TEL AVIV UNIVERSITY אוניברסיטת תל-אביב

The Lester and Sally Entin Faculty of Humanities
The Chaim Rosenberg School of Jewish Studies
Department of Archaeology and Near Eastern Cultures

RECONSTRUCTION OF THE ECONOMY OF THE CHALCOLITHIC PERIOD IN ISRAEL BY ASSESSMENT OF PRESERVATION AND DEGRADATION PROCESSES OF ORGANIC SUBSTANCES ADSORBED TO ARCHAEOLOGICAL CERAMIC VESSELS

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

BY: DVORA NAMDAR

UNDER THE SUPERVISION OF: **PROF YUVAL GOREN**

PROF STEVE WEINER (WEIZMANN INSTITUTE OF SCIENCE)

PROF RONNY NEUMANN (WEIZMANN INSTITUTE OF SCIENCE)

SUBMITTED TO THE SENAT OF TEL AVIV UNIVERSITY

JUNE 2007

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List of publications

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Abstract

In this work we would like to present an opportunity in solving complex archaeological questions using residue analysis methods. We demonstrate the possible contribution of this method by using it to solve one of the mysteries of the Chalcolithic Period in the Levant - the content and use of cornets. Cornets are cone-shaped ceramic vessels, characteristic of the Chalcolithic period (*ca.* 4700 - 3700 B.C.E.) in Israel and Jordan. Their contents and use are unknown. They were believed to function as cultic icons, to contain fluids and to be used in ceremonial practices. Analyzing the organic residues adsorbed inside the walls of the ceramic vessels led us to different conclusions as to the aforementioned functions. Using gas chromatography with flame ionization and mass selective detection, cornets from five different sites with different related activities (domestic, habitation cave and a cultic complex) were analyzed. The results indicate that all cornets contained the same assemblage of organic compounds adsorbed within their walls. This content differs from the contents of other types of ceramic vessels from the same sites, as well as from the residues found within the associated sediments.

We show that the cornets once contained beeswax. Beeswax is composed of fatty acids, odd numbered *n*-alkanes and wax esters. Focusing on the most stable components of beeswax, namely the *n*-alkanes, we have found by gas chromatography and gas chromatography-mass spectrometry analyses of combs from twelve colonies from Israel and Jordan that as beeswax ages and darkens its *n*-alkane composition changes. The amount of even numbered *n*-alkanes (C_{22}-C_{32}) is significantly higher in darker colored beeswax as compared to light colored

beeswax. To date only light colored beeswax has been analysed and it is therefore widely accepted that beeswax only contains odd numbered alkanes. This study changes this perception. We attribute this in part to the accumulation of cuticular residues found in the darker colored comb cells. Cuticular residues are known to contain C_{23}-C_{32} odd and even numbered *n*-alkanes. Furthermore, we also performed experiments to show that the specific assemblage of molecules extracted from the cornets probably formed as a result of heating of the beeswax. The assemblage of odd and even numbered *n*-alkanes found in the cornets is almost identical to that found in the residues of beeswax heated on modern ceramic fragment. Thus, we conclude that the cornets contained heated beeswax and were probably used for illumination, a notion that is consistent with their widespread distribution. The presence of beeswax in the cornets contributes to our understanding of the Chalcolithic period; a time when secondary products such as milk, olive oil and possibly wine came into use.

We strengthen our conclusion with one example of the analysis of extracts from the walls of an ancient beehive composed of eight unfired clay cylinders and dated to the Iron Age IIA (10-9th centuries BCE). It was recently unearthed at Tel Rehov in the north of Israel by Amihai Mazar and Nava Panitz-Cohen. The hives were found under one meter of heavily burnt sediment. They were identified as beehives based on ancient Egyptian pictorial depictions, classical literary sources and modern ethnographic parallels. These are the only beehives discovered so far in the Ancient Near East dating to pre-Classical periods. Analyses of the residues using same methods used for the analysis of the cornets' extracts revealed that the organic components adsorbed inside the hive walls contain relatively stable compounds that were not destroyed over the years, such as fatty acids and odd

and even *n*-alkanes containing 23 to 33 carbon chains. Their presence, reflecting degraded beeswax, is also consistent with the installation being ancient beehives.

This is the first time that a systematic analytical approach to residue analysis has been applied in Israel for solving archaeological questions. Preservation of organic molecules in this climate is generally poor. Here we establish the way that residue analysis can be applied in Israel.

1 Introduction and Methodology

1.1 Introduction

Ceramic artifacts are to be found in huge amounts and varieties in archaeological contexts. They were widely used for serving, storing, transporting and cooking organic materials in the past, starting in Asia around 15,000 years ago, and in the Levant about 7000 years ago. Traditional methods of pottery analysis include typologic, stylistic, ethnographic and technologic studies; the latter include various physical, mineralogical and chemical methods. These methods provide information about relative ages, clay sources, temper, firing temperatures, cultural affiliations, trade connections, and possible functions (e.g., Shepard, 1974; Rice, 1987). A valuable source of palaeodiet and cultural information may be found in the organic residues preserved within the walls or on the surfaces of archaeological vessels. Fired and unglazed clay often functions as a trap for organic biomolecules, which may be preserved over many millennia (Evershed et al. 1990; Englinton and Logan, 1991). As a result of the porosity of the clay matrix, residues of the original commodities processed in the pottery can be identified thousands of years later. This approach is known as "residue analysis" (Evershed, 1993; Heron and Evershed, 1993). Identification of biomolecules derived from the earlier use of the vessel can provide us with a lot of new data, but at the same time one must take into account their possible degradation through time (Evershed *et al*, 1997; Malainey *et al*, 1999).

The Chalcolithic Period of the southern Levant (4700-3500 BC) is one of the most interesting periods in this area, hence it was selected as the main focus of this study. This period is marked by the increasing exploitation of the environment

by human societies, the development of larger settlements with apparent social hierarchy and craft specialization, and the emergence of the so-called "secondary product revolution" (Gilead, 1995:463-480; Levy, 1995:226-243). The latter aspect reflects the exploitation of the environment in a sophisticated manner, not only for the raw material but also gaining the knowledge of producing secondary products . Examples of studies of this type include the detection of dairy product residues extracted from European Neolithic ceramic vessels), the use of beeswax for illumination , and the use of sheep wool (Davis 1984; Grigson, 1995, 2006). It is claimed that in this period also wine and olive oil were secondarily produced from cultivated olives and grapes (Sherratt 1981, 1983).

1.2 Residue Analysis

In this work we address some aspects of this topic using the analyses of the organic residues of foodstuff and other organic materials adsorbed into the walls of ceramic vessels. Such analyses could address questions regarding the "secondary product revolution" by providing data on vessel contents.

The most common class of molecules studied by residue analysis is the solvent extractable lipids. Lipids are major constituents of animal fats, plant oil, waxes, resins etc. of the natural world and they occur ubiquitously in plants and animals. These are among the most stable molecules and therefore the most likely to be preserved. The identification of these types of preserved molecules has never been systematically applied in the archaeology of the southern Levant, since it is generally thought that this region is not conducive to the preservation of organic materials. Thus our initial aim in this work was to try to determine whether, and if so under what conditions, significant preservation of organic residues could also

occur in archaeological ceramics from this region. A survey of extracted organic residues from ceramics of several archaeological sites in Israel was conducted in order to shed light on the extent of degradation / preservation in this region, and to serve as a basis for the discrimination between well and poorly-preserved samples for future studies.

Research of the last 15 years has shown that a significant proportion of the ceramics examined yielded lipid residues. Notable findings include the identification of remnants of plant products such as oil, resin, and wax derived from various transported, stored or processed commodities (Mills and White, 1989; McGovern *et al*, 1996; Serpico *et al.* 2003; Stern *et al.* 2003). Preserved animal fats, however, are the most commonly observed components of fatty residues recovered from archaeological ceramics (Rottlander, 1990; Dudd and Evershed, 1998). This reflects not only the utilitarian nature of the pottery but also the importance of fats to past cultures. In addition to their nutritional value, they would have been important as illuminants, sealants, lubricants polishes, binders, bases for perfumes, medicinal and cosmetic ointments used in religious rituals and burial practices, and as art materials. Almost all of these activities would have involved the use of pottery containers (Dudd *et al*, 1999). These studies were mostly conducted in Europe and our aim was to determine if adequate conditions exist for its application to our region.

Condamin *et al.* (1976) were the first to show that fatty acids can be preserved in the porous matrix of archaeological sherds. This study, and others that followed, were based on the distribution of fatty acids obtained after extracting the lipids and subsequently analyzing them using Gas Chromatography (GC). The determination of the origin of the lipids was done based on the distribution of saponified free fatty acids in present day reference fats (Röttlander and Schlichtherle, 1979; Patrick et

al 1985; Röttlander, 1990). However, as decomposition changes the relative proportions of fatty acids such direct comparisons can not be trusted (Skibo, 1992; Tauber, 1998). Malainey at al (1999) tried to overcome this problem by analyzing the fatty acid composition of 130 native Canadian plants and animals. Several foodstuffs were thermally and oxidatively degraded, showing that after degradation several foodstuff fatty acid compositions were similar. Obviously, this approach cannot be applied in general as it is impossible to imitate all the possible degradation mechanisms for all foodstuffs suggested for one vessel use.

Where almost no component of the original triglycerides or other esters was left to enable identification of the source material, and the distribution of the fatty acids failed to help, others tried to solve the problem with a different approach; the measurement of the stable carbon isotope ^{13}C ratios of the saturated $C_{16:0}$ and $C_{18:0}$ fatty acids (Evershed et al, 2001). The use of the $\delta^{13}C$ method is based on the differences in diet and metabolism of several classes of animals that result in different concentrations of ^{13}C (Evershed et al, 1997; Woodbury et al, 1998). However, in order to obtain reliable reference data one needs to raise animals with fodder similar to that used in the ancient periods from which the vessels were used. This technique was also used to distinguish between lipids derived from plants with different carbon fixation pathways, namely C_3 and C_4 plants (Woodbury et al, 1998). Most of the terrestrial plants are C_3. As indicated above, it is hard to obtain definitive results using this method.

1.3 Our Strategy

Our first step was to locate the best geographical environment for obtaining archaeological samples. The differences in conditions of the surroundings turned

out to have a major impact on degradation and therefore, on the preservation state of the organic residues adsorbed into the vessel walls. Based on the fact that this is a region well known for spectacular preservation of organic matter in caves at least, we focused our investigation on the Dead Sea region and compared preservation there with preservation at sites in the Negev desert and in the lowland of Israel. The results of this investigation are presented in Chapter 2.

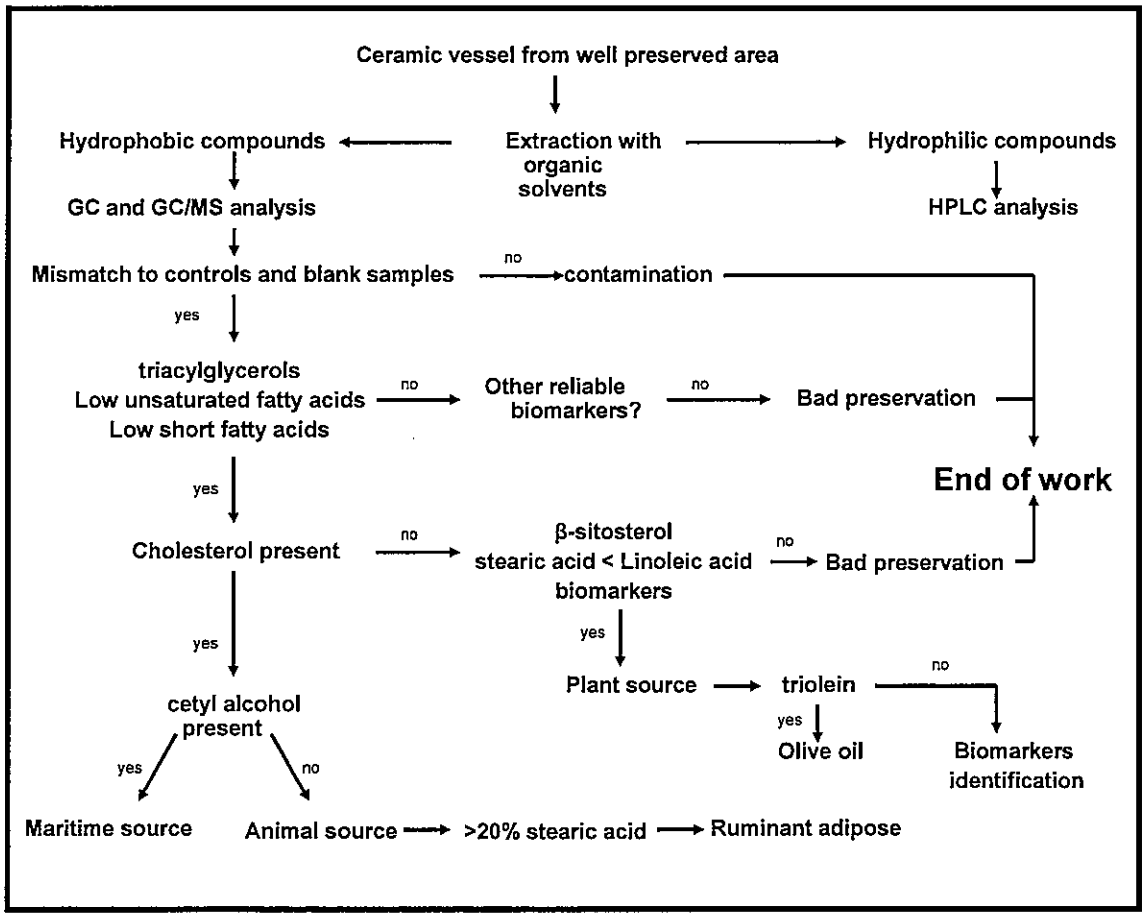
The next step, after determining the presence of well preserved organic material, was to detect for possible contamination, either from the burial environment by transfer from the soil or due to other sorts of alteration processes. The evaluation of possible contamination sources involved (1) sampling of sediments from the immediate surrounding of the vessels; (2) comparison of extracts from different parts of the same vessel such as the rim, handle or any outer decoration compared with the vessel's body or base; (3) comparison of extracts of different vessel types from the same site containing several items for inter-comparison to detect also post-excavation contamination; (4) extraction of blank samples to detect any laboratory contamination; and (5) duplication and sometimes triplication of each sample in order to detect contamination during preparation or treatment of the samples. We reduced the risk of contamination of the samples during laboratory preparation by keeping the work environment as clean as possible, using only well cleaned glassware and high grade solvents, and keeping the samples after extraction in an anaerobic, cold and dark environment. This step is also discussed and presented in the next chapter of this thesis.

The last step was the identification of the substances in the vessels. We analyzed different materials that could serve as the source of the extracts, elaborated on their modern occurrence. Thus, we were able to identify not only the original material but also its mode of use in antiquity. The methodology we applied

in the identification of the source material is demonstrated in chapter 4. The verification of this identification and one other application of it is thoroughly discussed in chapter 5.

In this thesis we apply all these approaches for residue analysis of an enigmatic vessel type, namely the Chalcolithic cornet. The analyses of these vessels led us to investigate the chemical composition of modern wild beeswax sampled in different places in Israel and Jordan. This enabled us to detect the presence of heated beeswax, in the Chalcolithic cornets and Iron Age beehives excavated at Tel Rehov. In this work we showed that under the right conditions, application of residue analysis in the Levant is not only feasible but can assist in solving long-lasting archaeological questions.

Flow chart of the identification of organic materials adsorbed into ceramic vessels



2 Experimental A: The Content and Use of the Chalcolithic Cornets

2.1 Introduction

The Chalcolithic period (~ 4700 to 3800/3700 B.C.E) in the Levant represents a marked material cultural shift from the preceding Neolithic period, with the emergence of metallurgy (hence the term "Copper-Stone age" or Chalcolithic), as well as the production of olive oil, wine, milk and other secondary products (Sherrat 1981). This was also a period when ceramic vessels with novel shapes were produced. One of the most enigmatic ceramic vessel types found only in the Ghassulian culture of the southern Levantine Chalcolithic period, is the cornet, a cone-shaped vessel (Commenge 2006; Gilead and Goren 1995). Early forms of these vessels are parabola-shaped, while later forms are longer and have an attached solid cigar-shaped extension of their base (Fig. 1A). Though unevenly distributed among sites, cornets constitute a characteristic component of the Chalcolithic Ghassulian culture within the southern Levant. The relative abundance of cornets varies in different sites (Gilead 2002). Because of their abundance in public structures, often with religious attributes (Teleilat Ghassul, En Gedi shrine, Gilat sanctuary), it was suggested that they were used for some ritual function (Amiran 1981; Gilead 2001). They are however also present in other, apparently domestic contexts, such as the sites of Grar (Gilead and Goren 1995: 158-163), Nahal Besor (site O) (Macdonald 1932), Horvat Hor (Govrin 1987) and Arad (Amiran 1978). Their functions are however not known.

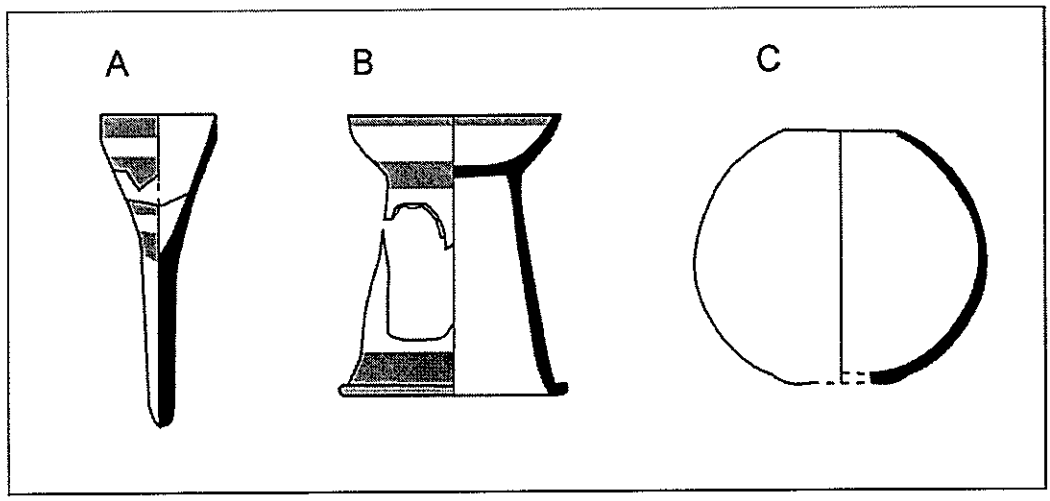


Figure 1 Schematic illustration of the sampled items. **A**, cornet. **B**, fenestrated pedestal bowl. **C**, holemouth jar. The right side depicts the inside of the item and the left side the outside surface. The black line describes the wall section. The dotted line depicts the red paint on the vessel surface.

This chapter presents the results of the analyzes of the lipid assemblages extracted from the ceramic walls of cornets from selected sites, using gas chromatography (GC) and GC-mass spectrometry (GC/MS). The sites from which the vessels were obtained represent different archaeological contexts: the En-Gedi shrine in the Judean Desert (Ussishkin 1980), the Moringa Cave near the En-Gedi shrine, which was possibly used for habitation (R. Porat and U. Davidovich, pers. comm.), the habitation site of Grar in the northern Negev (Gilead 1995), the habitation site of Nahal Qomem (Gat-Guvrin; Wadi Zeita) in the Shephelah lowlands of central-western Israel (Fabian in press; Perrot 1961), and the recently excavated cemetery of Horvat Qarqar located in the same region (Fig. 2). The study also included samples of two other vessel types from the same sites in order to examine the possibility of post-depositional contamination, including bowls on high fenestrated pedestal and holemouth jars (Fig. 1B, C respectively). Sediments from around the items were also analyzed for the same purpose.

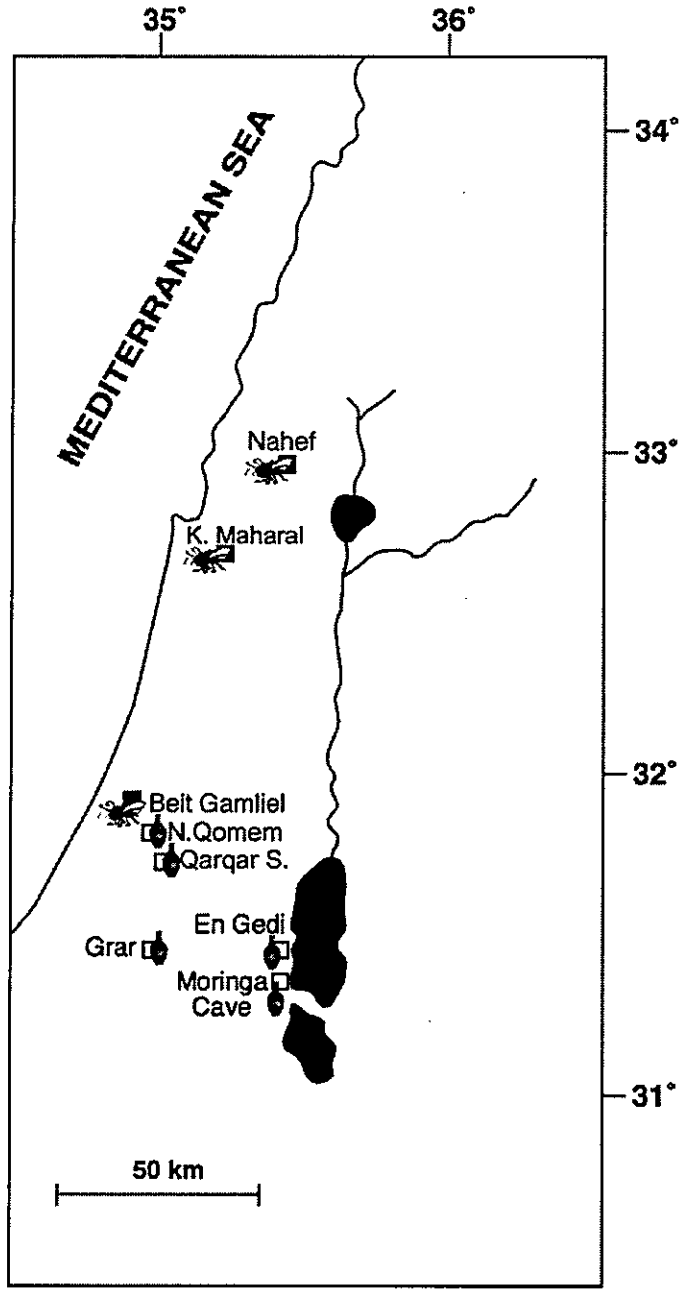


Figure 2 Schematic map of Israel and its surroundings. Modern beeswax sample sites are marked with a bee; archaeological sites are marked with a jar.

2.2 MATERIALS AND METHODS

2.2.1 Materials

Analyses were performed on the following vessels: six cornets, two bowls on high fenestrated pedestal and two holemouth jars from the En-Gedi shrine; two cornets and four bowls on high fenestrated pedestal from the Moringa Cave; two cornets from Grar; six cornets from Nahal Qomem and four cornets from Horvat Qarqar (Fig. 2). Some of the samples from En Gedi and all the samples from Grar were washed after the excavation. Sediments associated with the vessels were also sampled as controls at all the above sites except for Grar where the entire assemblage of vessels had been washed. A fresh soil sample from the site could not be obtained due to environmental changes resulting from repeated planting and the use of fertilizers. Wild bee combs were sampled; two from Kerem Maharal, one from Bet Gamliel and one from Nahef (Fig. 2).

2.2.2 Methods

2.2.2.1 Residues extraction method

The extraction and analysis procedures of the lipids from the ceramic vessels followed Evershed *et al.* (Evershed, et al. 1990) and Charters *et al.* (Charters, et al. 1993). All glassware was pre-treated with 1N HCl, soaked in fuming nitric acid, washed five times with distilled water, and then washed twice with acetone, followed by chloroform and dried under a heating lamp. Fragments of the sherds were broken off the ceramic vessels with pliers. They were cleaned under a stream of clean compressed air, fragmented with a hammer and then ground to a powder in an agate mortar and pestle. About two grams of the powder was weighed and then split equally into clean glass centrifuge tubes. Duplicate blank samples composed of loess from the Negev heated to 600 °C for 24 h were analyzed with

each batch of samples. 10 ml of chloroform and methanol (2:1 v:v) were added to each tube and the mixture was sonicated for 15 min. The tubes were centrifuged for 10 minutes at 3500 rpm. The supernatant was removed to another glass centrifuge tube and solvents were evaporated using a CS110 Speed-vac Plus (ThermoSavant). *N,O*-bis(trimethyl)silyltrifluoroacetamide, BSTFA, (50-150 µL) containing 1% trimethylchlorosilane (TMC) were added to each tube and heated at 60 °C for 1 h. After derivatization, the samples were dried under a gentle stream of nitrogen and 100-150 µL of cyclohexane was added to each tube. Five µL of each sample were injected into the gas chromatograph (GC) with either flame ionization (FID) or mass selective (MSD) detectors. The amounts were calculated from the average peak areas of a series of seven *n*-alkane standards (nC_{26}-C_{32}) compared with the total peak area of compounds extracted from the ceramics and analyzed by the mass spectrometer.

2.2.2.2 Simulated degradation experiments

Pieces of modern ceramic were washed with dichloromethane for two days. After being dried and heated to 600 °C to remove all associated organic compounds, a known amount of modern beeswax was placed on a ceramic fragment and then heated to 400 °C for 2 hours. The organic residues adsorbed into the ceramic matrix were extracted and analyzed using the methods described for archaeological samples. Each beeswax sample was prepared and analyzed three times.

2.2.2.3 Gas Chromatography (GC)

GC analysis was carried out using a HP6890 GC equipped with a flame ionization detector (FID) and using a split injection mode with a 1:10 split ratio.

When the amount of extracted material was small, a splitless injection mode was used. Thirty meter and 15 m, 0.32 mm ID 5% cross-linked phenylmethyl siloxane capillary columns (HP-5) with a 0.25 µm film thickness were used for the separation. The longer column was used for the analyses of archaeological samples and for the analysis of the *n*-alkanes composition of beeswax. The shorter column was used for the analysis of the whole composition of modern beeswax. Helium was used as a carrier gas at a constant flow of 1.1 ml/s. Two methods were used. First a slow analysis for screening and then a faster analysis were made in order to improve the separation of the identified material. In the slow analysis the initial oven temperature was 50 °C and a heating gradient of 6 °C/min was started after 2 min injection, while in the fast analysis a heating gradient of 10 °C/min was applied; all other parameters were the same for both methods. Upon reaching 350 °C, the run was continued for an additional 10 min. The injection temperature was 220 °C and the FID detector temperature was 350 °C. The identification of individual compounds was based on the elution order and comparison to reference standards.

2.2.2.4 Gas Chromatography/ Mass Spectrometry

GC/MS measurements were carried out on another gas chromatograph (HP6890) with a mass-selective detector (HP5973; electron multiplier potential 2 KV, filament current 0.35 mA, electron energy 70 eV, and the spectra were recorded every 1s over the range m/z 50 to 800). The same capillary column noted above (30 m) was used. Peak assignments were based on comparisons with library spectra (NIST 1.6), reported in the literature (Tulloch 1970, 1971; Tulloch and Hoffman 1972) and by comparison of retention times of reference standards.

2.2.2.5 Thermogravimetric Analysis (TGA) and Differential Thermal Analysis (DTA)

Samples of dark and light colored beeswax were analyzed using a SDTQ600 TGA instrument (TA Instruments). A five mg sample was placed in an alumina pan and introduced into a micro-furnace. The heating rate was 20 °C/min with a flow of 100 ml/min air. The final temperature was 1000 °C. The weight loss and the heating temperature were continuously recorded as a function of time.

2.3 RESULTS

Lipid assemblages were detected in the analyzed cornet extracts from En Gedi, Moringa Cave and Grar. They are dominated by long chain *n*-alkanes (paraffins) with 21 to 32 carbon atoms (nC_{21} – nC_{32}) and fatty acids (palmitic acid, $C_{16:0}$, stearic acid, $C_{18:0}$ and lignoceric acid, $C_{24:0}$) with traces of a long chain alcohol, *n*-triacontanol (C_{30}ol) (Fig. 3A). The distributions of the *n*-alkanes normalized to the most abundant component in the extract are fairly uniform, whereas the relative proportions of the fatty acids and alcohols are more variable (Fig. 4). The lipid extracts from all the bowls on high fenestrated pedestals contain slightly lighter *n*-alkanes (C_{17} to C_{25}) together with 5-10 carbon fatty acids ($C_{5:0}$ to $C_{10:0}$) and with lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitic and stearic acids, cetyl alcohol (C_{16}ol) and stearyl alcohol (C_{18}ol). Isopropyl myristate and 1-H-indole-2-carboxylic ester were also detected (Fig. 3B). All the holemouth jar extracts contain *n*-alkanes (nC_{20} to nC_{27}), short chain saturated fatty acids ($C_{5:0}$ to $C_{9:0}$) as well as myristic acid, palmitic acid and stearyl alcohol (Fig. 3C).

Clearly, each vessel type has a distinctly different assemblage of lipids. Furthermore, extracts from sediments sampled from the vicinity of the vessels

showed completely different lipid assemblages. Some ceramic extracts contained no lipids at all. We conclude that lipids extracted from the ceramic vessel walls indeed reflect their contents and not post-depositional contamination from the associated surroundings, nor are they derived from any post-excavation treatment. In our present study we focused on the lipid assemblages from the cornets. We leave the interpretation of the molecular assemblages from the other vessel types to a future investigation that will involve comparison of the same items found in other sites and attributed to later periods.

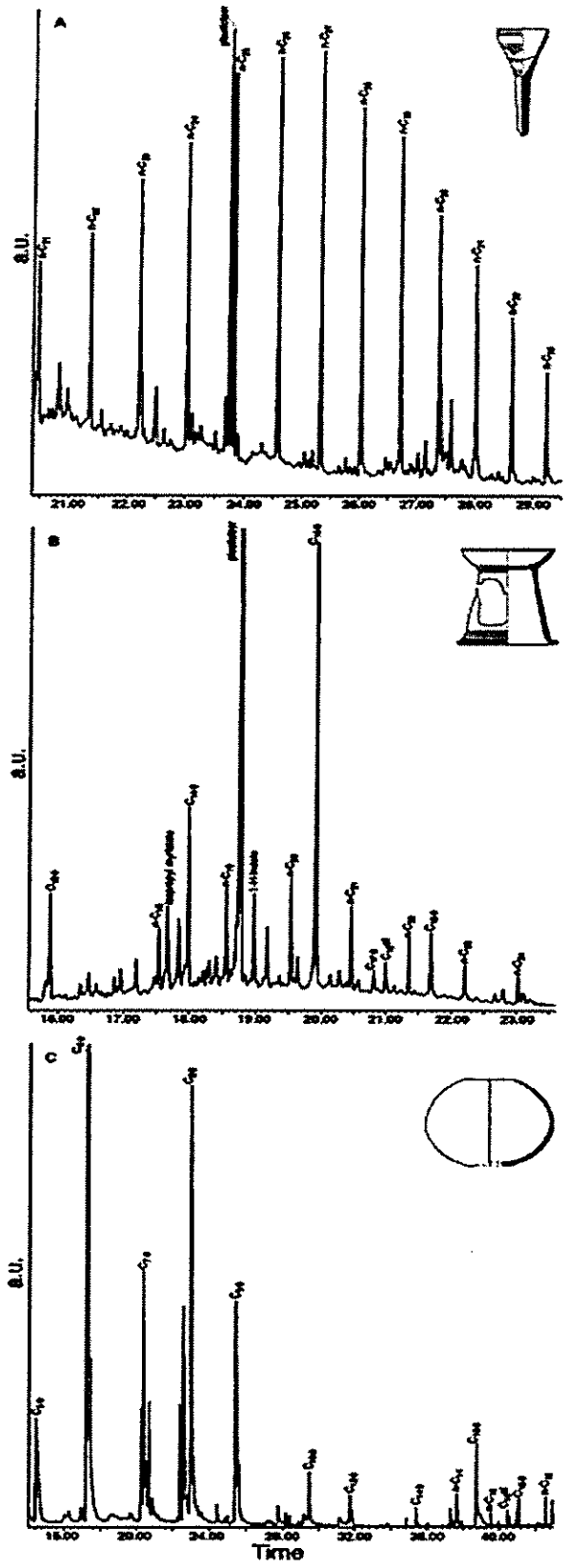


Figure 3 Representative gas chromatograms of the organic extracts from the different ceramic items sampled in En-Gedi. **A**, cornet EGC6/1. **B**, fenestrated pedestal bowl EGM20/1. **C**, holemouth jar EGP3/1. The *x*-axis represents retention times. The *y*-axis represents arbitrary units.

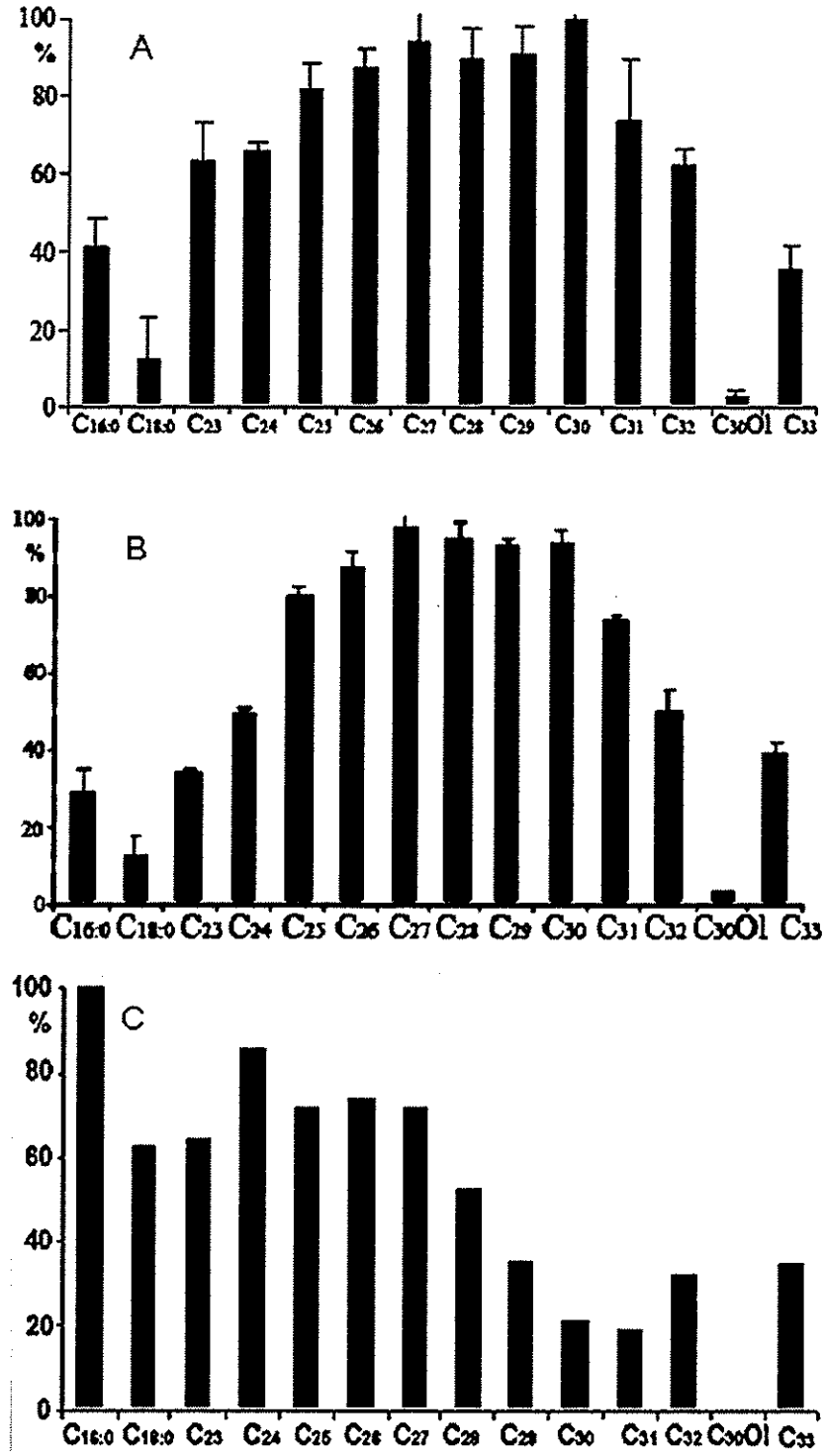


Figure 4 Histograms of averaged peak areas of the extracts from the cornets according to their site of origin: A, En-Gedi, B, Moringa cave and C, Grar. C_X refers to *n*-alkane with x carbons in its chain; $C_{X:0}$ refers to fatty acid with x carbons in its chain and 0 degree of unsaturation; $C_X$ol refers to *n*-alcohol with x carbons in its chain. The *y*-axis represents the relative proportions (percentage) compared to the total peak area.

The amounts of identifiable organic components preserved within the ceramic walls of the cornets are relatively high, namely between 200 and 505 µg/g in the En-Gedi cornets and 300 to 340µg/g in the Moringa Cave specimens. The two cornets from Grar also produced lipid assemblages resembling those of the cornets from the other two sites, however, only 30-35 µg/g hydrophobic organic material was obtained. In the ten cornets sampled from Nahal Qomem and Horvat Qarqar no detectable traces of organic residues were found. The different quantities of organic residues preserved in the cornets from the different sites may be attributed to the different environments in which the vessels were buried. Organic material tends to be better preserved in the arid zone of the Dead Sea region as compared with the semi-arid zones of the northern Negev and is certainly better preserved than in the sub-humid climatic zones of the Shephelah foothills of central Israel.

Since the fatty acids and alcohols present in the cornet extracts are not indicative of their source as they can be derived from different materials or processes, we focused on identifying the source of the alkanes. Closer examination of the *n*-alkanes reveals that there is a clear predominance of *n*-C_{27} amongst the odd numbered *n*-alkanes (ONA) in all the cornets analyzed and that the relative proportions between all ONAs normalized to the total alkanes peak area are similar (Fig. 5A). This is not the case for the even numbered *n*-alkanes (ENAs). The relative abundance of the ENAs is more varied (Fig. 5B). It should be noted that the lipid assemblages do not contain biomarkers such as organo-sulfur compounds that would be indicative of oil shale or bitumen (Koons, et al. 1965) or of terpenoids that would be indicative of floral resins (Asperger, et al. 1999; Downing, et al. 1961; Kolattukudy 1969; Regert 2004; Stern, et al. 2003). A possible source of the lipids in the walls of the cornets is beeswax.

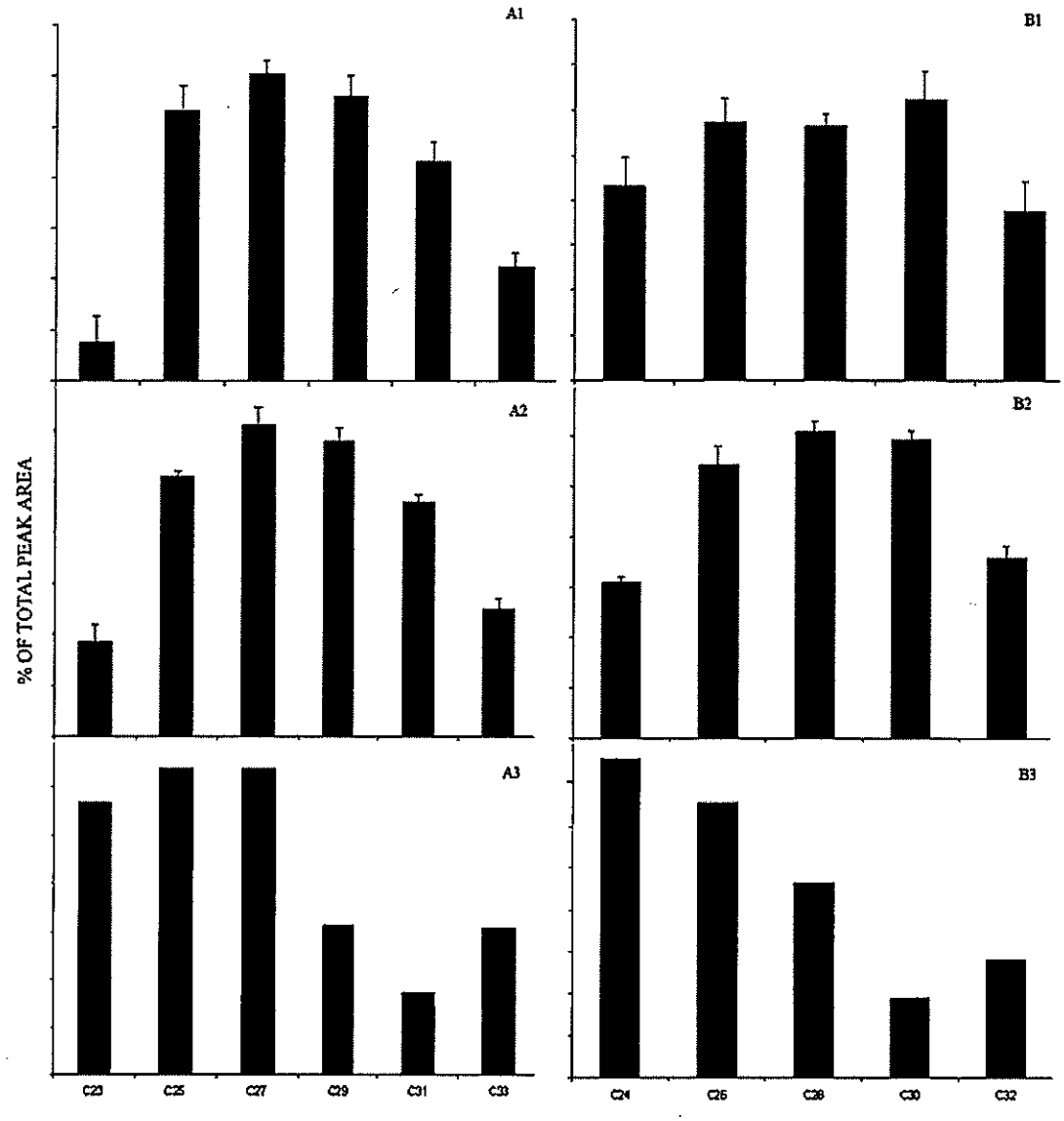


Figure 5 Histograms of the averaged amounts of **A,** odd and **B,** even numbered alkanes ratios in all the analyzed cornets extracts (1- En Gedi; 2- Moringa Cave; 3- Grar). C_X refers to *n*-alkane with x carbons in its chain. The *y*-axis represents the relative proportions (percentage) compared to the total peak area.

It is well established that beeswax contains three major groups of compounds: (i) fatty acids, $C_{16:0}$, $C_{18:0}$ and $C_{24:0}$; the latter serves as a biomarker; (ii) ONAs (C_{23} – C_{31}) and (iii) wax esters, mostly of palmitic acid in the range of C_{40} to C_{52} (Tulloch 1970). The absence of wax esters is probably due to poor preservation conditions

and/or heating in antiquity. The varied amounts of *n*-triacontanol ($C_{30}H_{62}OH$) and palmitic acid that are present in the cornet extracts are consistent with hydrolysis of wax esters (Tulloch 1971). Although wax ester hydrolysis should, in principle, have produced additional odd numbered *n*-alcohols in the range of 24 to 36, only *n*-triacontanol was detected. This observation is consistent with previous reports that "only very small amounts of even-numbered long-chain linear alcohols (C_{26}-C_{34}) appeared in beeswax when heated at 100°C" (Regert, et al. 2001), since the dominant palmitic ester in beeswax contains 46 carbons, and thus the alcohol most abundant after hydrolysis is triacontanol. Therefore, the presence of ONAs dominated by *n*-C_{27} and the significant presence of $C_{16:0}$, $C_{18:0}$, $C_{24:0}$ fatty acids and *n*-triacontanol in the cornets extracts are all consistent with the possibility that the original material preserved in the cornets is indeed beeswax. The presence of ENAs is not.

Namdar et al (2007; see chapter 2) analyzed modern wild hives of *Apis mellifera* from eleven different locations in Israel and one from Jordan. As all the samples analyzed in the literature were from light colored beeswax, Namdar et al (2007; see chapter 2) analyzed the *n*-alkanes from both dark and light colored beeswax. In all the light colored parts of the honeycombs analyzed there was indeed a dominance of ONAs as reported (Evershed, et al. 1997; Evershed, et al. 1999; Regert, et al. 2001). All other components of beeswax, such as $C_{16:0}$, $C_{18:0}$ and $C_{24:0}$ fatty acids and wax esters in the range of 40 to 54 carbons were also detected. Dark colored beeswax however contained up to six times more ENAs in their lipid extract, compared to light colored beeswax. Further it Namdar et al (2007; see chapter 2) concluded that the source of these ENAs was not from the beeswax itself, but another component. One possibility is cuticles, as these are present in fairly large amounts in the dark colored parts of the hive. The amounts

of ENAs in the dark colored beeswax are, however, on average still significantly less than those observed in the cornet ceramics. We therefore conclude that the *n*-alkane distribution observed here should be related to some other secondary process that took place in the cornet ceramic.

We therefore considered the possibility that burning or heating beeswax in a ceramic vessel could change the relative amounts of alkanes, based on the hypothesis that the source of the ENAs would be less likely to burn off especially when adsorbed into the ceramic. TGA/DTA analyzes of light and dark colored hive material indeed showed that 90% of the material was lost at temperatures below 400 °C (probably the beeswax) while other components burned at higher temperatures (Fig. 6). We then experimentally demonstrated that the relative proportions of ENAs dramatically increased by heating. We placed light and dark colored beeswax on clean ceramic fragments and heated them in an oven at 400 °C for 2 hrs. The compounds that were adsorbed into the ceramic were extracted and analyzed. In both cases, the extract indeed contained more ENAs than the starting material, and the ENAs and ONAs extracted were in proportions similar to those found in the cornets (Fig. 7A). No significant differences were noticed between the extracts of the light and dark colored beeswax. We also placed bees' remains on clean ceramic fragments, heated them in the same manner. Here too, the ENAs were significantly concentrated in the ceramic fragment (Fig. 7B).

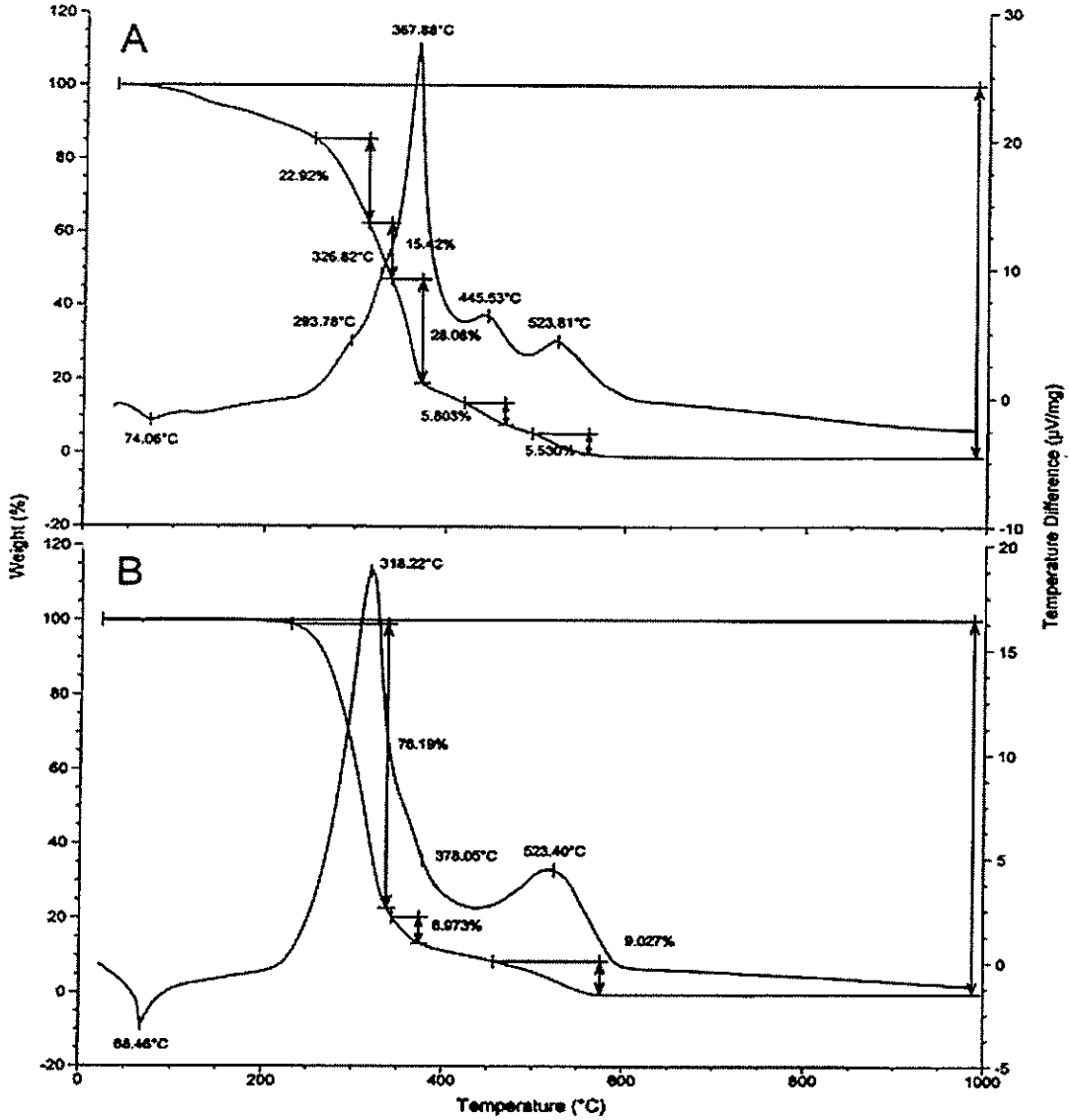


Figure 6 Thermo Gravimetric Analysis and Differential Thermal Analysis spectra of the weight loss and temperature changes of **A**, dark and **B**, light colored beeswax.

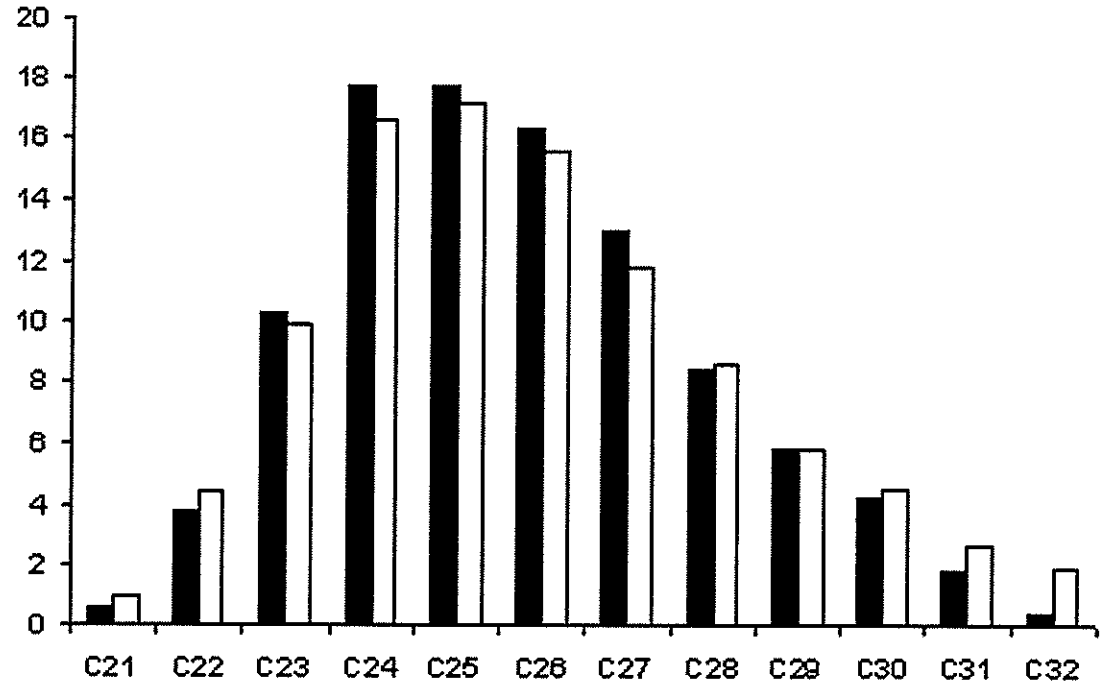


Figure 7 Histograms of the alkanes' ratios of heated modern beeswax (white columns) and bees' cuticles (black columns) in the ceramic extracts. C_x refers to *n*-alkane with x carbons in its chain. The *y*-axis represents the relative proportions (percentage) compared to the total peak area.

2.4 DISCUSSION

We show here that the assemblages of compounds extracted from cornets from different sites in Israel are dominated by *n*-alkanes. These alkanes are clearly not contaminants from the environment, as each vessel type contains a distinct molecular assemblage and other vessel types do not contain the *n*-alkane assemblage present in the cornets. The most likely source of these compounds is from beeswax, as well as another component that accumulates in the hives that contains both ONAs and ENAs.

Salvy et al (2001) have shown that the waxy coating present on the surface of the bees' cuticles contains both ONAs and ENAs. This is certainly one likely source of the *n*-alkanes in the cornet extracts. There may well be other sources,

particularly in the dark colored parts of the hive where brooding takes place and various non-waxy residues accumulate. These ENAs are clearly associated with and/or protected by materials/structures that are more stable at elevated temperatures than the beeswax itself. Hence they were concentrated inside the ceramic fragments heated together with beeswax.

Reports of ancient beeswax residues were based on the presence of wax esters together with only ONAs (Evershed, et al. 2003; Heron, et al. 1994). In these cases the beeswax was clearly better preserved than in the material we analyzed. There have however been reports of residues from ceramic vessels that contained both ONAs and ENAs (Regert 2004), but as it was not known then that beeswax under some circumstances can contain both even and odd-numbered alkanes, the authors could not conclude that these residues were derived from beeswax.

It is interesting to consider whether or not the presence of both even and odd-numbered alkanes in the cornet ceramic walls necessarily implies that the beeswax was heated. As the ENAs are derived from more stable (at least with regard to temperature) components of the hive material, it is conceivable that diagenetic processes at ambient temperatures caused the differential loss of the ONA dominated wax components, leaving mainly the more stable components with their ONA and ENA lipid assemblages. We note however that some of the other vessels at the archaeological sites we examined, such as the bowls on high fenestrated pedestal, contained relatively unstable and more volatile compounds. It is unlikely that preservation conditions were different for the cornets. It is more likely that absence of esters and particularly of alcohols in the cornets, as well as the presence of high levels of ENAs, is due to heating of the beeswax.

This raises the possibility that the beeswax in the cornets was used for illumination. The conical shape and the variable size of the cornets, the presence or absence of solid appendages and the fact that they were found in different contexts in the Ghassulian are indeed all consistent with the cornets having been used as vessels for beeswax candles. Beeswax was used as an illuminant in antiquity (Evershed, et al. 1997). The absence of soot on the inner surfaces of the cornets can be attributed to the fact that slow-heated beeswax does not produce soot (Asperger, et al. 1999). It was suggested that during the Chalcolithic period small shallow bowls were used as oil lamps containing olive oil as fuel (Gilead, 2001). This assumption has not, to date, been validated.

In the Chalcolithic period in the southern Levant, another use of heated beeswax may be in the copper industry, where the "lost wax technique" was widely employed. In fact the oldest known assemblage of copper objects from Nahal Mishmar contains elaborate "prestigious items" (mace heads, standards, crowns, etc.) that were produced by the "lost wax technique" (Key 1980; Tadmor et al. 1995; Shalev 1995; Namdar, et al. 2004). This industry may have required large quantities of beeswax. It is interesting to note that Ussishkin (Ussishkin 1970) associated the Nahal Mishmar hoard with the nearby Chalcolithic shrine at En-Gedi, raising the possibility that the shrine might have been the source for these items. Cornets are however rare or absent in Chalcolithic sites with evidence for copper industry (Levy 1987; Perrot 1955; Shugar 2000). No evidence for casting by the lost wax technique was observed in these sites, where open casting seemed to be the normal practice. In the sites investigated here no evidence for copper-casting activity has been reported. Therefore, the relation between the copper industry and cornets should be further investigated.

The highest concentrations of residues were from vessel walls excavated in the Dead Sea area. Lower concentrations were obtained from vessels from the northern Negev No organic compounds were preserved in the ceramic vessels sampled in the inner coastal plain region. This is consistent with the general observation that organic materials are relatively well preserved in the Dead Sea region (for example the Dead Sea Scrolls themselves (de Vaux 1961), the papyrus scrolls in the Cave of Letters (Yadin 1963) and the spectacular findings of Nahal Hemar, dating to the first half of the eighth millennium B.C.E. cal (Bar-Yosef and Alon 1988). The presence of preserved lipid assemblages outside the Dead Sea region does open up the possibility that these compounds can be more widely utilized for determining the contents of different shaped vessels in the Levant. This information would be invaluable for reconstructing aspects of ancient economies in this region.

Dvory Namdar, Ronny Neumann, Yuval Goren, Nizar Haddad, Yossi Sladezki, Isaac Gilead and Steve Weiner. The Content and Use of Enigmatic Ceramic Vessels ("Cornets") from the Chalcolithic Period (6000 years ago), Israel. Submitted for publication in *American Journal of Archaeology*.

3 Experimental B: Monitoring Natural Degradation of Beeswax

3.1 Introduction

As mentioned in the previous chapter, the most common components of beeswax are wax esters, mostly of palmitic acid in the range of C_{40} to C_{52} (Tulloch 1970). These wax esters constitute about 70% by weight of the beeswax and have been extensively studied (Tulloch 1980, Mills and White 1994, Asperger et al. 1999, Aichholz and Lorbeer 2000, Jimenez et al. 2003, Jimenez et al. 2004). The remaining components are mainly fatty acids ($C_{16:0}$, $C_{18:0}$ and $C_{24:0}$; the latter serves as a biomarker) and *n*-alkanes (C_{23} – C_{31}). The *n*-alkanes are reported to be composed almost entirely of odd numbered carbon chains with only traces of even numbered *n*-alkanes. Notably, all the reported analyses of alkanes in beeswax were from light colored material (Tulloch 1970, Regert et al. 2001). The color of beeswax however varies from light yellow to dark brown and even black. The different colors roughly correspond to the different activities taking place in the cells. The newly built cells ("white") and honey-storing cells ("yellow") are located in the most peripheral areas of the hive. The brood areas ("black") are in the center of the comb. These are surrounded by the pollen-storage cells ("brown") for the nutrition of the larvae (DeGrandi-Hoffman, 1989) (Fig. 1). The darker color of the central area is attributed to the presence of the pupal lining residues left in this area (Hepburn and Kurstjens 1988). The darker and hence older beeswax is also heavier and more brittle (Berry and Delaplane 2001). Here we analyze the *n*-

alkane contents of differently colored beeswax from 12 natural bee hives of *Apis mellifera ligustica* and *Apis mellifera syrica* collected in Israel and Jordan respectively.

Figure 1. Photograph of the different activity areas in a wild beehive from Kerem Maharal, Israel: **A**, the brooding area, **B**, the pollen storing area, **C**, the honey storing area, **D**, new cells still not used

3.2 MATERIALS AND METHODS

3.2.1. Materials

Twelve wild bee colonies were sampled from the following locations in Israel: Bet-Gamliel, Rehovot (5 different colonies of different ages), Nahef, Juara, Hayogev and Kerem Maharal (2 different colonies) and from one location in Jordan: Abu Zead valley (Fig. 2). We sampled and analysed several combs per hive, according to their color. We generally sampled one dark and one light colored comb within each wild beehive. If the hive had a uniform color, only one comb was sampled. In the beehives from Rehovot 2 and 3, Juara, Hayogev and Kerem Maharal 2, light and dark colored beeswax were present. In the beehives from Beit Gamliel and Rehovot 4 no dark parts were found while in the hives from Rehovot 1 and 5, Nahef, Kerem Maharal 1 and Jordan very light colored parts were absent. The beehive from Jordan is a traditional clay mud hive maintained free of human introduction of wax foundations. *Apis mellifera syrica* was identified using morphometric and DNA methods as the native bee that lived in this region before the introduction of imported bees (Haddad and Fuchs 2004).

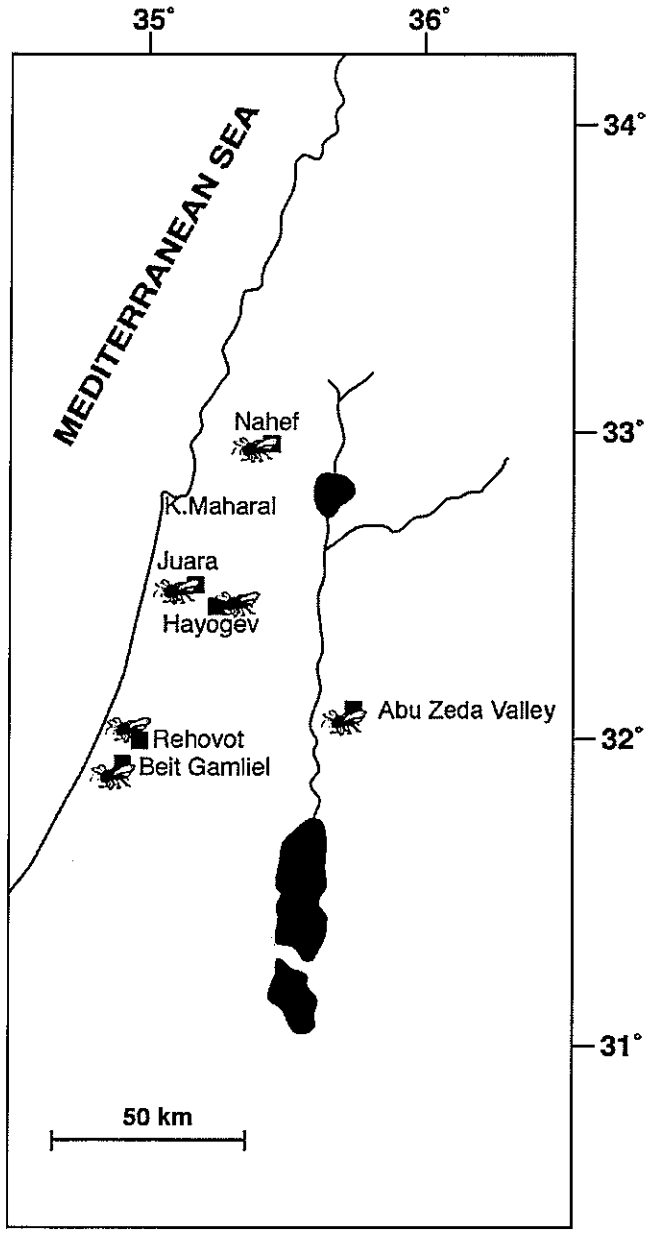


Figure 2 Map of the Southern Levant. Beeswax sample sites are marked with a bee.

3.2.2. Extraction of alkane fraction in beeswax

All the comb beeswax samples were analysed without any additional purification steps. Two methods were used for the analysis of beeswax in order to determine whether or not the results are influenced by analytical procedures. These were based on the methods reported by Regert et al. (2001) ("method A") and Evershed et al. (1997) ("method B"). As we were interested also in detecting compounds other than alkanes, the silylation step (see below) was included. When

analyzing only the *n*-alkane composition of wild beeswax, methods A and B were used without carrying out the silylation step.

Method A. 5 mg of beeswax was transferred to a sterile glass screw-top vial and 5 mL of dichloromethane were added and then sonicated for 5 min. 100 µL of the solution was transferred to another glass vial, and the solvent was evaporated under a stream of argon. 50 µL of *N,O*-bis(trimethyl)silyltrifluoroacetamide, BSTFA, containing 1% trimethylchlorosilane (TMC) was added and the sample was heated to 65 °C for 30 minutes. After the vial cooled to room temperature, the silylating reagent was evaporated under a stream of argon, and 50 µL of dichloromethane was added; 5 µL were injected into the gas chromatograph (GC).

Method B. Approximately 5 mg of beeswax was transferred to a sterile glass screw-top vial and 2 mL of chloroform: methanol (2:1 v:v) solution were added and then sonicated for 10 min. 200 µL of the solution was transferred to another glass vial, and the solvent was evaporated under a stream of argon. 150 µL of BSTFA containing 1 % TMC was added and the sample was heated to 65°C for 30 minutes. After the vial cooled to room temperature, the silylating reagent was evaporated under a stream of argon, and 100 µL of cyclohexane were added; 5 µL were injected into the GC.

n-Alkanes standards C_{10}-C_{25} were obtained from Restek (A032194 Diesel Range Organics mix, Tenn/Miss). The C_{26}-C_{32} standard was custom made.

3.2.3. Analysis of alkanes by Gas Chromatography (GC) and Mass Spectrometry (MS)

3.2.3.1 Gas Chromatography (GC)

The GC analysis was carried out using a HP6890 GC equipped with a flame ionization detector (FID) and using a split injection mode with a 1:10 split ratio.

When the amount of extracted material was small, a splitless injection mode was used. A 30 m, 0.32 mm ID 5% cross-linked PhMe siloxane capillary column (HP-5) with a 0.25 µm film thickness was used for separation. Helium was used as a carrier gas at a constant flow of 1.1 mL/s. The initial oven temperature was 50 °C and a heating gradient of 10 °C/min was started 2 min after injection. Upon reaching 345 °C, the run was continued for an additional 10 min. The injection temperature was 220 °C and the FID detector temperature was 350 °C. The identification of individual compounds was based on the comparison of retention times to reference standards.

3.2.3.2. Gas Chromatography/ Mass Spectrometry

GC/MS measurements were carried out on another gas chromatograph (HP6890) with a mass-selective detector (HP5973; electron multiplier potential 2 kV, filament current 0.35 mA, electron energy 70 eV, and the spectra were recorded every 1s over the range mass to charge (m/z) 50 to 800). The same capillary column noted above was used. Peak assignments were based on comparisons with library spectra (NIST 1.6), spectra reported in the literature (Tulloch 1970, 1971, Tulloch and Hoffman 1972) and by comparison of retention times of reference standards.

3.2.4. Analysis of chitin by Fourier Transform Infrared Spectroscopy (FTIR)

FTIR was performed using a Midac Corporation (Costa Mesa, CA, USA) instrument. The spectra were obtained by mixing about 0.1 mg of sample with about 80 mg of KBr. The spectra were collected at 4 cm^{-1} resolution.

3.2.5. Amino Acid Analysis

Aliquots of the dry insoluble phase were hydrolyzed under 6 N HCl vapor in vacuum for 24 h. Following evaporation of the HCl, the hydrolyzates were derivatized with Waters AccQ·Fluor reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) that stabilizes and fluorescently labels both primary and secondary amino acids. The derivatives were then analyzed with an automatic amino acid analyzer (HP Aminoquant system). The identification and quantification of the separate amino acids is based on external standard retention times and peak areas respectively.

3.2.6. Analysis of absorbance by Ultra Violet -Visible spectrophotometry (UV-vis).

UV-vis spectra were taken on an HP 8453 diode array spectrometer. The measurements were carried out by diluting 50µL of a 0.01 M solution of the dissolved beeswax in 2 mL of dichloromethane. The absorption intensity was measured at 500 nm.

3.3. RESULTS

3.3.1 Alkane composition

Beeswax from natural honeycombs was sampled by color and analysed using GC and GC/MS. Analyses of light colored beeswax showed the presence of odd numbered *n*-alkanes (ONAs) in the range of C_{23}- C_{33} with a clear predominance of the C_{27} alkane with only very small amounts of even numbered *n*-alkanes (ENAs) in the range of C_{22}- C_{32}. In the 9 light colored honeycombs the distribution patterns of the alkanes are consistent with reported results (Tulloch 1970, Hepburn and Kurstjens 1988, Heron et al. 1994, Evershed et al. 1997,

Regert et al. 2001). All other features that are attributed to beeswax were detected, but not analysed in depth (Fig. 3). The alkanes were identified according to their mass to charge (m/z) ratios, their typical m/z fragmentation patterns and retention times in comparison to standard alkanes. GC and GC/MS analyses of dark colored beeswax showed an assembly of alkanes containing both odd and even numbered alkanes (Fig. 4).

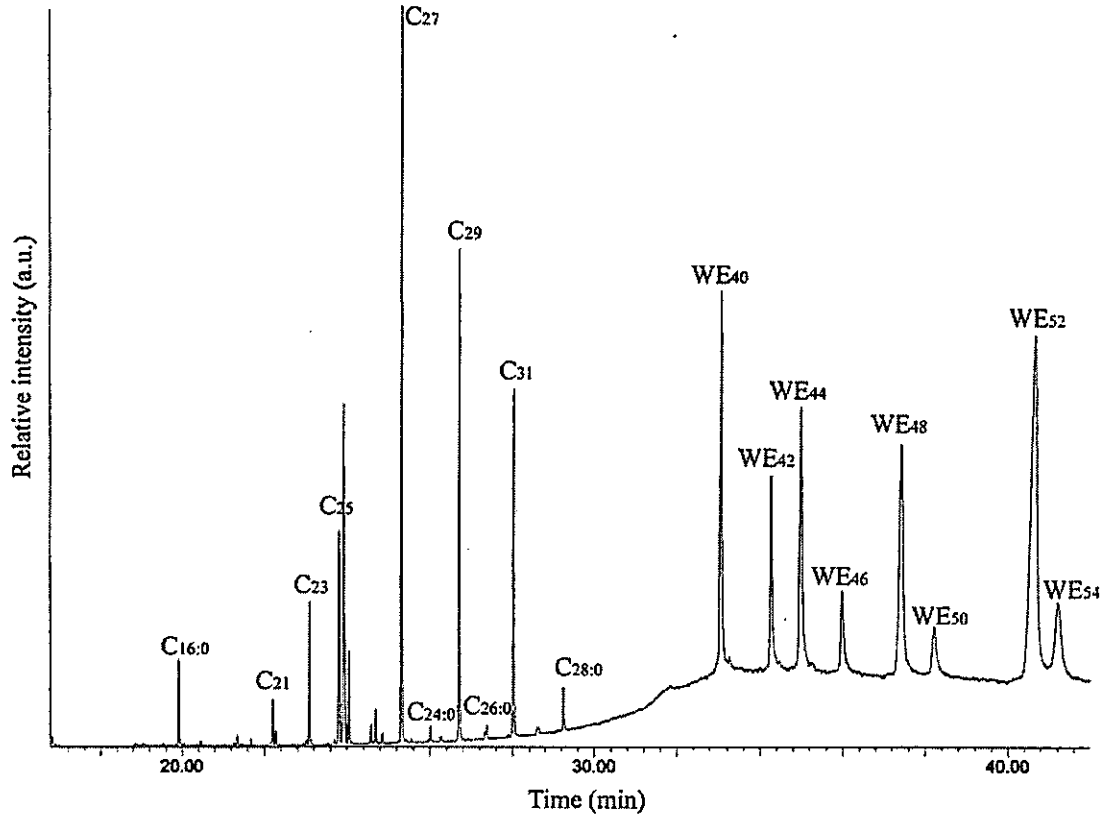


Figure 3 Representative gas chromatogram of the organic extracts from light colored beeswax from bee comb from Israel. C_X refers to *n*-alkane with x carbons in its chain; $C_{X:0}$ refers to fatty acid with x carbons in its chain and 0 degree of unsaturation; WEx refers to palmitic wax esters with total of x carbons in the molecule; a.u. = arbitrary units.

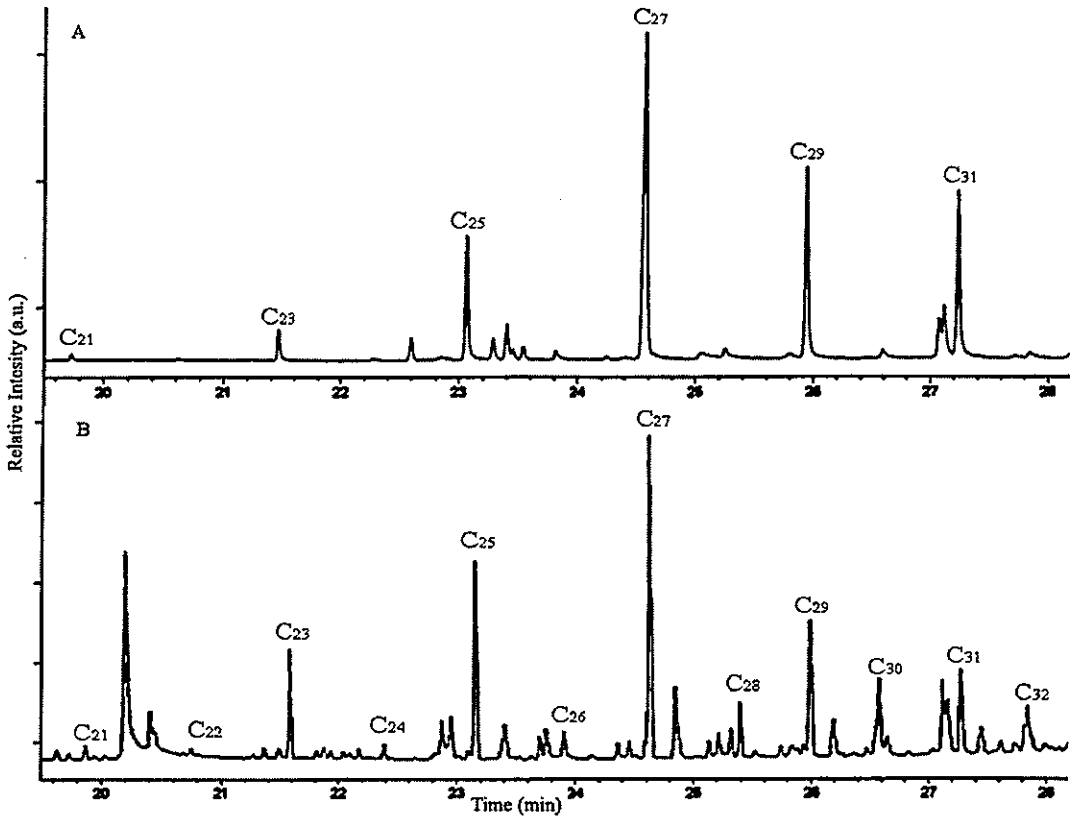


Figure 4 Gas chromatograms of the range of *n*-alkanes in the organic extracts from **A,** light colored and **B,** dark colored beeswax from Israel. C_X refers to *n*-alkane with x carbons in its chain.

Darker colored beeswax (based on visual observations and absorption at 500 nm) contains on average about 3 times more even numbered *n*-alkanes (ENAs) than lighter colored beeswax (Table I and Fig. 5). The ratios of ENAs to ONAs in different colored beeswax from the same hive also show that darker beeswax contains higher amounts of ENAs in all the hives analysed (Fig. 6). No differences in *n*-alkane composition were observed due to the extraction method used. From this we infer that the ENAs in the beeswax might be derived from a different source, as compared to the ONAs which are derived mainly from the beeswax itself. To investigate this possibility further, we analysed other properties of beeswax by color.

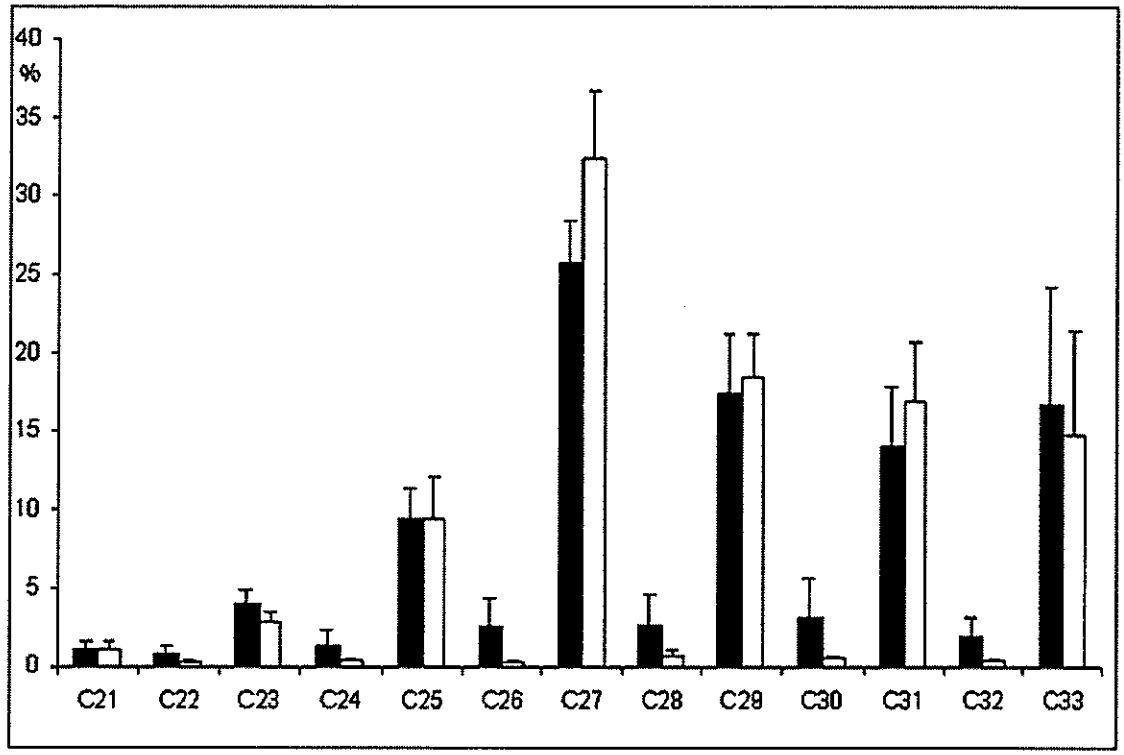


Figure 5 Histograms of averaged peak areas of the alkanes extracted from all the light colored (white columns) and dark colored (black columns) beeswax samples. The relative peak areas are normalized to the most abundant alkane. C_X refers to *n*-alkane with x carbons in its chain. Y axis = %.

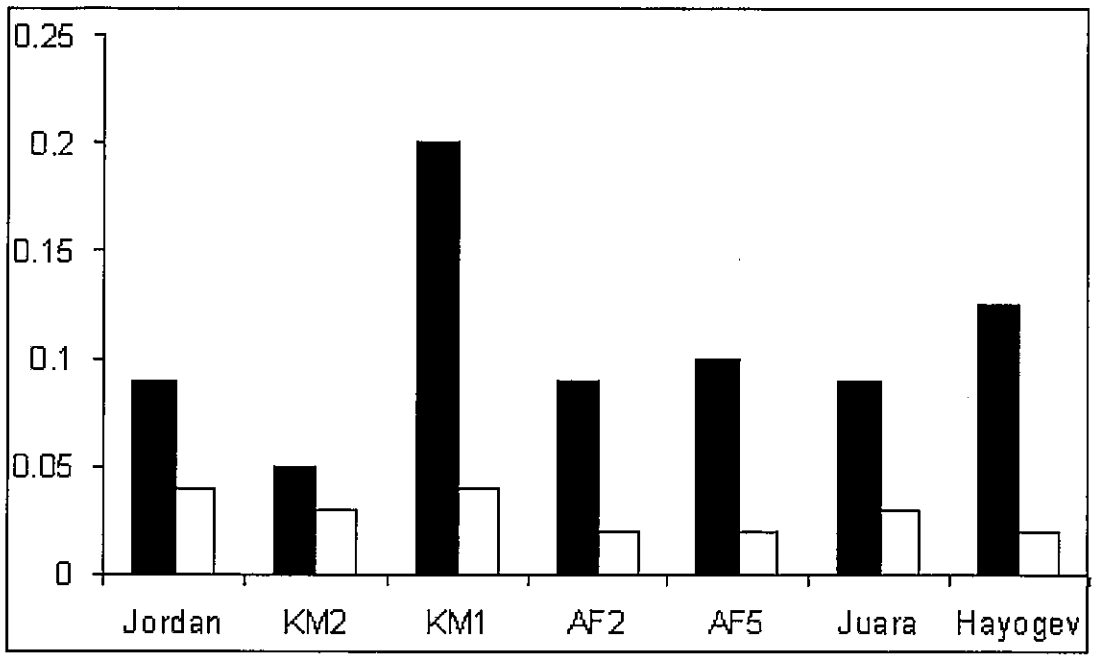


Figure 6 Histograms of the total ENAs\ONAs ratios in beeswax from seven hives in Israel and Jordan. White columns = ratio in light colored beeswax. Black columns = ratio in dark colored beeswax. KM= Kerem Maharal. AF = Rehovot (Faculty of Agriculture).

Table I. Odd to even numbered alkanes ratio, weight loss percentage and spectrophotometric analyses of dark and light colored beeswax

Dark colored beeswax					
Origin of comb	% ONAs	% ENAs	ENAs/ONAs	% weight loss	Absorbance
Jordan	91.8	8.2	0.1	33	0.21
Kerem Maharal 2	95.2	4.8	0.05	21	0.11
Kerem Maharal 1	83.2	16.8	0.2	24	0.14
Rehovot 2	91.9	8.1	0.1	24	0.12
Rehovot 1	94.0	6.0	0.1	26	0.04
Rehovot 5	91.0	9.0	0.1	28	0.05
Juara	91.6	8.4	0.1	32	0.2
Hayogev	89.3	10.7	0.1	34	0.25
Nahef	94.0	6.0	0.1	35	0.1
average	91.3	8.7	0.1	28.5	0.14
Std. deviation	3.5	3.5	0.04	5.1	0.04
Light colored beeswax					
Jordan	96.1	3.9	0.04	2	0.01
Kerem Maharal 2	96.9	3.1	0.03	2	0.02
Kerem Maharal 1	95.8	4.2	0.04	3	0.01
Rehovot 2	97.6	2.4	0.02	2	0.01
Rehovot 3	96.8	3.2	0.03	2	0.01
Rehovot 5	98.3	1.7	0.02	2	0.02
Juara	97.2	2.8	0.03	4	0.01
Beit Gamliel	97.2	2.8	0.03	3	0.01
Hayogev	98.3	1.7	0.02	3	0.01
average	97.1	2.9	0.03	2.5	0.01
Std. deviation	0.9	0.8	0.01	0.7	0.004
Cuticles	93.1	6.9	14	40	0.02

Table I %ONAs and %ENAs was calculated from the calibrated peak area of the different alkanes present in the beeswax extract. % weight loss is the amount (mg) of insoluble pellet left after dissolving the beeswax sample with organic solvents compared to the initial weight of the beeswax sample. Absorbance is calculated at 500nm wavelength.

3.3.2 Other properties

The absorbance of the soluble phase of the different colored beeswax varied only slightly (Table I). Thus we report, regarding all parameters we analyzed, solely on the two color extremes (dark and light). More than 95 weight % of the light colored beeswax dissolved in dichloromethane or in a chloroform:methanol mixture, while only 70 weight % of the dark colored beeswax dissolved in these solvents. We also noted that when two separate samples (approximately 40 mg each) of light and dark colored beeswax were maintained in separate open vials at 80 °C in an incubator for 30 days, the dark and light phases separated after the samples were rapidly cooled. The heated light colored beeswax contained mostly a white phase with a very thin dark layer at the bottom of the vial. The heated dark colored beeswax contained mostly dark viscous material with a thin hard white crust on top. These observations imply that non-waxy material is present in significant amounts in the dark colored beeswax, whereas it is present in only small amounts in the light colored beeswax.

FTIR analysis of the insoluble fraction of all the beeswax samples showed the presence of chitin (Amide I at 1650 cm^{-1}, Amide II at 1544 cm^{-1}, Amide III at 1318 cm^{-1}, sugar ring at 1073 cm^{-1}) (Fig. 7). Amino acid analyses of samples from various parts of the hives showed larger amounts of amino acids in the darker parts of the hive.

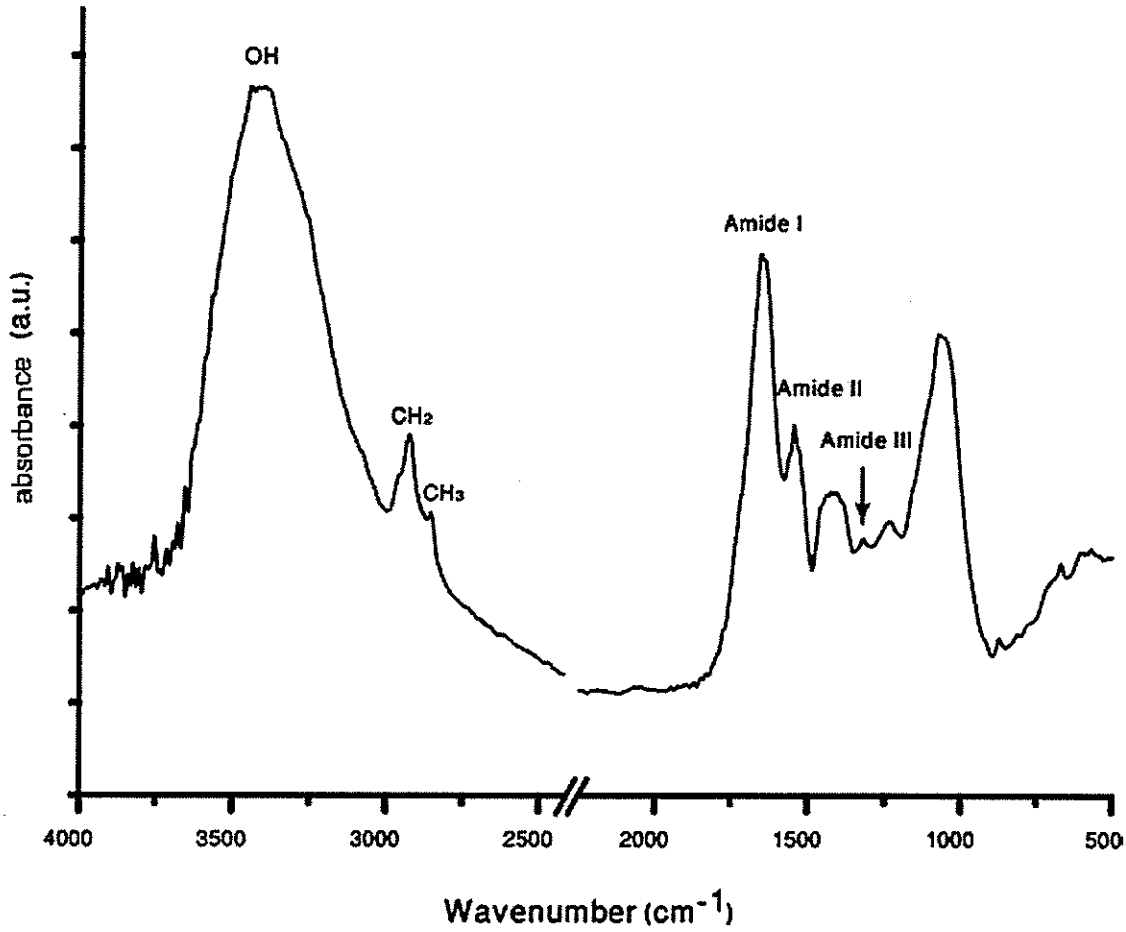


Figure 7 Infrared absorption spectrum of the insoluble fraction of dark colored beeswax showing the presence of chitin.

3.4 DISCUSSION

The chemical composition of light colored beeswax has been extensively investigated and its predominantly odd numbered *n*-alkanes assemblage is well documented (Tulloch 1980, Lockey 1988, Hepburn et al. 1991, Asperger et al. 1999, Aichholz and Lorbeer 2000). The fact that bees use different parts of the comb for different purposes and that the central part is usually used for brood rearing is also well known (Hepburn and Kurstjens 1988, Hepburn et al. 1991, Berry and Delaplane 2001). Furthermore, hydrocarbon assemblages containing both odd and even numbered *n*-alkanes are known to be deposited on the

surfaces of the cuticles of bees' exoskeletons (Jay 1963, Blomquist and Jackson 1973, Hepburn and Kurstjens 1988). Salvy et al. (2001) report using GC and GC/MS analyses that extracts of waxes from the surface of bees' cuticles contain odd and even *n*-alkanes in the range of $C_{22} - C_{32}$. We confirmed this observation using cuticles obtained from two dark colored beeswax samples. We however did not obtain the relatively high concentrations of ENAs compared to ONAs that Salvy et al (2001) reported. This could be due to pest infestation of these bees by *Varroa jacobsoni* (Salvy et al 2001). Cuticular hydrocarbons are synthesized during the nymphose phase in the haemolymph of the bees, secreted immediately after molting and are incorporated into their cuticular lipids (Blomquist and Jackson 1973, Lockey 1988). Therefore, we can conclude that the larger amounts of ENAs present in the dark colored beeswax compared to light colored beeswax can be correlated to the accumulation of cuticles in these parts of the hives. This conclusion is consistent with the presence of chitin and protein in the fraction extracted from dark colored beeswax. Nevertheless, the absorption of different colors at 500 nm by beeswax, showed no correlation with the ONA\ENAs ratios (Table I). This lack of correlation indicates that there may be other factors that contribute to the dark color in the brood rearing areas. The amino acid composition of both light and dark colored beeswax is not dominated by Glycine and Alanine, showing that silk was not a major component of the protein fraction we analysed.

We thus envisage the following scenario for the presence of larger amounts of ENAs in dark colored beeswax. Cuticular hydrocarbons are synthesized during the pupal phase in the haemolymph of the bees. During the regular use of the comb by the bees, cuticles with their waxy layers accumulate, leaving their traces in the beeswax (Jay 1963). As a result the beeswax characteristics change. Its color darkens, it becomes brittle and heavier, the cell size decreases and the

chemical composition of the comb changes (Hepburn and Kurstjens 1988). The ENAs secreted by the bee onto its cuticle, are incorporated into the beeswax. These are extracted from the whole lipid assemblage of the dark colored beeswax. The decrease in cell size causes the bees to lay their eggs in lighter colored cells, and thus the darkened areas in the hive enlarge with time (McLellan 1978, DeGrandi-Hoffman 1989). In the old comb ENAs are found all over the hive in increasing amounts. Because the source of the ENAs in the beeswax is additive in nature, the relative amounts of ENAs compared to the ONAs are variable, as we have observed. One implication of this observation is that in order to obtain representative analyses of parts of the comb or the whole comb, it is essential to homogenize the sample well prior to analysis.

Dvory Namdar, Ronny Neumann, Yossi Sladezki, Nizar Haddad and Steve Weiner. 2007. Alkane Composition Variations Between Darker and Lighter Colored Comb Beeswax. *Apidologie*, accepted for publication.

4 Experimental C (Control): Iron Age Beehives from Tel Rehov

4.1 The Discovery

In a recent excavation conducted at the site of Tel Rehov in northern Israel under the supervision of Amihai Mazar and Nava Panitz- Cohen, an intriguing installation containing eight unfired clay cylinders lying in a consecutive row was unearthed (Figs. 1 and 2).

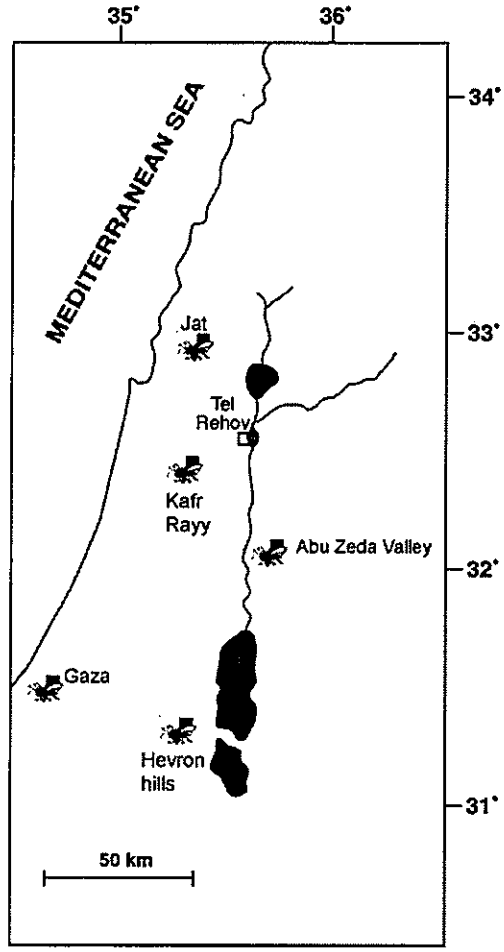


Figure 1 Map of the Southern Levant. Ethnographic hives are marked with a bee. Tel Rehov is marked with a jug.

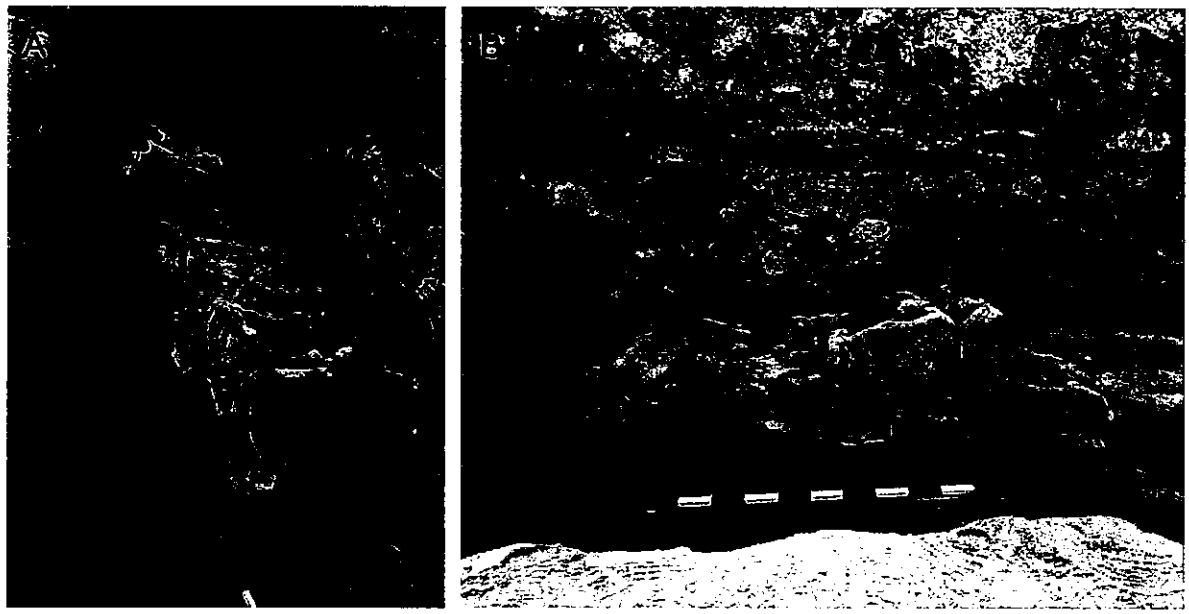


Figure 2 View of the beehives found at Tel Rehov: **a** – looking northeast; **b** - frontal view, looking east.

Tel Rehov (Arabic: Tell es-Sarem) is a 10 hectares mound located about 5 km south of Beth-Shean and 7 km west of Pella in the alluvial Beth-Shean valley, which is a segment of the Jordan valley. It was one of the largest Iron Age cities known in the Land of Israel. Eight excavation seasons since 1997 revealed a prosperous and well-planned city of the 10-9th centuries BCE (Mazar 1999; Mazar et al. 2005; Mazar, in press). The installation was discovered in Area C, located at the north-western corner of the city (Fig. 3). The intriguing installation included eight clay cylinders arranged along a north-south line. Each cylinder is about 0.8 m long and about 0.44 m in diameter and is made of unfired clay walls about 8 cm thick. The cylinders were placed on a wooden beam that also ran on a north-south axis; burnt traces of this beam were revealed especially near the southern end of the installation (Fig. 2A). The best preserved cylinders had a clay wall blocking their western end, in the centre of which was a small hole, with black signs along its perimeter. The opposite, eastern end of the cylinder remained open, but could be blocked by lumps of clay, some of which were found *in situ*. Few remnants of a

second row of such cylinders above the bottom row were also found. There was a bench than ran at least 9.2 m long, made of compact earth, about a half meter east of the hives.

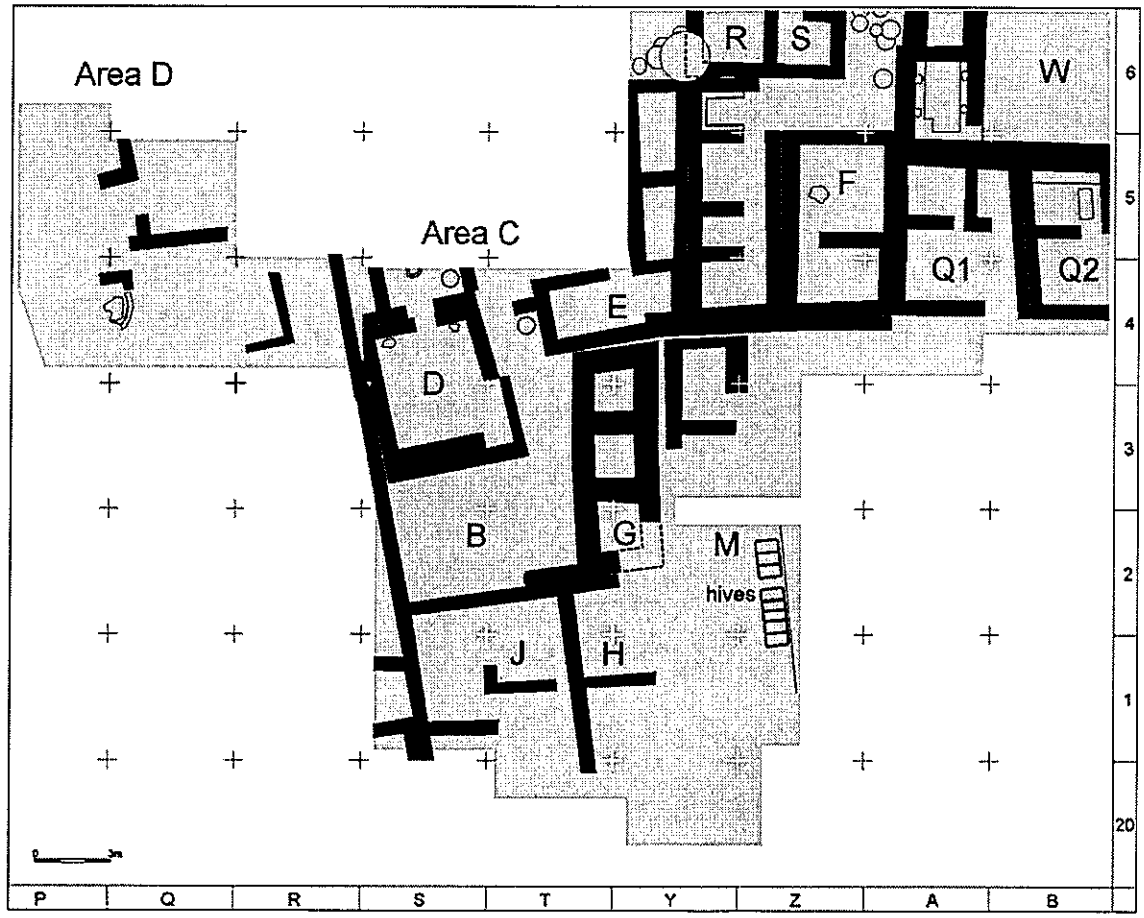


Figure 3 Plan of Tel Rehov Area C Stratum V, showing the beehives in Building H.

The entire area was covered by a ca. 1 m thick destruction layer, including many fallen bricks, burnt beams and a large amount of restorable pottery vessels. This destruction layer was tilted from the west to the east. Some of the intact and restorable pottery vessels from this layer appear to have fallen from a second floor (Mazar 1999; Mazar et al. 2005). Thus it was concluded that the area was probably roofed and had a second floor above what seems to be a basement in which the beehives were located. Locating unfired clay or mudbrick installations under a roof

makes sense, as the winter rains would damage the construction considerably. This is also confirmed by some of the ethnographic parallels cited below and by literary descriptions (Crane and Graham, 1985).

Here we use several independent sources of information to confirm that these structures are indeed beehives: pictorial, literary and ethnographic parallels, as well as analyses of residues extracted from the walls of the structure.

4.2. Pictorial, Literary and Ethnographic Parallels

The identification of the Tel Rehov installation as a beehive is based on pictorial depictions, literary evidence and ethnographic analogies. The only archaeological sources relating to beekeeping in the Ancient Near East are four Egyptian pictorial depictions: one from the tomb of Niuserra of the 5th Dynasty, two from the 18th Dynasty and one from the 7th century BCE (Kuény 1950; Crane 1983: 34-39; Crane and Graham 1985: 24-27; Serpico and White 2000: 410; Kritsky 2007). They show cylindrical horizontal hives stacked in several layers. No other pictorial depictions of hives are known until Medieval times, but there are literary references to beehives in Hittite documents, where they seem to have been a valuable commodity (Crane and Graham 1985: 31-34).

No such hives have been recorded from archaeological excavations from this or earlier ages. The main direct archaeological evidence for hives comes from Classical Greece where a good number of pottery hives were found; they are all shaped as cylindrical jars with a base, open on one side only, with extension rings and lids (Crane 1983: 45-47; Crane and Graham 1985: 149-153). Beekeeping is described by various Greek and Roman authors, who sometimes include a detailed description of the hives (for references see Crane and Graham 1985: 31-

39). Columella's description is particularly comprehensive and is cited by later authors. Notably, he defines unfired clay as the most unsuitable material for constructing hives, since it would be too cold in the winter and too hot in the summer; tree bark and wood are considered by him to be the preferred materials for constructing hives.

Beehives shaped as cylinders made of unfired clay, fired pottery, bark, wood or basketry and placed in horizontal rows are well known in many traditional cultures throughout the Mediterranean, the Middle East, East Africa, India and the Far East (Crane 1983). In traditional Palestinian villages, hives made of unfired clay are common (Avitsur 1976). Some of these are similar to those at Tel Rehov and are located in the courtyard of village houses and sometimes even inside a farm building (Fig. 4). Unbaked clay hives were used by Palestinian cave dwellers in the southern Judean Hills; their front walls were decorated with clay mouldings (Havakook 1985, photo between pages 144-145). A wall composed of such hives was recorded near Kefr Raay, north-east of Shechem (Zertal and Mirkam 2000). The rows of hives had front walls with small openings in the centre, similar to those in the Tel Rehov Iron Age installation; these openings could be closed by a piece of wood. The ethnographic museum at Kibbutz Yif'at in Israel exhibits unbaked clay hives from the Druze village of Jat in western Galilee; they have a movable door composed of a clay disc with a protruding handle. Also exhibited fired clay hives produced in the pottery workshops of Gaza, made as cylinders opened on both sides. Other traditional hives in that museum are made of wood and basketry. Unfired clay hives are well-known in modern Egypt, where 300-500 hives were recorded in single stacks (Mellor 1928; Crane 1983; Kritsky 2007).

Figure 4 Photograph of an ethnographic modern parallel from Wadi Zeita, Jordan. The photograph was taken by Dr. Nizar Haddad, the head of the Bee Research Center in Amman.

In many of these hives, the cylinder's front was closed by either a permanent wall or a removable lid; in both cases, a small hole was left for the bees to enter and exit and yet prevent larger insects or animals from entering the hive. Such small round openings are found in the Tel Rehov hives, as mentioned above (Fig. 2). The back was usually left open and sometimes blocked with removable materials in order for the beekeeper to be able to smoke the hive and thus force the bees to fly out through the opening in the front, and thus enable the harvesting of the honey combs. As Crane and Graham maintained (1985), this procedure is well-illustrated in an Egyptian pictorial depiction from the 7th century BCE (in Tomb TT279 at Thebes) and is practiced in all traditional beehives (though missing in Greek hives which are closed at one end). In Tel Rehov, the bench along the eastern edge of the hives, which faced the edge that was found open or

temporarily blocked, could have served for the hive caretakers and for harvesting the combs.

4.3. Analytical Identification of Degraded Beeswax Residues

4.3.1 Materials and Methods

4.3.1.1 Materials

Two different cylinders from the Tel Rehov installation were analyzed, as well as the sediments associated with the installation. The organic residues extracted from the unfired clay walls were analyzed using Gas Chromatography (GC) and GC/ Mass Spectrometry (GC/MS) (See supplementary material).

4.3.1.2 Residues extraction method

The extraction and analysis procedures of the lipids from the ceramic vessels followed Evershed et al. (1990) and Charters et al. (1993). All glassware was pre-treated with 1N HCl, fuming nitric acid and then washed with distilled water, and then washed with acetone, followed by chloroform and dried under a heating lamp. Pieces of ceramic were broken off the hives with pliers, cleaned under a stream of clean compressed air, fragmented with a hammer and then ground to a powder in an agate mortar and pestle. About 2 g of the powder was weighed and then split equally into clean glass centrifuge tubes. Duplicate blank samples composed of loess from the Negev heated to 600 °C for 24 h were analysed with each batch of samples. 10 mL of chloroform and methanol (2:1 v:v) were added to each tube and the mixture was sonicated for 15 min. The tubes were centrifuged for 10 minutes at 3500 rpm. The supernatant was removed to another glass centrifuge tube and solvents were evaporated using a CS110 Speed-vac Plus (ThermoSavant). *N,O*-bis(trimethyl)silyltrifluoroacetamide, BSTFA,

(50-150 µL) containing 1% trimethylchlorosilane (TMC) were added to each tube and heated at 60 °C for 1 h. After derivatization, the samples were dried under a gentle stream of nitrogen and 100-150 µL of cyclohexane was added to each tube. 5 µL of each sample were injected into the gas chromatograph (GC) with either flame ionization (FID) or mass selective (MSD) detectors. The amounts were calculated from the average peak area of a series of 7 *n*-alkane standards (nC_{26}-C_{32}) compared with the total peak area of compounds extracted from the unfired clay walls and analyzed by the mass spectrometer.

4.3.1.3 Gas Chromatography (GC)

The GC analysis was carried out using a HP6890 GC equipped with a flame ionization detector (FID) and using a split injection mode with a 1:10 split ratio. When the amount of extracted material was small, a splitless injection mode was used. A 15 m, 0.32 mm ID 5% cross-linked PhMe siloxane capillary column (HP-5) with a 0.25 µm film thickness was used for separation. Helium was used as a carrier gas at a constant flow of 1.1 mL/s. The initial oven temperature was 50 °C and a heating gradient of 10 °C/min was started after 2 min injection. Upon reaching 345 °C, the run was continued for an additional 10 min. The injection temperature was 220 °C and the FID detector temperature was 350 °C. The identification of individual compounds was based on the elution order and comparison to reference standards.

4.3.1.4 Gas Chromatography/ Mass Spectrometry

GC/MS measurements were carried out on another gas chromatograph (HP6890) with a mass-selective detector (HP5973; electron multiplier potential 2 kV, filament current 0.35 mA, electron energy 70 eV, and the spectra were

recorded every 1s over the range m/z 50 to 800). The same capillary column noted above was used, 30 m long. Peak assignments were based on comparisons with library spectra (NIST 1.6), spectra reported in the literature (Tulloch 1970, 1971; Tulloch and Hoffman 1972) and by comparison of retention times of reference standards.

4.3.2. Analytical Results

Analysis of the lipids extracts from two of the eight Iron Age IIA beehives from Tel Rehov detected lipid assemblages that are dominated by long chain *n*-alkanes with 23 to 33 carbon atoms (nC_{23} – nC_{33}) and fatty acids (palmitic acid, $C_{16:0}$, stearic acid, $C_{18:0}$ and lignoceric acid, $C_{24:0}$) (Fig. 5). Extracts from sediments sampled from the vicinity of the vessels showed completely different lipid assemblages (Fig. 6), leading us to conclude that lipids extracted from the beehive walls indeed reflect the contents of the hives and have not been contaminated by the associated surroundings.

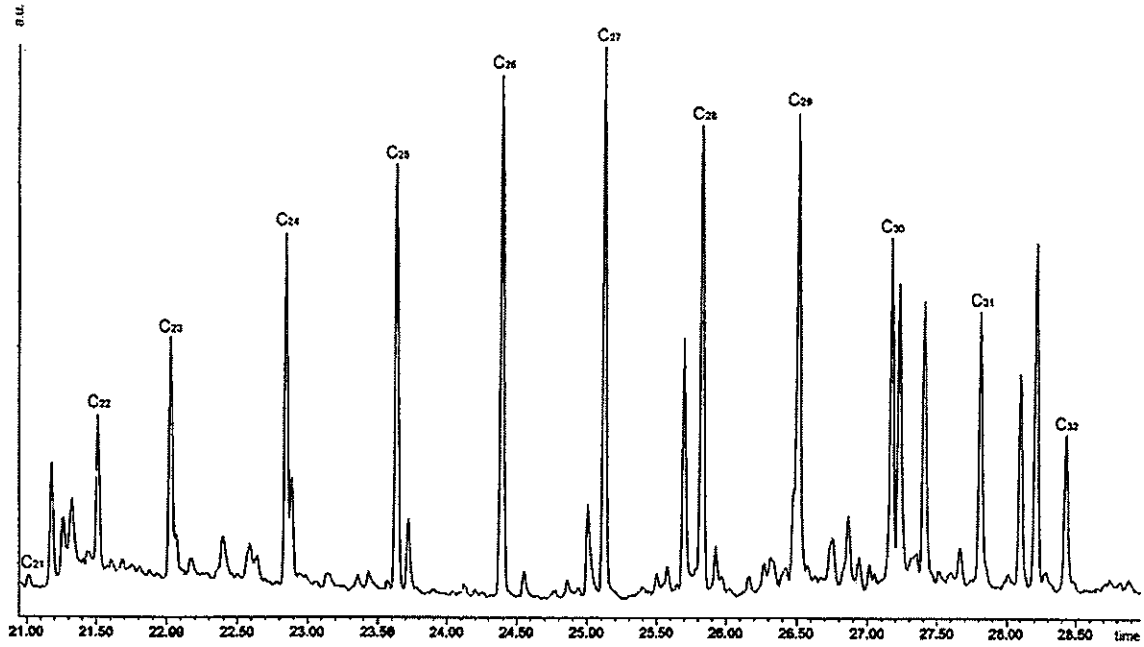


Figure 5 Gas chromatogram of the organic extracts from the installation walls found at Tel Rehov. C_X refers to *n*-alkane with x carbons in its chain; $C_{X:0}$ refers to fatty acid with x carbons in its chain and 0 degree of unsaturation.

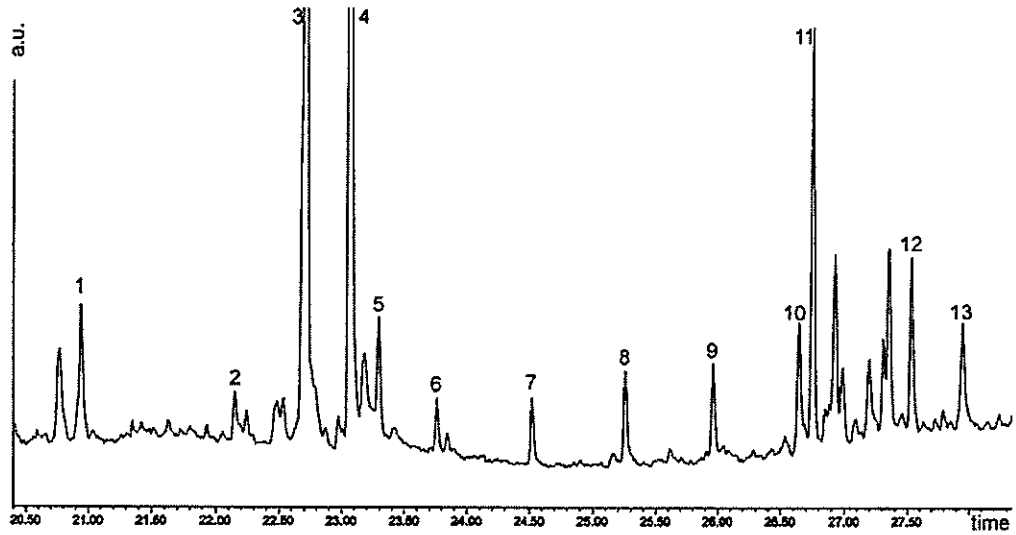


Figure 6 Gas chromatogram of the organic extracts from the sediments from the installation surroundings. C_X refers to *n*-alkane with x carbons in its chain; $C_{X:0}$ refers to fatty acid with x carbons in its chain and 0 degree of unsaturation.

The lipid extracts from the beehive show a clear predominance of *n*-C_{27} amongst the odd numbered alkanes (ONAs) and that the relative proportions between all ONAs normalized to the total peak area are similar. This is not the case for the even numbered n-alkanes (ENAs). The relative abundance of the ENAs is more varied (Fig. 7).

Based on ethnographic similarities mentioned above, this installation is believed to have functioned as a beehive and thus is expected to contain beeswax residues. As already mentioned in the previous chapters, it is well established that modern beeswax contains 3 major groups of compounds: (i) fatty acids, $C_{16:0}$, $C_{18:0}$ and $C_{24:0}$; the latter serves as a biomarker; (ii) ONA (C_{21} – C_{33}) and (iii) wax esters, mostly of palmitic acid in the range of C_{40} to C_{52} (Tulloch 1970). The presence of ONAs dominated by *n*-C_{27} and the significant presence of $C_{16:0}$, $C_{18:0}$, $C_{24:0}$ fatty acids in the Tel Rehov beehive extracts are all consistent with the identification of beeswax in the installation. The varied amounts of palmitic acid that are present may be due to the hydrolytic breakdown of wax esters known to be present in beeswax (Tulloch 1971). Although wax ester hydrolysis should have produced odd numbered *n*-alcohols in the range of 24 to 36, no alcohols were detected in the beehive extract. The hives at Tel Rehov were covered by about one meter thick debris of destruction layer that was severely burnt. Due to that, the hives could have been exposed to high temperatures which would cause both the breakdown of wax esters and the evaporation of long chain alcohols (Namdar et al., submitted).

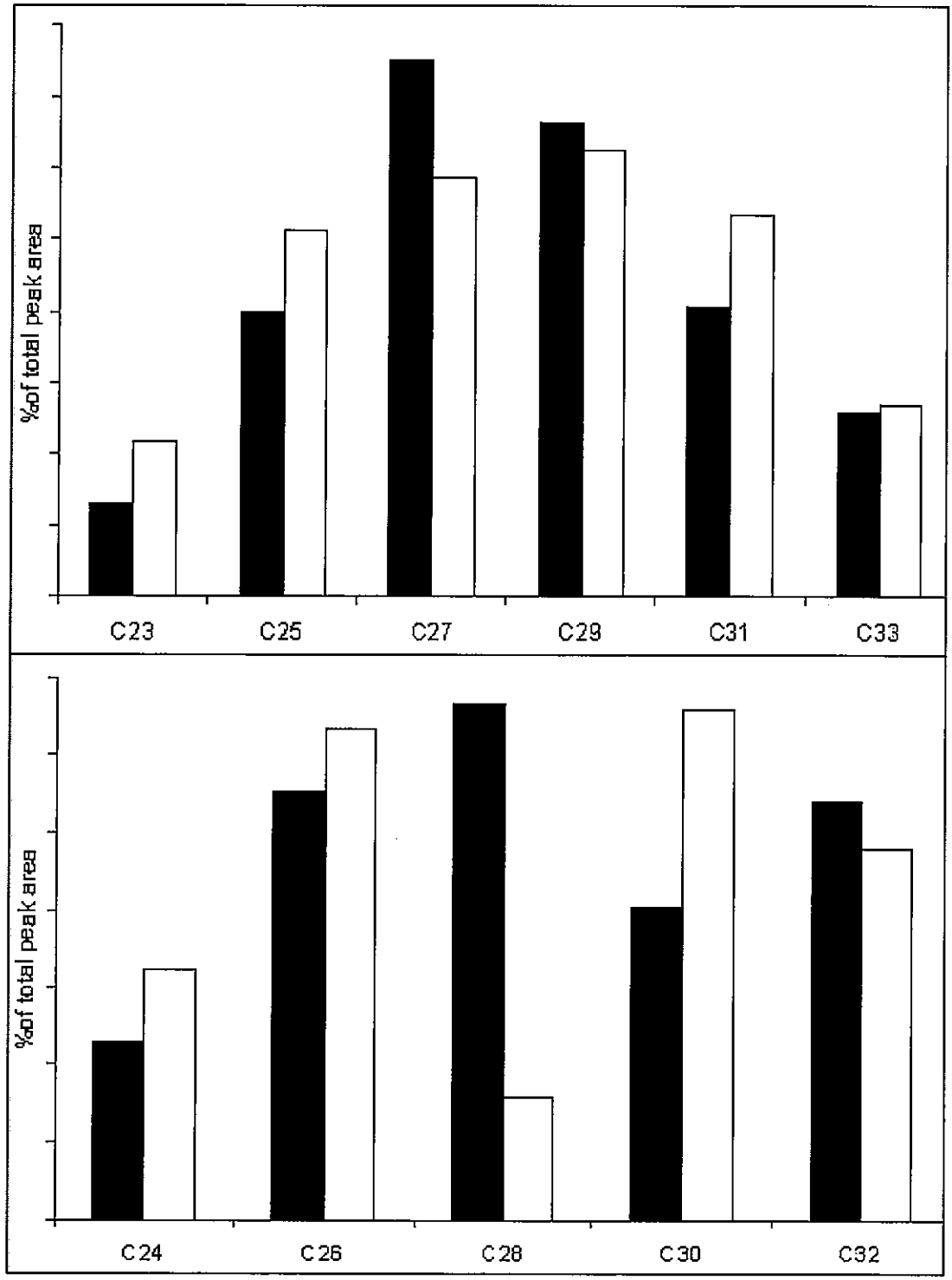


Figure 7 Histograms of the peak area of **a,** even numbered and **b,** odd numbered *n*-alkanes extracted from the walls of two cylinders of the installation found at Tel Rehov. The relative peak areas are normalized to the total peak area of alkanes present in the extract. C_X refers to *n*-alkane with x carbons in its chain.

The most stable components of beeswax, the ones that are predicted to survive through time, are the *n*-alkanes. The main difference between the ancient beehive extracts and modern beeswax regarding the alkane composition is that the latter is known to contain large amounts of ONAs and only negligible amounts of ENAs (Evershed et al. 2003; Kolattukudy 1969; Tulloch 1970). Since we found significant amounts of ENAs in the extracts of the cylinders, we investigated modern beeswax and found an enrichment of ENAs compared to ONAs in dark colored beeswax. This is attributed to the accumulation of bees' remains during the occupancy of the hive. As mentioned in the previous chapters, the coatings of the bees' cuticles are rich in ONAs and ENAs (Salvy et al., 2001), and these could contribute *n*-alkanes to the beeswax (Namdar et al, accepted for publication). Namdar et al (submitted) also showed that another change in the *n*-alkane composition of beeswax occurs when modern beeswax from *Apis mellifera* is placed on a clean ceramic shred and heated to 400 °C. The relative proportions of ENAs compared to ONAs dramatically increases. In fact the proportions of ENAs and ONAs are a similar to those analyzed in the cylindrical clay containers from Tel Rehov.

4.4. Discussion

Beehives are one of the lesser known subjects in the archaeological record. Perhaps due to their fragility and location outside settlements, no actual remains of beehives are known, to the best of our knowledge, in the entire ancient Near East prior to the Hellenistic period. Here we present the only beehives excavated so far in the ancient Near East. They were found at Tel Rehov in a thriving urban center of the 10-9th centuries BCE, in a context of a large building that was severely burnt most probably at the end of the 10th century BCE. Finds in the vicinity of the

beehives point to cultic activity perhaps related to the honey and beeswax production. In Egypt, honey was used for various medical treatments, and thus beehives were considered important and were under state supervision. The function of honey in ancient Israel is less well known, since the Biblical references mentioning honey are unclear: many of them perhaps refer to honey made of fruits such as dates and figs, and the Bible or any other written document do not mention beekeeping. Only, two literary stories mention wild honey combs (Judges 14:8; and possibly I Samuel 14: 25-29). The Tel Rehov beehives are thus unique in their demonstration of the importance of honey and beeswax production in the local economy and perhaps in ritual and medicine as well.

The presence of beehives in Tel Rehov raises the inevitable question: why and for what purpose were beehives located in a closed structure in the middle of a densely built city? The importance of the production of honey and beeswax in this complex is demonstrated by the discovery in the same space as the beehives of two outstanding cult objects: a decorated four horned pottery altar and an elaborately painted large chalice. The latter was covered by a mottled rust-like coating, similar to the rust-like coating found on the inside of the hives. These were discovered just to the south-west of the hives, together with several pottery chalices to the west of the hives. These objects could have been used in cultic activities related to the manufacture of honey and beeswax. Such a relationship between cult and industry is known in various cases, such as the affinity between metal production and cult in the Late Bronze Age copper production centres at Timna' valley (Rothenberg 1983) and Cyprus (i.e. Karageorghis 1973; Dothan and Ben-Tor 1983; Artzy 2000), as well as the 7th century BCE olive oil industry at Tel Miqne - Ekron (Gitin 1989). In ancient Egypt, beeswax was thought to possess magical powers, and this could also have been the case in ancient Israel. Such

attributes may explain the rather surprising location of the hives inside the city and so close to built structures. It might be that the beehives were part of a larger complex which had a specialized economic and perhaps religious function. Part of this complex was a granary structure (Building G) attached to Building H on the west.

The period to which this beehive is related to, the second half of the 10th century BCE, corresponds with the Solomonic era, the invasion of Shoshenq I and the split of Israel into two states: southern (Judah) and northern (Israel). Rehov was a flourishing city during that time and the beehives are just one aspect of the economic and religious practices in this city. The results of archaeological, pictorial, ethnographic and scientific analysis are integrated in this study to compose a lively picture that extends a new meaning to the Biblical definition of the Land of Israel as a "Land of Milk and Honey". Though the Bible does not mention apiaries, the example from Tel Rehov shows that they were known at that time and functioned very much like those from Egypt that are dated much earlier, as well as like the much later beehives known from the ethnographic record.

Amihai Mazar, Dvory Namdar , Nava Panitz-Cohen, Ronny Neumann and Steve Weiner. The Iron Age Beehives at Tel Rehov in the Jordan Valley: Archaeological and Analytical Aspect. Submitted for publication in *Antiquity*.

5 Conclusions and Prospect for the Future

Analyzing numerous ceramic items from different sites in the Levant and also Europe, with different shapes and related uses, made me understand both the difficulties and the potential that residue analysis is concealing.

With some of these difficulties we dealt during this work, i.e. limiting down the number of samples and the amount of each sample needed for reliable and reproducible results; the differentialpreservation of organic material in samples coming from different sites and from different localities within one site; the changes that the raw material might go through during its use in antiquity or while deposited; and the extent to which burial contamination affect the adsorbed organic residues.

In this thesis we demonstrated that the existing residue analysis techniques previously applied elsewhere, can also be applied systematically in the area around the Dead Sea where preservational conditions for organic matter are relatively good. In this area we clearly show that different vessel types have different assemblages of residues and that these most probably reflect the contents of the vessels when in use. This study therefore shows that systematic residue analysis investigations can be undertaken in this area of the southern Levant, where there are many archaeological sites dating back to the beginning of pottery production.

Indeed, our work showed that in general it is correct to say that preservation of organic substances in other areas outside the Dead Sea region is poorer. But we believe that the ability to identify organic residues in other areas in Israel still remains. Even within a particular site, the preservational conditions can vary from

one location to another depending upon the sediment type, the local hydrological regime, the amount of oxygen available and probably many other factors. For example, Weiner and Bar-Yosef (1990) noted that collagen in bones was better preserved in localities where the clay component of the sediments was high. It is well known that the presence of clay reduces the flow of water through the sediments and hence the rate of degradation of organic matter. We thus do not conclude that residue analysis will not prove to be useful in areas outside the Dead Sea area, but applying it in a systematic manner will require a different sampling methodology than the one which was regularly used. In order to alleviate this problem it could be useful to use independent screening methods for identifying areas where preservational conditions may be better, before starting the residue analysis of ceramic fragments.

Thus, understanding the full potential of residue analysis to the archaeological research is still left to be explored. We demonstrated the potential of solving long debated issue that could not be resolved in any other archaeological mean. We also demonstrated the need of independent proof when archaeology and ethnography seem to have a solid indication of the possible use of an item or installation. We showed that relying on known chemical composition of natural substances is not enough for this complicated matter of identification of ancient exploitation of raw materials. On top of that, we note that understanding the chemical and physical relations between the ceramic matrix and the adsorbed organic material is a highly reed knowledge which we only began exploring in this work.

Furthermore, the application of residue analysis for the identification of specific vegetable oils, cereals, wine, beer, floral-based drugs, dairy products and the use of fish remains a long term objective. Their diagnostic lipids are less stable than

the components we detected in this work and therefore, further progress will require use of other methods and techniques, such as high performance liquid chromatography with mass selective detection and proteomics.

The archaeological questions that residue analysis can help resolve are too many to count. Providing the right controls and screening methods it might help placing the archaeological research in Israel at the cutting-edge of this promising and continuously progressing field.

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אוניברסיטת תל-אביב TEL AVIV UNIVERSITY
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בית הספר למדעי היהדות ע"ש חיים רוזנברג
החוג לארכיאולוגיה ותרבויות המזרח הקדום

שחזור הכלכלה בתקופה הכלקוליתית בארץ ישראל באמצעות ניתוח מצבי שימור ותהליכי בליה של חומרים אורגאניים ספוחים בכלי חרס עתיקים

חיבור לשם קבלת תואר דוקטור לפילוסופיה

מאת: דבורה נמדר

מנחים: **פרופ' יובל גורן**

פרופ' סטיב ויינר (מכון וייצמן למדע)

פרופ' רוני נוימן (מכון וייצמן למדע)

הוגש לסנאט של אוניברסיטת תל אביב

יוני 2007

אוניברסיטת תל-אביב TEL AVIV UNIVERSITY
הפקולטה למדעי הרוח ע"ש לסטר וסאלי אנטין
בית הספר למדעי היהדות ע"ש חיים רוזנברג
החוג לארכיאולוגיה ותרבויות המזרח הקדום

שחזור הכלכלה בתקופה הכלקוליתית בארץ ישראל באמצעות ניתוח מצבי שימור ותהליכי בליה של חומרים אורגאניים ספוחים בכלי חרס עתיקים

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יוני 2007

תקציר

מטרתה של עבודה זו היא לבחון את האפשרות לניתוח שרידים אורגניים שנספחו לכלי חרס ככלי לפתרון שאלות בארכיאולוגיה של ארץ ישראל. זאת באמצעות ניתוח מקרה מבחן עיקרי, שאלת השימוש בבזיכים (גביעי קרן) מן התקופה הכלקוליתית בדרום הלבאנט, ומקרה מבחן משני הנובע ממנו. הבזיכים הם כלים קוניים המהווים "מאובן מנחה" לתרבות העיסולית בתקופה הכלקוליתית (4700-3700 לפנה"ס). בישראל ובירדן. זהות תכולתם והשימוש שנעשה בהם בתקופה זו אינם ידועים. הדעה הרווחת בנוגע לשימושם טענה כי הם שימשו להכיל נוזלים והיו ככל הנראה חלק מכלי פולחן טקסי. פתרון סוגיה זו ישמש כאבן בוחן ליכולת למימוש שיטת מיצוי וזיהוי חומר אורגני ספוח באזורנו.

מניתוח השרידים האורגאניים שנספחו לדפנות החרס של הבזיכים שנתגלו באתרים ארכיאולוגיים שונים עולה למסקנה שונה מזו שהוצעה לעיל. הבדיקות נעשו באמצעות שיטות הפרדה כימיות (הפרדה גזית וספקטרומטריית מאסות) לניתוח הרכב התכולה של הבזיכים שהתגלו בחמישה אתרים ארכיאולוגיים שונים המייצגים סוגי פעילויות נבדלות – אתר מגורים, מערת מגורים ומבנה פולחני. תוצאות הבדיקות הצביעו על כך שכל הבזיכים שנבדקו הכילו את אותו הרכב מולקולות אורגניות ספוחות. תכולה זו שונה מהתכולה האורגאנית שהתגלתה בטיפוסי כלי חרס אחרים שהתגלו באותם אתרים עצמם, כמו גם מהרכב המולקולות האורגניות שנתגלו בקרקע מסביבת הקבורה של הכלים.

מתוצאות העבודה עולה כי הבזיכים הכילו בעבר שעוות דבורים. שעוות דבורים מורכבת מחומצות שומן, אלקאנים רוויים אי זוגיים ומולקולות אסטריות. המרכיב היציב ביותר מבחינה כימית בהרכב מולקולארי זה, ולפיכך זה שלו הסיכוי הגבוה ביותר לשרוד שנים רבות ללא שינוי כימי, הם האלקאנים ולפיכך מרבית המחקר התמקדה בהם . באמצעות שיטות הפרדה גזית שבוצעו בשתים עשרה כוורות פרא מודרניות מן הארץ ומירדן, התברר כי עם הזדקנותה והתכהות צבעה משתנה גם הרכבה הכימי של השעווה. כמות האלקאנים בעלי שרשראות הפחמנים הזוגיות (C_{22}-C_{32}) עולה בצורה משמעותית עם התכהות השעווה. יש לציין כי בחקר ההרכב הכימי של שעוות דבורים שנעשה עד עתה, נבחנו רק שעוות בהירות ונקיות ולפיכך מקובל היה לחשוב כי הרכב שעוות דבורים אינו מכיל אלקאנים זוגיים. מחקרי משנה תפיסה זו, כיון שמתברר כי שינוי ההרכב הכימי הנדון נובע מהצטברות שברים של חלקי גוף מתים (קוטיקולות) המופיעים בתאים הכהים של השעוות השונות. ידוע כי קוטיקולות דבורים

מכוסות שכבה שעונית המכילה אלקאנים זוגיים ואי זוגיים בטווח של 23-32 פחמנים בשרשרת ואלה מהוות את מקורן בחומר הנבדק. יתרה מזאת, מן הניסויים שנערכו במסגרת עבודה זו עולה כי המולקולות האורגאניות שמוצו מדפנות הבזיכים הן תוצר של פעולות חימום של שעוות דבורים על גבי החרס. הרכב האלקאנים הזוגיים והאי-זוגיים שהתגלו בבזיכים זהה כמעט לחלוטין לזה שהתגלה במיצוי של שבר חרס נקי שעליו חוממו שעוות פרא מודרניות. לפיכך, הבזיכים הכילו שעוות מחוממות ושימשו ככל הנראה למאור, תפיסה שמתאימה גם לפיזור האתרים הרחב המשויכים לתרבות העיסולית, בהם התגלו הבזיכים (איציק גלעד, תקשורת אישית). הנוכחות של השעווה בבזיכים תורמת להבנתנו את התקופה הכלקוליתית, תקופה המאופיינת בשימוש בחומרים משניים מן הטבע כדוגמת חלב, שמן הזית ומוצרי תסיסה של ענבים שבוצע ככל הנראה לראשונה.

תוצאות המחקר של מקרה המבחן מתחזקות על סמך מקור נוסף. כמקרה מבחן משני שמשו שמונה מיכלים צילינדריים מן המאה העשירית לפנה"ס שהתגלו בחפירות תל רחוב שבעמק הירדן התיכון בחפירותיו של פרופ' עמיחי מזר ובניהולה בשטח של נאוה פניץ-כהן. המיכלים התגלו כשהם שוכבים זה לצד זה ומכוסים בשכבה עבה של עפר שנשרף בטמפרטורות גבוהות. מיכלים אלו זוהו ככוורות דבורים על סמך תיאורים וציורים מצריים עתיקים, מקורות ספרותיים קלאסיים והתאמות אתנוגרפיות מודרניות. אלו הן הכוורות היחידות שנתגלו עד כה במזרח הקדום ומתוארכות לתקופות קדם- קלאסיות. אנליזות של ההרכב הכימי של החומרים האורגאניים שנספחו לדופנותיהן באזורים הבלתי צרופים , שנעשו באותן שיטות שיושמו על הבזיכים הכלקוליתיים, גילו שההרכב האורגאני מכיל חומצות שומן ואלקאנים זוגיים ואי זוגיים בעלי 23-32 פחמנים בשרשרת. נוכחות הרכב אלקאנים זה, שמשקף כאמור שעוות דבורים שרופה, מתיישב יפה עם זיהוי המכלים ככוורות דבורים ומאשר את המסקנה בנוגע לתכולתם של הבזיכים הכלקוליתיים.

מחקר זה מייצג נסיון ראשון מסוגו לבדיקה בכלים אנליטיים של שרידים אורגנים שנספחו לדפנות כלים מן הלבאנט לשם הגדרת השימוש האפשרי שנעשה בהם בזמן העתיק. מתוצאותיהן של עבודות קודמות הוצע כי שימורן של מולקולות אורגאניות באזורנו הוא גרוע בדרך כלל ולכן הוכחת היכולת המדעית לבצע מחקר שכזה מהווה כלי חשוב למחקר הארכיאולוגי ופתח לשימושים רבים נוספים בו בעתיד.