It has been proved that fat residues can survive for centuries since pottery retard oxidative and hydrolytic degradation of lipids [3] which, in addition, are not easily leached upon burial [4,5].

Since the 1970s the analytical procedures used to characterize organic residues from archaeological finds are based on chromatographic techniques [6,7] often coupled with mass spectrometry [2,4,8,9]. Both techniques are microinvasive since imply the extraction of the residual compound from small amount of grinded potsherd in contact with the original content of the vessel [2,4,9,10].

In several cases, preliminary FTIR spectroscopic analyses were also performed providing a fingerprint and general information on the nature of the organic remains [9,11] or to study the materials, such as pitch and wood tar, used in waterproofing archeological amphorae [12].

Despite to the great effort made in the last 20 years in detecting residues in archeological samples and identifying their nature through the knowledge of proper biomarkers, the distinction between animal fats and plant oils is not always straightforward. The use of gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) [13] or gas chromatography followed by nanoelectrospray mass spectrometry [14] can be quite helpful since general class of biomarkers are present in fats: unsaturated fatty acids in plant oils, long-chain polyunsaturated fatty acids in fish oils, short-chain fatty acids in diary fats, plant sterols in plants oil [15]. It is worth observing that animal fats in ancient residues

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http://dx.doi.org/10.1016/j.talanta.2013.09.060

Table 1
Vibrational wavenumbers (cm^{-1}) and proposed assignments for olive oil, clay and calcium carbonate.

Wavenumbers (cm^{-1})	Proposed assignment	Technique
3468	Glyceride ester 2 × C=O stretching	Transmission
3394	O–H stretching of H_2O molecules and\or of O–H of clay	Transmission, DRIFT, Reflection
3006	C–H stretching *cis*-double bond fatty acid	Transmission
2925	Aliphatic CH_2 asym. stretching	Transmission, DRIFT, Reflection
2855	Aliphatic CH_2 sym. stretching	Transmission, DRIFT, Reflection
2516	$2 \times CO_3{}^{2-}$ stretching of $CaCO_3$	DRIFT, Reflection
1796	$2 \times CO_3{}^{2-}$ bending of $CaCO_3$	DRIFT, Reflection
1746	Ester C=O stretching	Transmission, DRIFT, Reflection
1654	*Cis*-olefines C=O stretching	Transmission
1630	α,β Unsaturated aldehydes and ketones C=O stretching	Transmission
1627	H–O–H bending of H_2O molecules and\or of O–H of clay	Transmission, DRIFT
1575/1538	C–O stretching of carboxylic salts	Transmission
1464	Asym. bending of aliphatic CH_3 and CH_2 groups	Transmission, DRIFT
1428	$CO_3{}^{2-}$ stretching of $CaCO_3$	Transmission, DRIFT
1377	Sym. bending of the Aliphatic CH_2 group	Transmission
1087	Si–O stretching of silicates	Transmission, DRIFT, Reflection
967	Bending of trans-olefines CH group	Transmission
877	$CO_3{}^{2-}$ bending of $CaCO_3$	Transmission, DRIFT, Reflection
798/778	Si–O stretching of quartz	Transmission, DRIFT
723	CH_2 rocking and the out-of-plane bending of cis-disubstituted olefins	Transmission, DRIFT
714	Sym. deformation of $CO_3{}^{2-}$ of $CaCO_3$	DRIFT

have been more extensively investigated than plant fats whose presence has been ascertained not only in vessel for food but also in roman lamps [15,16] sometimes together with olive oil in traces [17].

The aim of this research was to check the capability of infrared spectroscopy to identify oil residues applying microinvasive or for the first time noninvasive techniques. Fragments of the broken amphorae which constitute the artificial mound called Monte dei Cocci (Roma) have been analyzed and results compared with data obtained from experimental samples [18].

2. Experimental

2.1. Reagents

Chloroform (Pharmaceutical grade) supplied by Carlo Erba (Milano, Italy) and methanol (HPLC-gradient grade) purchased from Panreac (Nova Chimica, Cinisello Balsamo (Mi) Italy) were used without further purification.

2.2. Samples

2.2.1. Oil samples

Nine samples of extra-virgin cold pressed olive oil from different southern and central areas of Italy but from the same harvesting year (2012) were spectroscopically investigated before and after the artificial ageing.

2.2.2. Archeological samples

Five fragments of broken amphorae, of average size $10 \times 8 \times 2\ cm^3$, came from the external layer of the artificial mound called "Monte Testaccio" in Rome and were therefore dated to the 3rd century A.D. Potsherds came preferentially from near the base and therefore had an optimal contact with the content. All the shards upon excavation were cleaned with a brush using distilled water.

2.2.3. Reference samples

Thirty shards of not waterproofed pottery from different manufacturers constituted the reference set and were analyzed before and after soak them in one of the olive oil sample for 24 h. Twenty oil impregnated fragments were artificially aged.

2.3. Analytical procedures and instrumentation

2.3.1. Extraction

Samples of finely ground ancient and reference potsherd (5 g) were extracted in a Soxhlet apparatus with 30 ml of a chloroform/methanol 2:1 (v:v) mixture for 3 h. The extracted solutes (1 ml) were recovered after evaporation of the solvent under reduced pressure in a rotary evaporator at temperature below 40 °C.

2.3.2. Artificial ageing process

Olive oil specimens and replicas soaked in oil for 24 h were artificially aged using a weatherometer QUV Accelerated Weathering Tester (Q-Lab, Cleveland, OH (USA)) fitted with A310 UV lamps set at an irradiance of $0.67\ W/m^2$. During the cycles the temperature was controlled at 45 °C and the humidity was 58%. The rate of photodegradation was followed by using FTIR spectroscopy. The ageing process ranged from 10 to 60 days. After 60 days the spectra matched those of specimens weathered with different procedures reported in literature [19]. Therefore, the process was considered concluded. Table 1.

2.3.3. FTIR

An Alpha FT-IR Spectrometer (Bruker Optics, Ettingen, Germany) with globar source and DTGS (deuterated triglycine sulfate) detector was used in the spectroscopic range 7500–375 cm^{-1} with 200 scans or better, resolution 4 cm^{-1}. The instrument is equipped with exchangeable sampling modules. Therefore, different approaches were adopted depending on the status of the sample examined.

Olive oil samples, before, during and after the ageing process, were uniformly spread on CsI window transparent up to 200 cm^{-1} and studied in absorbance. Spectra of liquid extracts from the archeological and reference potsherds were studied in the same experimental conditions.

Weighted finely ground microamounts from ancient and modern ceramic samples were dispersed in KBr in excess in different concentrations (sample: KBr ranged from 1:100 to 1:1000) and studied by using the Diffuse Reflection Infrared Fourier Transform

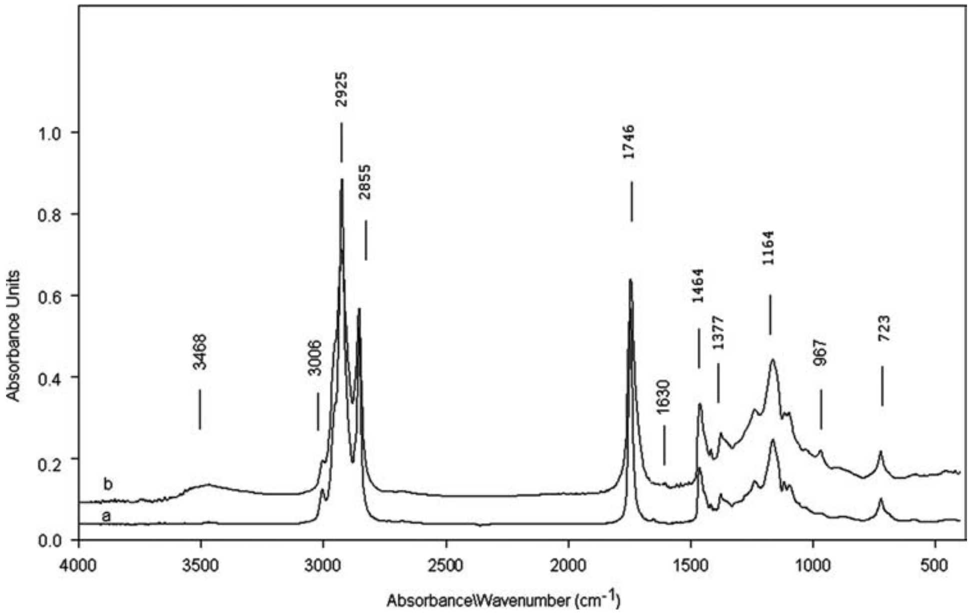

Fig. 1. FTIR spectra of olive oil from Sabina (Lazio, Italy) before accelerated ageing (a) and after 60 days of treatment (b).

(DRIFT) sampling accessory. The spectrum of the same amount of pure KBr was used as background.

The external reflection module was employed for the spectroscopic investigation of ancient and modern solid samples without preliminary manipulations. Many spots (about 3 mm diameter) of both sides of each sample were analyzed, for sake of comparison, controlling and monitoring the sampling areas with an integrated video camera.

3. Results and discussion

3.1. FTIR spectroscopy

The first step of the work consisted in the characterization of the spectral changes of pure olive oil samples before, during and after the accelerated weathering. In spite of the different origin, spectra collected did not show appreciable differences, probably because sharing the year of harvesting and the manufacturing procedures. Therefore, for sake of example, only the spectra of the olive oil sample from Sabina (Lazio) before and after 60 days of artificial ageing are compared in Fig. 1a and b respectively. As no overtones and\or combination bands were observed at higher frequency (7500–4000 cm^{-1}) the figure shows only the fingerprint region (4000–400 cm^{-1}).

The *cis/trans* isomerization of unsaturated fatty acids during the oxidation process has been successfully monitored employing FTIR spectroscopy [19,20].

The spectral changes observed in this work match perfectly with those reported in the literature in spite of the different degradation processes adopted.

The band at 3468 cm^{-1}, hardly observed in olive oil, attributed to the overtone of the of the carbon–oxygen stretching of the carbonyl group of the glyceride ester [19] appears intense and broad after degradation. The weak absorption band at 3006 cm^{-1} assigned to the C–H stretching mode of *cis*-double bond fatty acids looses intensity [20,21]. The C–H asymmetric and symmetric stretching modes of the aliphatic CH_2 functional groups are clearly detected at 2925 and 2855 cm^{-1} respectively. The CH_3 functional groups give symmetric and antisymmetric stretching bands at 2962 and 2872 cm^{-1} respectively. The first causes a shoulder of the absorption band at 2925 cm^{-1} towards high frequency side and the second determines a diminution of the valley between the two CH_2 modes [21]. The intense absorption band at 1746 cm^{-1} due to the carbon–oxygen stretching of the carbonyl functional group of the ester [21] broadens towards low frequency side. This change can be associated with the appearance of saturated aldehyde functional groups or secondary oxidation products that cause an absorption at 1728 cm^{-1} which overlaps with the band of the ester functional group [22,23]. The weak band at 1654 cm^{-1} assigned to the carbon–carbon double bonds of *cis*-olefines disappears while a very weak band at 1630 cm^{-1} is observed confidently attributed to α,β- unsaturated aldehydes and ketones [24].

No significant changes are observed in the 1500–1300 region where the asymmetric bending vibration of aliphatic CH_3 and CH_2 groups is detected at 1464 cm^{-1} and the symmetric bending mode of the aliphatic CH_2 group is observed at 1377 cm^{-1} [21].

Also the weak bands at 967 cm^{-1} due to the bending vibration of the CH functional group of *trans*-olefins and at 723 cm^{-1} associated to the overlapping of the methylene rocking vibrations and the out-of-plane bending vibrations of the cis-disubstituted olefins remain practically unchanged [25].

Spectra of specimens extracted from modern ceramic samples soaked in olive oil and from ancient ceramic fragments of amphorae confidently used for oil storage were obtained in the same experimental conditions of olive oil samples. As an example, Fig. 2 shows the spectra of the residues extracted from a modern ceramic sample soaked in olive oil and weathered for 20–60 days (Fig. 2b and c respectively) together with the spectrum of the product extracted with identical procedure from a potsherd from Monte Testaccio used for oil storage (Fig. 2d). For sake of comparison, the spectrum of the oil sample artificially weathered for 60 days in presence of clay is also reported (Fig. 2a). As said before, this last spectrum matches perfectly with that obtained from treating pure oil for the same lapse of time shown in Fig. 1.

The spectra of samples extracted from experimental potsherds after 20–60 days of ageing do not suggest the presence of clay components, even in traces, and illustrates the progress of the treatment. For example, the broad band around 3500 cm^{-1} gains intensity while the weak feature at 3006 cm^{-1} looses intensity until disappearing.

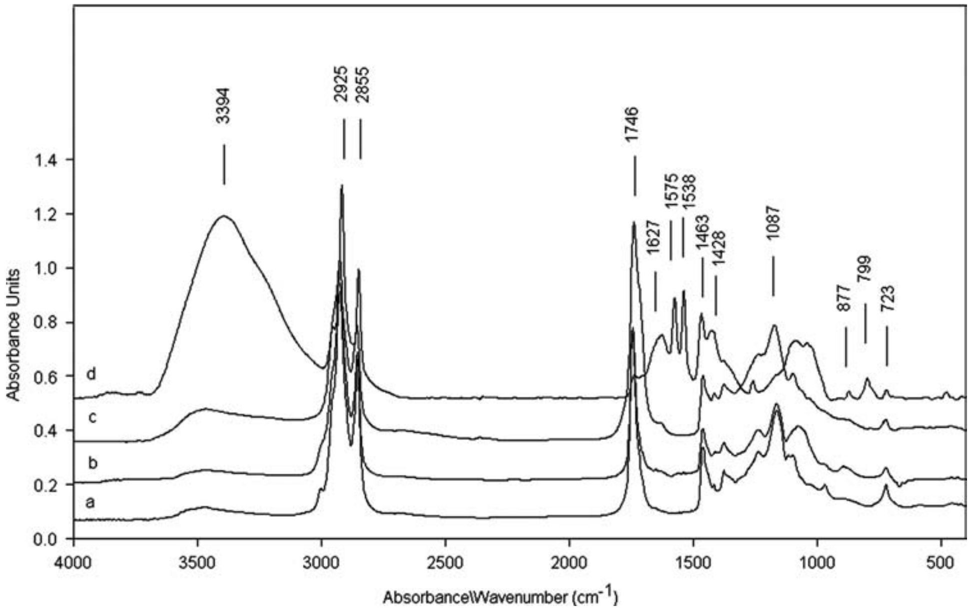

Fig. 2. FTIR spectra of olive oil artificially weathered for 60 days in the presence of clay (a), extracts from clay replicas after 20 (b) and 60 days of ageing (c), extract from one of the potsherd from Monte dei Cocci (Rome, Italy) (d).

In the spectra of liquid extracted from ancient fragments (Fig. 2d) the weak absorption band at 1746 cm^{-1} together with features around 2900 cm^{-1} demonstrate the existence of oil traces. In these residues, however, water, silicate components of clay and calcium carbonate are clearly present. In fact, a quite intense broad band at 3394 cm^{-1} together with a medium intensity broad band at 1627 cm^{-1} are readily assigned to the O–H stretching mode and H–O–H bending mode respectively of H_2O molecules and\or to hydroxyl groups of the clay [26]. The Si–O stretching mode around 1090 cm^{-1} belongs to the silicate component of clay and soil [27] and, finally, the bands at 1428 and 877 cm^{-1} are attributed to the CO_3^{2-} stretching and bending of calcium carbonate respectively [28]. According to archeologists, in fact, in order to avoid the smell of rancid oil, broken pots were covered by lime which reacts quite easily with fatty acids. Therefore, the appearance of the doublet at 1575, 1538 cm^{-1} assigned to C–O stretching of carboxylic salts of fatty acids [29] seems to support this old habit and suggests the transformation of the palmitic acid into adipocere, possibly due to microbial activity [30] or into carboxylic salts due to saponification reaction [31].

3.2. Drift

Micro amounts of all archeological and reference samples (Sections 2.2.2 and 2.2.3) have been spectroscopically analyzed employing the DRIFT technique which does not require the extraction of the residue. The results obtained on old and ancient samples are compared in Fig. 3. The bottom spectrum (Fig. 3a) belongs to a specimen taken from the external side of an ancient potsherd that reasonably had short or no contact with the content. In fact, only the aforementioned features belonging to silicate ($\sim$1090 cm^{-1}) and water or hydroxyl groups are observed (3394 and 1627 cm^{-1}). A moderate content of calcium carbonate is suggested by the detection of absorption bands approximately at 1430 and 877 cm^{-1}. The same features are observed in the replica sample soaked in oil and artificially aged for 60 days (Fig. 3b). which also shows a small content of quartz (doublet at 798 \778 cm^{-1}) [32]. In addition, the peaks at 1746 and the doublet at 2925\2855 cm^{-1} support the existence of olive oil traces.

The main difference between the spectra of specimens taken from the inner sides of the archeological potsherd (Fig. 3c) and from the inner side of replicas consists in the extremely high content of calcium carbonate in the former sample. Beside the stretching and bending modes of the CO_3^{2-} group, in fact, also the symmetric deformation of the carbonate group is observed at 714 cm^{-1} normally too weak to be detected. In addition, the overtones of the stretching and bending modes of the CO_3^{2-} group are clearly detected at 2516 and 1796 cm^{-1}respectively. It is reasonable to infer that carbonate was in powdered state since last features are normally absent in calcite or aragonite spectra [33].

3.3. Reflection

As a last step, many spots of modern and old potsherds were also spectroscopically examined in external reflection in order to check the possibility of ascertaining the presence of oil residues avoiding pretreatment and\or manipulation of the specimens. When employing this technique, bands in the high frequency spectral region ($>$1400 cm^{-1}) appear reversed due the restrahlung phenomenon, whose explanation is at present not completely settled [34].

The results obtained are collected in Fig. 4. The spectra of internal and external portions of modern and old clay fragments not in contact with oil are reported in Fig. 4a and c respectively. They appear quite similar showing only the absorption peak due to the silicate component of clay in the range 1100–1000 cm^{-1}. As replica vessels were surely not waterproofed we can reliably assume that ancient pottery had also no lining. The most intense peak of olive oil at 1746 cm^{-1} together with C–H stretching modes in the 2900–2800 cm^{-1} region are detectable in the spectrum of the modern fragment in prolonged contact with oil and artificially weathered (Fig. 4b). Unfortunately, remaining oil absorption bands are overlapped by the intense and broad absorption peaks of the clay silicate. On the contrary, clay components are completely lost in the spectrum of ancients potsherds (Fig. 4d) since hidden by the very intense fundamental and overtone modes of calcium carbonate in agreement with the observations made on DRIFT spectra. The survival of oil traces, however, is confidently suggested by the presence of a very weak absorptions at 1746, 2925 and 2855 cm^{-1}.

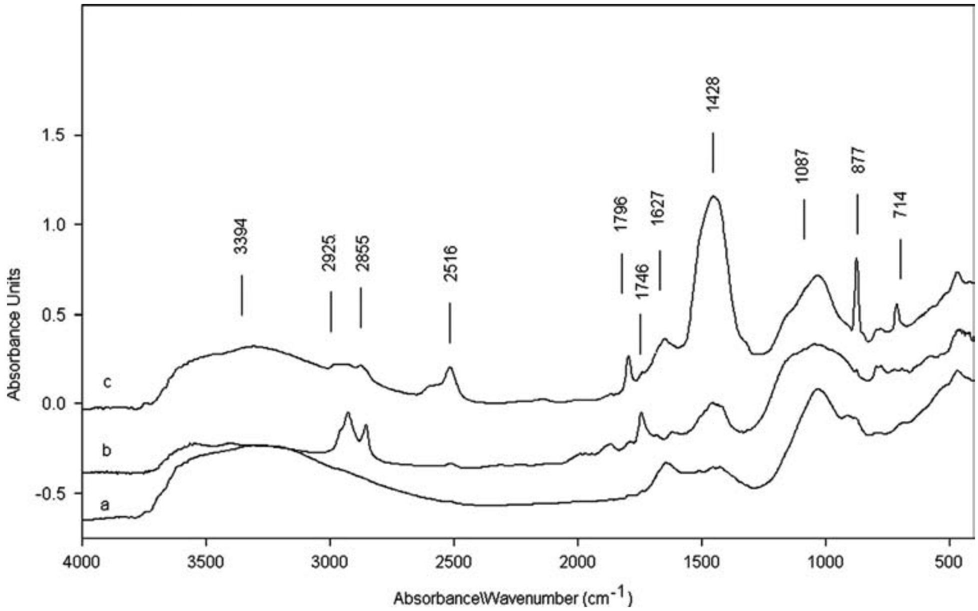

Fig. 3. DRIFT spectra of grinded micro amounts of the external (a) and internal sides of a reference vessel (b), the inner side of a potsherd from Monte dei Cocci (Rome, Italy) (c).

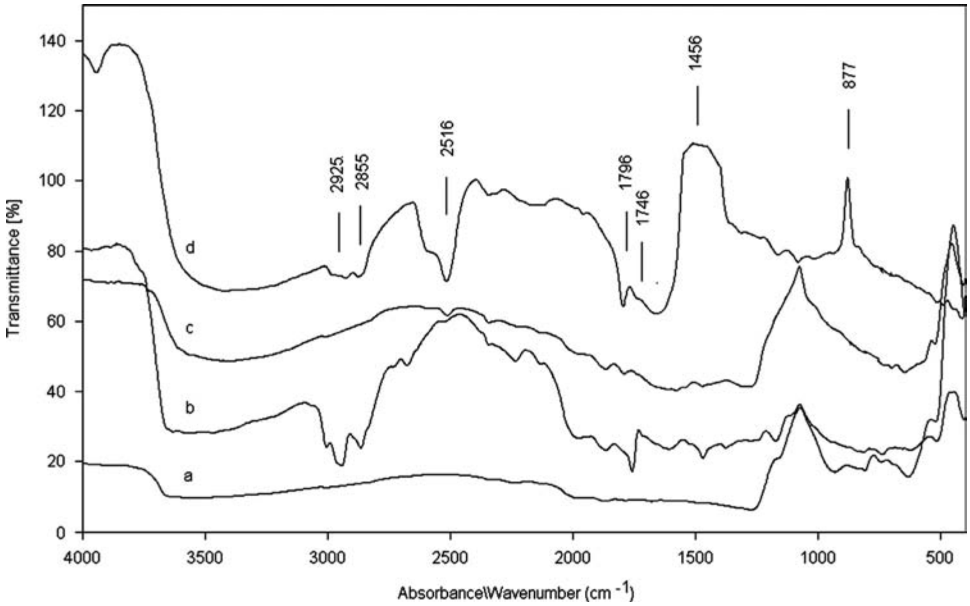

Fig. 4. Reflectance FTIR spectra of the external and internal sides of replica vessel (a and b), the external and internal sides of an archeological fragment from Monte dei Cocci (Rome, Italy) (c and d). Internal sides were in contact with oil.

4. Conclusions

FTIR spectroscopy is a quite fast and economic technique, normally employed for preliminary investigations only, that can give information about the nature of the content and\or the inside coating of archeological vessels. Normally it requires invasive or microinvasive approaches such as the extraction of the residues or the analysis of microfragments of the vessel.

In this study FTIR microspectroscopy has been employed, for the first time, in order to identify oil residues in roman amphorae of the 3rd century A.D. from Monte Testaccio (Rome). This experimental approach is particularly valuable when applied to archeological items whose manipulation is forbidden.

The results, compared with those obtained on replicas, gave clear indication of the existence of oil residues in the inner side of potsherds and evidenced the reaction of fatty acids, with the carbonate contained in the lime sprinkled on the mound in ancient times.

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