First Evidence for Vanillin in the Old World: Its Use as Mortuary Offering in Middle Bronze Canaan

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Abstract

Four small ceramic juglets that had been used as containers for offerings in an elite Middle Bronze Age III (ca. 1650-1550 BCE) masonry tomb uncovered at Tel Megiddo in the Jezreel Valley, Israel were tested using organic residue analysis. Notably, residues of vanillin, 4-hydroxybenzaldehyde, and acetonvanillone were identified in three of the four juglets examined. These are the major fragrance and flavour components of natural vanilla extract. To date, it has been commonly accepted that vanilla was domesticated in the New World and subsequently spread to other parts of the globe. Our research first ruled out all possibility of sample contamination and then conducted a post-organic residue analysis investigation of various species within the plant kingdom from which these principle compounds could have been exploited. The results shed new light on the first known exploitation of vanilla in an Old World context, including local uses, the significance and employment in mortuary practices, and possible implications for understanding trade networks in the ancient Near East during the second millennium BCE.

Keywords: Organic Residue Analysis, Gas Chromatography- Mass Spectrometry, Middle Bronze Age III, Masonry Tomb, Vanillin, 4-hydroxybenzaldehyde, acetonvanillone, Juglet.

1. Introduction

Vanillin (4-hydroxy-3-methoxy-benzaldehyde) is a significant aromatic and flavour compound that is found naturally and abundantly within the pods of the vanilla orchid plant (Radulovic et al., 2010). Vanillin is the major component of natural vanilla, which is one of the most commonly used and important flavouring materials worldwide (Walton et al., 2003: 505). The earliest documented exploitation of domesticated vanilla is known from the Aztecs of Mexico, who cultivated one particular vanilla orchid species (among 110 different vanilla orchid species), *V. Planifolia* Andrews, that was gathered and grown for its aromatic properties as well as a flavouring for coffee and chocolate (Walton et al., 2003: 505; Bythrow, 2005: 129). The Spanish colonisers brought this particular vanilla species to Europe after 1520 CE. Today, it is hand-cultivated in a number of tropical countries (Fouché and Jouve, 1999: 690). Vanillin possesses an important chemical property, allowing it to retain strong antimicrobial properties that counter against a number of yeast and mould strains. Therefore, it has the ability to destroy fungi and prevent bacteria from multiplying and thus acts as a preservative (López-Malo et al., 1995; Fitzgerald et al., 2004).

Using the method of organic residue analysis (ORA), we found significant amounts of 4-hydroxy-3-methoxy-benzaldehyde (vanillin), 4-hydroxybenzaldehyde, as well as lower concentrations of acetonvanillone in three small pottery containers excavated at Tel Megiddo (Israel) that were placed in an elite intramural tomb dating to the Middle Bronze III. These chemical compounds are the principle components of natural vanilla. This finding is the first archaeological evidence for the exploitation of vanilla in the ancient Old World and most probably worldwide, as early as *ca.* 1650-1550 BCE. The most likely source of these compounds is from vanilla orchid species endemic to a variety of tropical regions across the globe that are no longer widely exploited today. This discovery is also noteworthy as it informs on international trade networks that operated in the Old World during the middle of the second millennium BCE.

1.1. Tel Megiddo

Tel Megiddo is a Bronze and Iron Age site, located in the Jezreel Valley, Israel (Fig. 1; see supplementary Fig. S1 online). It is strategically situated on the international road connecting Egypt with Syria and Mesopotamia. In the second millennium BCE Megiddo served as the hub of a Canaanite city-state that controlled the western sector of the Jezreel Valley. Remains of Middle Bronze palaces, a temple and fortifications were unearthed at the site in past and present excavations (Ussishkin, 2018: 171-199). The elite who ruled from Megiddo were part of the international trade networks in the 2nd millennium BCE.

Figure 1: Aerial photo of Tel Megiddo showing Area H, facing south (courtesy of the Megiddo Expedition).

Area H of the Tel Aviv University-led excavations is located immediately to the west of the late Middle Bronze and Late Bronze palace complexes. It features a stratigraphic sequence ranging from the Iron IIB-C (Level H-1) to the MB III (Level H-16) (Finkelstein, Ussishkin and Cline, 2013). During the 2016 excavation season, an undisturbed, monumental, masonry-constructed chamber tomb (Fig. 2; Tomb 16/H/50; hereafter Tomb 50) dated by its ceramic contents to the Middle Bronze III period (*ca.* 1650-1550 BCE) was uncovered. The tomb, accessed by a dromos, is associated with Level H-16. This type of tomb is known from Middle and Late Bronze sites in the Levant (Gonen, 1992: 41; Ilan, 2002; Cradic, 2017). Three primary inhumations were found in the tomb, consisting of an adult male, adult female and sub-adult, who were lying beside at least six secondary inhumations, together with a luxurious assemblage of elite grave goods, which included gold, silver, bronze and bone items as well as an assemblage of ceramic vessels.

Figure 2: Tomb 50 entrance of interior, facing northwest (courtesy of the Megiddo Expedition).

1.2. Materials: Ceramic Assemblage

The ceramic assemblage of Tomb 50 includes 26 vessels, two of which were imported from Cyprus: A Middle Cypriot II Red-on-Red decorated bowl and a large globular Middle Cypriot III White Painted jug. The rest of the ceramic assemblage appears to be local. Twenty-three of the vessels were sampled for ORA but this research focuses on the ORA results of only the subset of four locally produced juglets (Fig. 3). The juglets were specifically sampled on the assumption that the preservation of residues in small, semi-closed containers would presumably be more abundant, and less exposed to exogenous contamination. The juglets were sampled from the base or near the base of the vessel and stored in paper bags in a controlled environment prior to sampling. Table S1 (found in supplementary online information) lists the vessels, and describes how they were handled during and after the excavation.

Figure 3: Photo and drawing of dipper Juglet TM-056.D, Locus 16/H/62, vessel number 14, pottery basket 18. This Juglet represents the same juglet type as juglets TM-116.D and TM-063.D as listed in Table 1. Juglet TM-140.D is not of the same type (courtesy of the Megiddo Expedition).

2. Results

Overview of Biomarker Analysis. The compounds identified in the four juglets analyzed by ORA are presented in Table 1. The organic content that was detected by gas chromatography revealed vanillin (4-hydroxy-3-methoxy-benzaldehyde), 4-hydroxybenzaldehyde and acetonvanillone in three of the four juglets (TM-063.D, TM-116.D, TM-056.D) (Fig. 4a, see supplementary Fig. S2a and S3a online) recovered from two different loci, in significant quantities (9-27 μg vanillin per gram ceramic, along with smaller amounts of 4-hydroxybenzaldehyde and acetonvanillone). None of the sediment controls analyzed for each vessel contained either vanillin, 4-hydroxybenzaldehyde, or acetonvanillone (Fig. 4b, see supplementary Fig. S2b and S3b online). Other compounds detected in the juglet extracts were monoacylglycerides (MAG), fatty acids - palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$), and stearic acid ($C_{18:0}$), as well as long chain primary alcohols 1-hexadexanol (C_{16ol}) and 1-ocatdecanol (C_{18ol}), along with B-sitosterol, and cholesterol. These compounds are present in both animal fats and plant oils (Copley et al., 2005; Baeten et al., 2013; Debono Spiteri et al., 2016). The P/S ratios ($C_{16:0}/C_{18:0}$; 4:1) within the samples along with B-sitosterol are characteristic of the presence of plant oil (Copley et al., 2005), while the presence of cholesterol indicates an animal/human fat origin (Copley et al., 2005; Baeten et al., 2013).

A vanillin standard compound was tested under the same analytical conditions as the archaeological samples, and confirms the presence of vanillin, therefore ruling out the possible presence of related compounds e.g. methylvanillin or ethylvanillin (Fig. 5).

Table 1: Molecular components in the total lipid extracts of the juglets

Lab no.	Major components	Fatty acids	AGs	Alcohols	Others	Quantification µg/g vanillin
TM-063.D crushed	4-hydroxybenzaldehyde Vanillin Acetovanillone	$C_{16:0}$	MAG_{16} MAG_{18}	C_{18ol}	Cholesterol	27 µg
TM-116.D crushed	4-hydroxybenzaldehyde Vanillin Acetovanillone	$C_{16:0}$, $C_{18:1}$ $C_{18:0}$,	MAG_{16} MAG_{18}	C_{16ol}, C_{18ol}	B-sitosterol	10.5 µg
TM-056.D Interior	4-hydroxybenzaldehyde Vanillin acetonvanillone	$C_{16:0}$, $C_{18:0}$	MAG_{16} MAG_{18}	C_{16ol}, C_{18ol}	cholesterol B-sitosterol	9 µg
TM-140.D Interior	None	$C_{16:0}$, $C_{18:0}$	MAG_{16} MAG_{18}	C_{16ol}, C_{18ol}	alpha tocopherol acetate cholesterol,	–

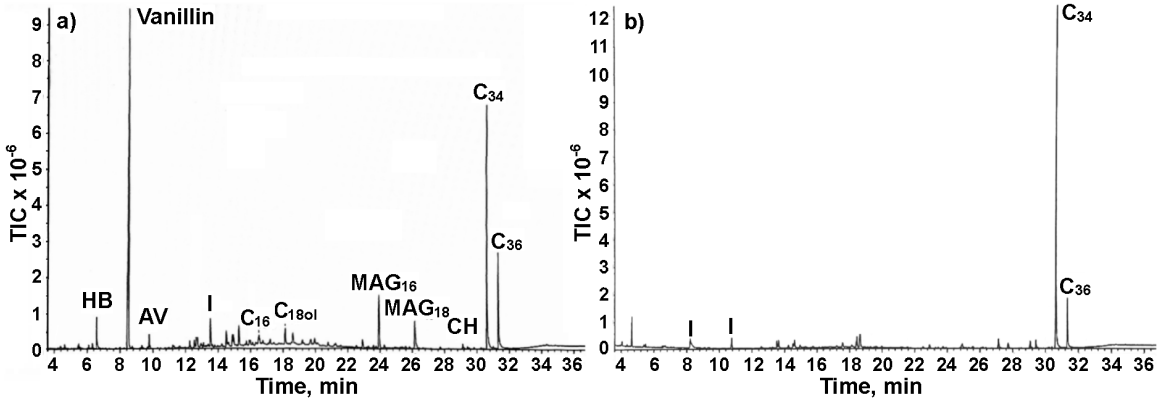

Figure 4: Total ion chromatogram of the lipid extracts analysed after trimethylsilylation of a) Juglet TM-063.D containing 4-hydroxybenzaldehyde, vanillin and acetonvanillone. b) sediment control for Juglet TM-063.D showing the absence of vanillin and other compounds. The chromatograms for the other juglets and sediment controls can be found in the supplementary material. (HB - 4-hydroxybenzaldehyde; AV - acetonvanillone; C_{16} - palmitic acid; C_{18ol} - 1-octadecanol; MAG_{16} – 1-palmitoyl-glycerol; MAG_{18} – 1-stearoyl-glycerol; CH - cholesterol; I – Impurity; Internal Standards: C_{34} -tetratriacontane and C_{36} hexatriacontane. Peaks not annotated are unidentified).

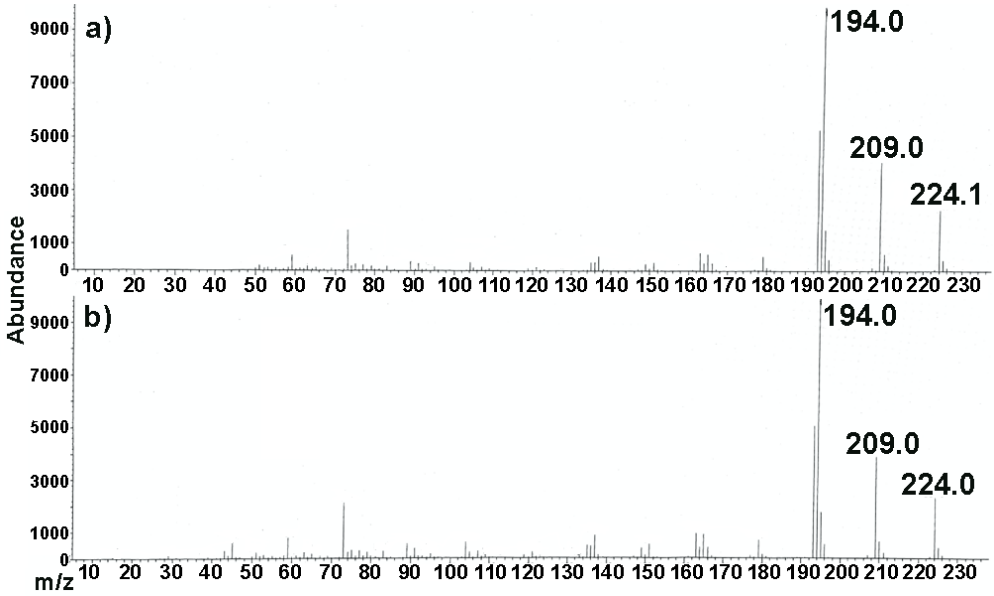

Figure 5: Mass spectra showing the fragmentation of a) silylated vanillin identified in the archaeological extract of Juglet TM-063.D b) silylated vanillin standard. The mass spectra show that both the standard silylated vanillin and the silylated vanillin detected within the archaeological extract have the same retention time (8.501 min), and the same molecular ion and fragmentation pattern in the mass spectrum.

3. Discussion

Vanillin, 4-hydroxybenzaldehyde, and acetonvanillone were extracted from three of the four juglets analyzed. A strong emphasis was placed on eliminating all possibilities of contamination. First, these compounds were nonexistent in all of the sediment controls and laboratory blanks. Therefore, the possibility of modern contamination can be ruled out. Second, vanillin, 4-hydroxybenzaldehyde, and acetonvanillone were not found in the extract of juglet TM-140 D. (found in the same locus of two of the juglets TM-63D. and TM-116 D.; see supplementary Fig. S4 online) thus, cross contamination from the burial context and/or during excavation is unlikely. Third, the three juglets that contained vanillin, 4-hydroxybenzaldehyde, and acetonvanillone came from two different loci and are of the same juglet type. In light of the above, it can be confirmed that the compounds identified, more specifically, vanillin, 4-hydroxybenzaldehyde, and acetonvanillone were present in the three juglets in antiquity and were not likely to have been introduced during deposition, excavation or post-excavation activities. Thus, these compounds genuinely reflect the past commodity stored in the juglets. Both vanillin and 4-hydroxybenzaldehyde are major components of natural vanilla extract (Huesgen, 2011). Acetonvanillone is a minor compound of vanilla, which however contains high intensity vanilla-like sensory notes (Perez-Silva, 2006). A detailed search for other major aromatic constituents of vanilla beans, namely vanillic acid, p-hydroxybenzoic acid, p-hydroxy benzyl methyl ether and acetic acid (Ranadive, 2006), was carried out, however none of these compounds were present. Thus, our results reveal the existence of natural, albeit degraded and only partially preserved vanilla essence. The other compounds identified in the juglets point to the presence of plant oil, perhaps constituents of a single product of which vanilla was an ingredient, although this is difficult to confirm due to the possibility of vessel reutilization.

3.1. Properties of Vanillin

Vanillin is an aromatic aldehyde that oxidizes slowly on exposure to moist air to the corresponding carboxylic acid. It is susceptible to photo degradation in air but rather stable to hydrolysis in water. Vanillin is readily biodegradable under aerobic and anaerobic conditions. Soil invertebrates can also metabolize vanillin (O'Neil, 2013: 1843). However, the burial and the basic nature of the ceramic fabric (calcite) tend to stabilize these compounds, allowing for the vanillin to be preserved. Furthermore, the absence of oxidizing conditions due to the fact that the tomb was sealed and the vanillin was found in semi-closed containers that would have protected it from these factors, would have favoured the preservation of vanillin. As noted above vanillin exhibits strong antimicrobial properties, which actively work against a number of yeast and mould strains (López-Malo et al., 1995; Fitzgerald et al., 2004), thus its ability to preserve and to be used as a possible preservative. The pleasant aromatic vanilla odour and sweet taste (Lewis, 2004: 3661; 2007: 1313; O'Neil 2013: 1843) make vanillin a desirable additive, as a flavouring agent in food and as a fragrance, thus increasing its possible medicinal and preservative benefits. Additionally, its properties as a fragrance combined with its antifungal and antibiotic properties make it ideal for use in the embalming of the dead.

3.2. Possible Source of Vanillin and Related Compounds

Vanillin is a minor component found in many plants as a decomposition product of woody tissue (all terrestrial plants), as a result of bioconversion of lignin, ferulic acid, eugenol, and isoeugenol by microorganisms such as yeasts, fungi, and bacteria as production hosts (Randulovic et al., 2010; Lesage-Meessen et al., 1996; Hansen et al., 2009; Di Gioia et al., 2011). It is also found in trace amounts in ground ivy (*Glechoma hederacea*) from Eurasia. A study of volatile constituents released from ground ivy, belonging to the Lamiaceae family, showed that leaves of this plant release trace amounts of vanillin (Radulovic et al., 2010; Gallage et al., 2014: 11). Trace amounts of vanillin are also present in plants as a moiety bound to sugar as a glycoside, and it has been reported to be present in lesser quantities in several essential oils (Duru et al., 2002; Modugno et al., 2006). In the Megiddo juglets, the presence of vanillin, 4-hydroxybenzaldehyde, and acetonvanillone deriving from the decomposition product of lignin is highly unlikely. Lignin upon decomposition leaves over 50 biomarkers, many of which should be observable under similar analytical conditions (Faix et al., 1990).

Slightly more plentiful sources of vanillin are found in aromatic and balsamic resins, such as benzoe and storax resins from Turkey and the East Aegean Islands (*Styrax* and *Liquidambar* genus, species *L. Orientalis* Mill.; Duru et al., 2002; Modugno et al., 2006). According to Modugno et al. (2006), vanillin and 4-hydroxybenzaldehyde appear in minor amounts in Benzoe resin, a product of *Styrax* spp. (*Styraceae* family). In antiquity resin obtained from *Liquidambar orientalis (Hammamelidiacea),* was grown in Turkey. The composition of Benzoe and Storax was studied by chromatography and showed that although vanillin and 4-hydroxybenzaldehyde are characteristic to Benzoe resin, the major components of Benzoe and also Storax are cinnamyl cinnamate and 3-phenylpropanyl cinnamate, with significant amounts of free benzoic and cinnamic acids, 3-phenylpropanol and cinnamyl alcohols (Mondugno et al., 2006: Figure 1). These compounds were all absent in the lipid extracts of the juglets analyzed in the present study. With these major compounds missing, it can be deduced that vanillin and 4-hydroxybenzaldehyde in the juglets

studied here do not originate from the aromatic resin of Benzoe and or Storax. This is furthered supported by the presence of acetonvanillone in the juglets from Megiddo, which is not found in Benzoe or Storax (Mondugno et al. 2006).

The highest natural concentrations of vanillin, typically 1-3 % and sometimes higher, are found in the properly cured pods of the vanilla bean (Hocking, 1997: 1055; Gallage et al., 2014:1). This abundant amount of vanillin within the vanilla pods is consistent with the large amount of vanillin found within the archaeological samples. Gobley (1858) was the first to isolate and identify vanillin as a component of vanilla bean and to state that vanillin is the principal flavour component (Gobley, 1858: 247; Walton et al., 2003: 505; Sinha et al., 2008; Sharp et al., 2012). Natural vanilla extract contains approximately 200 different compounds (Huesgen, 2011). The most abundant compounds are vanillin, 4-hydroxybenzaldehyde, vanillic acid, and 4-hydroxybenzoic acid (Huesgen, 2011: Figure 1). Although the aromatic acids and esters of vanillic acid and 4-hydroxybenzoic acid were not identified in the Megiddo juglets, the two major components of natural vanilla extract (vanillin and 4-hydroxybenzaldehyde) were detected in significant amounts, along with minor compound acetonvanillone. Acetonvanillone contains high intensity vanilla-sensory notes as important as vanillin (Perez-Silva, 2006). Therefore, it can be postulated that the most probable source of vanillin, 4-hydroxybenzaldehyde, and acetonvanillone found within the juglets is the vanilla orchid.

3.3. Botanical Origins of Vanilla

Vanilla is a genus of orchids belonging to the *Vanilloidae* subfamily, which is a sister group to *Epidendroidae,* a large subfamily group of *Orchidoidae,* which in turn is the principal family of terrestrial flowering plants: this makes the vanilla orchid one of the most ancient orchids in the plant kingdom (Cameron, 2000; 2004; Lubinsky et al., 2003; 2006: 926). From morphological observations of flowers, Portères (1954) drew hypotheses for the origin of vanilla and proposed that the principal diversification centre for the genus could be Indo-Malaysian. The Indo-Malaysian stock diversified and evolved on one hand in Madagascar, Mascarhenas Islands and Africa, and on the other hand in oriental Asia and occidental Pacific Islands. Asian species have evolved with a migration towards the Pacific and from there, either directly towards America or indirectly towards continental Asia and Europe during the Tertiary (65.5-2.5 mya) (Portères, 1954; Bory et al., 2008). However more recent historical bio-geographical studies based on phylogeny data suggest a different development (Cameron, 2000). Cameron (2000) proposes that even if *Vanilla* is a pan-tropical genus, species from South America are sister to those from Africa and Asia. Vanillinae lineage evolved prior to the breakup of Gondwana (160 mya) in South America. Then, a migration to the Old World before 100 mya occurred by vicariance events rather than by long-distance dispersal. Consequently, Orchidacea may have evolved much earlier than is traditionally believed (Cameron, 2000).

According to the classification list of Portères (1954), vanilla orchid plants are terrestrial forest climbing vines that are divided into 110 currently recognized species of vine-like orchids which are endemic to pan-tropical regions between 27° latitude north and 27° latitude south of the equator on all continents, except for Australia (Dressler, 1993; Fouché and Jouve, 1999: 690). Under natural conditions, the vanilla plant climbs in tropical rainforests at 20°C- 30°C (Fouché and Jouve, 1999: 691). Most (52) of the species are endemic in tropical America, 31 species are native to

Southeast Asia and New Guinea, 17 in Africa, 7 in the Indian Ocean Islands, and 3 in the Pacific area (Portères, 1954; Bory et al., 2008)

According to Portères (1954), only 18 vanilla species are aromatic however Soto Arenas (2003) recognizes 35 known or expected aromatic vanilla species, which are mostly of American origin (Bory et al., 2008). Of the various 35 aromatic species of vanilla orchid presently identified, the species most widely exploited today is *Vanilla Planifolia* since it yields the highest concentration of vanillin and makes up for 95% of the world's commercial vanilla (Hanelt et al., 2001; Bory et al., 2008; Radulovic et al., 2010). *V. Planifolia* is native to the Mesoamerican tropical rainforest, and its first recorded cultivation was by the Aztecs in 1300 CE (Bythrow, 2005: 129). Although *V. Planifolia* is the most fragrant and cultivated aromatic species today, there are at least four possible known aromatic vanilla species that could have been exploited in the Old World as early as the second millennium BCE. These are:

a) *Vanilla polylepsis* Summerh, endemic to central east Africa (Portères, 1954; Fouché and Jouve, 1999: 691).
b) *Vanilla albida* Blume, endemic to Southeast Asia: India, Sri Lanka, Malaysia and Indonesia (Portères, 1954; Fouché and Jouve, 1999: 691; Hanelt, 2001).
c) *Vanilla abundiflora* J. J. Sm., endemic to Southeast Asia (Soto Arenas, 2003; Bory et al., 2008).
d) *Vanilla griffithii* Rchb. F. endemic to Southeast Aisa: India, Sri Lanka, Malaysia and Indonesia (Soto Arenas, 2003; Bory et al., 2008).

These particular aromatic vanilla orchid species are succulent-stemmed, perennial climbing plants, producing vanillin within its seedpods and flower. Ethnographic evidence shows that these orchids are grown locally and or harvested in the wild by the natives for use as a flavouring agent (similar to that of *Vanilla Planifolia*), spice, food, medicine, and aroma although the fragrance is weaker than that of the species *Vanilla Planifolia* (Lawler, 1984; Guzman and Siemonsma, 1999; Hanelt, 2001; Soto Arenas, 2003; Seidemann, 2005; Bory et al., 2008). In contrast to the general view, 'vanilla like extract' and or 'vanilla oil' is not limited to *Vanilla Planifolia.* We believe that these 'inferior' aromatic vanilla orchid species, that are not harvested for large-scale or economical use today, are the more likely source of vanillin, 4-hydroxybenzaldehyde, and acetonvanillone compounds found in the juglets of Megiddo Tomb 50. It is possible that not only the vanilla fruit was exploited but that the entire orchid flower was used in order to extract and produced a vanilla enriched oil by seeping. We have reached these conclusions taking into consideration the known properties of these aromatic vanilla species as well as their proximity to the Levant. It is important to mention that due to the growing and harvesting limitations of vanilla, reference material from these suggested species could not be carried out. Thus, there is still a lack in our knowledge with regard to the exact amount of vanillin content within the entire orchid (not limited to the fruit) among the many different aromatic species of vanilla. Lastly, we must keep in mind the possibility that unknown and/or extinct vanilla orchid species may have also been exploited and a source of vanilla during the middle of the second millennium BCE.

4. Archaeological Conclusions

The most probable source of the compounds vanillin, 4-hydroxybenzaldehyde and acetonvanillone (degraded vanilla essence) can be traced to either the regions of central east Africa or Southeast Asia in places such as India and Sri Lanka. The first half of the second millennium BCE saw the establishment of long distance trading

systems, the most famous being the Old Assyrian network that connected the city of Ashur and the Anatolian Plateau (Larsen 1987). Literary sources report on intense trade contacts between large urban centres in Mesopotamia (e.g. Ashur, Mari), and places as far away as Dilmun (modern days Bahrain), the Indus Valley and Afghanistan (Eidem and Højlundb, 1993; Khol and Lyonnet, 2008; Liverani, 2014). Although Egypt was divided between different ruling dynasties at the time discussed here, an African trade system should also be considered. Egypt held long-lasting connections with the 'Land of Punt" located in east Africa, which served as a source of gold and many exotic items, such as aromatic resins, black wood, ebony, ivory, and wild animals (Flammini, 2008; Breyer, 2016). In the early 12th dynasty of Egypt the granite blocks found at Mit Rahina (Memphis) detail the activities of Amenemhet II during two years of his reign including Egypt's commercial foreign relations with the Levant by land and by sea (Marcus, 2007). Another trade link to southern India/ Sri Lanka can be seen in the peppercorns used for the mummification of Ramesses II in the 13th century BCE (Lichtenberg and Thuilliez, 1981). In later periods in antiquity, long distance trade between the Far East, Egypt, and the Levant are well known (Tomber, 2000; Ritter, 2009). The identification of the vanilla compounds from the Megiddo juglets raises the possibility of trade between these regions even in earlier periods when shipping methods were relatively similar (Chong and Murphy, 2017).

If indeed the aromatic vanilla orchids were gathered and exploited in one (or both) of the regions identified above, it should not be a surprise that the product found its way into the main cultural and economic centres of the Old World. The elite of Megiddo, living by the main international artery, could have had access to vanilla either directly through Egypt, or other city-states or trade centres in the Southern Levant like Hazor (Maeir, 2000) or Ashkelon (Cohen, 2002; Stager, 2002). Since this is the first time vanillin and related minor compounds have been found in a burial context, it is difficult to assess whether the use of vanillin is unique to this tomb, or if it was part of the regular burial kit of elite groups in the Southern Levant (Baker, 2012). A recent study on Egyptian embalming recipes (Jones et al., 2018: 7-8), *ca.* 3700-3500 BCE, reveals the presence of plant oil, resin, and an aromatic plant extract component identified within funerary textiles used to wrap the bodies in both prehistoric burials, Pharaonic and Graeco-Roman mummies. Moreover, this recipe contained antibacterial agents employed by the Egyptian embalmers much like that of the vanilla-infused oil found in the juglets of the Megiddo masonry tomb. In both cases, one should not ignore the possibility that the Egyptians and the local residents of Megiddo recognized the high quality of vanilla and that they may have used this vanilla-infused oil for embalming rites, aromas in cultic practices, as a flavouring agent, and/or for medicinal purposes.

5. Methods

5.1. Field Sampling Techniques

5.1.1. Collection of Samples

Once the vessels were unearthed and chosen for organic residue analysis, they were taken from the field and set aside in cardboard boxes and sent to the Tel Megiddo offices at Tel Aviv University for further sampling. Using nitrile gloves and clean pliers, a sherd from the base of each vessel was isolated and placed in a paper bag before crushing/drilling to obtain a powdered sample. For juglets, the base (as well as the rim) is the preferred area for sampling as it is the most likely candidate for retaining the largest quantity of absorbed lipids (Charters et al., 1993)

5.1.2. Sediment Control

A sediment sample, taken from in and around the area where each vessel was unearthed, was used for cross-referencing as well as for detecting exogenous contamination.

5.2. Preparation of Samples for ORA

5.2.1. Drilled Samples

Approximately 1-2g of ceramic sherd was drilled from the interior surface of selected sherds using a Dremmel modelling drill with tungsten removable drill tips that were pre-washed by sonication with dichloromethane. The surface layer of the sherd was first lightly drilled and discarded to remove possible contamination introduced by handling, storage, and or exogenous contamination. The ceramic powder was weighed and transferred into labelled, sterile 20 mL glass vials.

5.2.2. Crushed Samples

Ceramic samples lacking a sufficiently large surface for drilling were crushed and grinded into homogeneous fine powder using an agate pestle and mortar that was pre-washed with dichloromethane. Both the exterior and interior surfaces were lightly drilled prior to crushing to remove exogenous contamination. One gram of powder was transferred into labelled, sterile 20 mL glass vials.

5.2.3. Sediment Controls

Sediment controls were crushed into a fine powder using a clean agate pestle and mortar. One gram of sediment was weighed and transferred into labelled, sterile 20 mL glass vials. The pestle and mortar were washed with dichloromethane after each sample was taken.

5.3. Standards and Reference Material Methods

To ensure a correct identification of the molecules based on mass spec identification and retention time of the archaeological samples, reference material was used. Authentic standards of the compounds (vanillin; 99% assay, Sigma-Aldrich) were treated with the same method applied to archaeological samples and analysed by GC-MS. A comparison of the molecular compositions of both archaeological samples and standards allowed for a more accurate identification of the compounds.

5.4. Total Lipid Extract Protocol

5.3.1. Lipid Extraction

Lipid extraction followed established protocols (Evershed et al. 1990; Charters et al. 1993). Prior to extraction, 20 μg of tetratriacontane (C_{34} *n*-alkane) internal standard (IS) was added directly to each of the powdered ceramic samples for quantification purposes. Five mL of dichloromethane: methanol (2:1; v/v) (HPLC grade; Fischer) were added to each of the ceramic samples. The samples were then sonicated for 15 min. and then centrifuged at 2000 rpm for 10 min. The supernatant was pipetted into clean 20 mL scintillation vials. The extraction was repeated twice more, combining the extracts. The solvent was then evaporated under a gentle stream of nitrogen gas and mild heating (40°C) until 2 mL of concentrated solvent remained.

5.3.2. Derivatization by Silyation

Forty-fifty μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS), as well as 3-4 μL of pyridine were added to each sample and then heated on a hot plate at 40°C for 30 min. The samples were then evaporated to dryness under a gentle stream of nitrogen at ~40°C. The samples were re-dissolved with 100 μL of dichloromethane containing a second internal standard,

hexatriacontane (c36 *n*-alkane; 0.02075 mg/mL) and vortexed for 30 s to ensure a homogeneous solution. The extract was then transferred into clean 2 mL GC vials with glass inserts for analysis.

5.5. Analytical Instrumentation, Gas Chromatography/ Mass Spectrometry (GC-MS)

Analysis was carried out on samples run as trimethylsilylated (TMS) derivatives on an Agilent Technologies 7890B GC coupled to an Agilent Technologies 5977A MSD. Splitless injection was performed using a GERSTEL Multipurpose Sampler and GERSTEL Cold Injection System (CIS) 4. The GC was fitted with an Agilent J&W DB-5HT column (15m × 0.32mm i.d.; 0.1μm film thickness). The inlet temperature was ramped from 30°C to 350°C at 12°C s^{-1} (5 min. isothermic hold). The oven temperature was initially set at 40°C (1 min. isothermic hold), ramped to 100°C at 15°C min^{-1}, and then to 240°C at 6°C min^{-1}, and finally increased to 350°C at 20°C min^{-1} (7 min. isothermic hold). Helium was used as the carrier gas. The split/splitless injection system was operated in splitless mode with a purge flow of 3.0 ml min^{-1} and a constant pressure at the head of the column of 8.3124 psi. The ion source temperature was fixed at 300°C and the transfer line was kept at 300°C. Electron Impact (EI) spectra were obtained at 70eV with a full scan from m/z 50 to 950. Data was acquired using an MSD ChemStation F.01.01.2317.

5.6. Peak Identification, Gas Chromatography-Mass Spectrometry

Compounds were identified based on their mass fragmentation patterns, and compared to published literature (add references used), authentic standards (add standards used), and the National Institute of Standards and Technology library mass spectra, (NIST 2.2, 2014).

5.7. Quantification of Lipids Using Gas Chromatography- Flame Ionization Detector (GC-FID)

Quantification of lipids was carried out using GC Agilent Technologies 6890N Gas Chromatograph equipped with a flame ionization detector (FID). The GC was fitted with a HP-5MS column (30m x 0.32 mm i.d.; 0.25 μm film thickness), The samples were run as TMS derivatives, and were injected in splitless injection mode. Helium was used as a carrier gas at a constant flow of 1.1 ml min^{-1}. The oven was set at 50°C (2 min. isothermic hold), followed by a heating gradient of 10°C min^{-1} to 345°C (10 min. isothermic hold). The inlet temperature was 220°C. Retention times were verified using identical column and conditions on an Agilent Technologies 6890 GC with an Agilent Technologies 5973 MSD.

Quantification of the amounts of lipids per unit weight ceramic was conducted on three samples that were found to contain vanillin. Vanillin was quantified separately based on calibration curves using silylated standards. Lipid yields were considered significant if more than 5 μg of lipid per gram of sherd (μg g^{-1}) was obtained, which ensured that archaeological lipid profiles could be securely distinguished from background contamination (Evershed, 2008).

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Author Contribution Statement

V.L and Y.G. wrote the main manuscript text. M.A.S.M prepared figure 1, I.F. prepared figures 2-3 and V.L. and R.N. prepared figures 4-5. All authors reviewed the manuscript.

Competing Interest Declaration

The author(s) declare no competing interest.

First Evidence for Vanillin in the Old World: Its Use as Mortuary Offering in Middle Bronze Canaan

Supplementary Information

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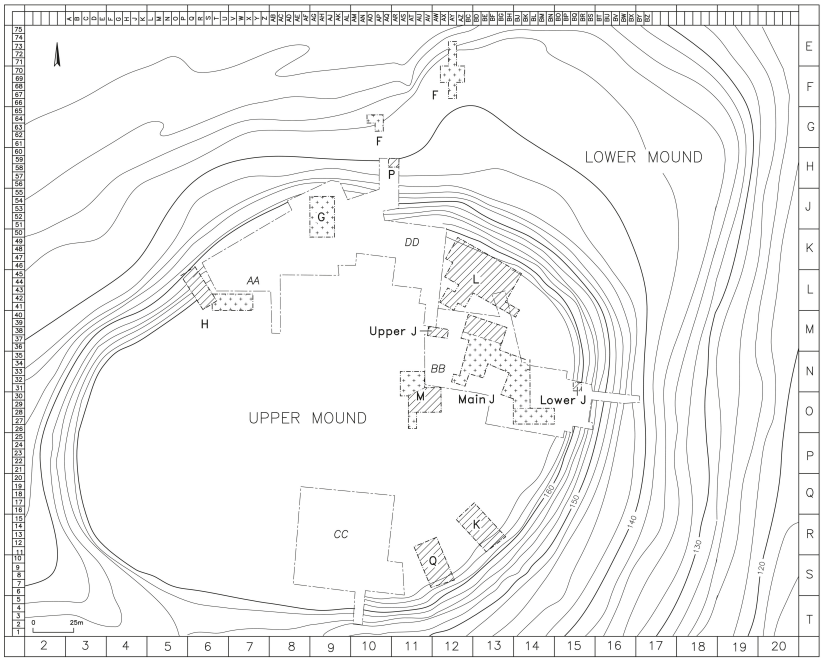


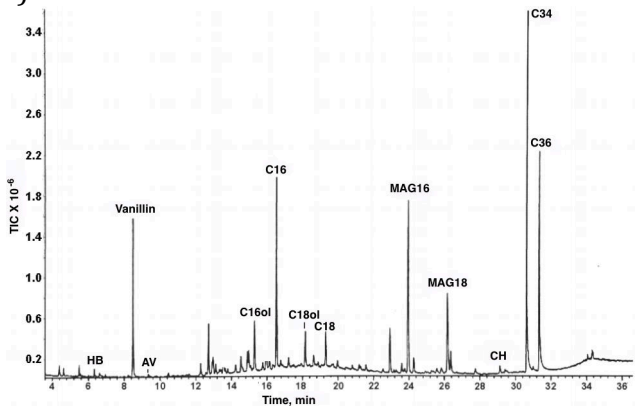
S1: Map of Tel Megiddo showing excavation areas (Finkelstein, Ussishkin and Halpern 2006: fig 1.1).

Table S1: Details of the juglets sampled

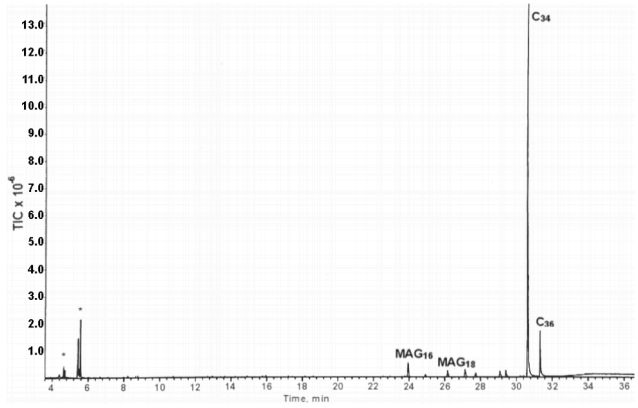
Lab no.	Locus	Basket	Vessel no.	Context	Condition and Treatment
TM-063.D Crushed	16/H/65	5	8 Dipper juglet.	Vessel was found *in situ* in the northernmost part of the tomb chamber, found in an upright	Fragmented juglet with all pieces recovered, sediment inside. Sample was broken off from the base and then

				position near a small assemblage of jewellery and an inhumation.	crushed for analysis.
TM-116.D Crushed	16/H/65	12-2	6 Dipper juglet	Vessel was found *in situ* in the northernmost part of the tomb chamber, found in an upright position near a small assemblage of jewellery and an inhumation.	Fragmented juglet with all pieces recovered, sediment inside. Sample was broken off from the base and then crushed for analysis.
TM-056.D Interior	16/H/62	18	14 Dipper juglet	Vessel was found *in situ*, sitting upright in the south-eastern part of the tomb chamber.	Fragmented juglet with all pieces recovered, sediment inside. Sample was broken off from the base and the interior surface drilled to obtain a powdered sample for analysis.
TM-140.D Interior	16/H/65	12-1	6 Juglet	Vessel was found *in situ* in the eastern part of chamber, found in an upright position near a small assemblage of jewellery and an inhumation.	Fragmented juglet with all pieces recovered, sediment inside. Fragment from near the base was chosen and the interior surface drilled to obtain a powdered sample for analysis.

a)

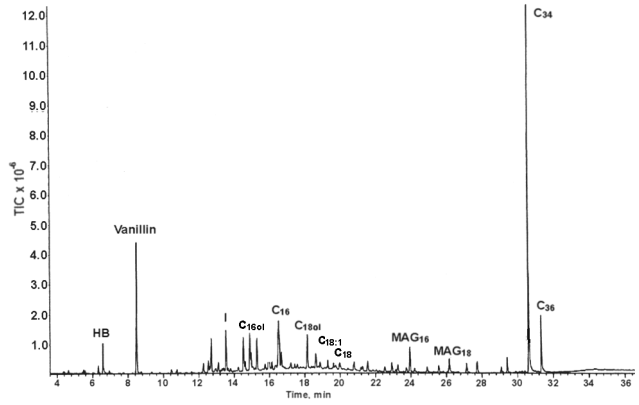


b)

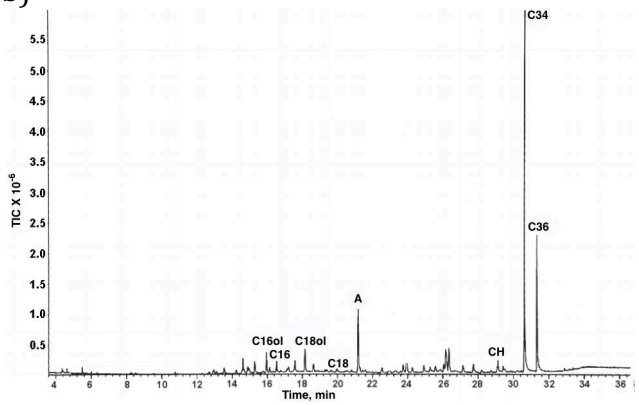


S2: Total ion chromatogram of the lipid extracts analysed after trimethylsilylation of a) Juglet TM-056.D; b) sediment control for Juglet TM-056.D. (HB- 4-hydroxybenzaldehyde; AV- acetovanillone; C_{16ol}- 1- hexadexanol; C_{16} - palmitic acid; C_{18ol} - 1-octadecanol; C_{18} - stearic acid; MAG_{16} – 1-palmitoyl-glycerol; MAG_{18}- 1 stearoyl-glycerol; CH - cholesterol; I- Impurity; Internal standards- C_{34} - tetratriacontane and C_{36} - hexatriacontane. Peaks not annotated are unidentified).

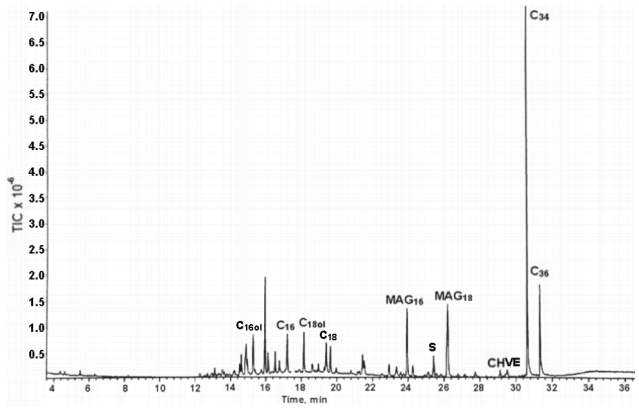
a)



b)



S3: Total ion chromatogram of the lipid extracts analysed after trimethylsilylation of a) Juglet TM-116.D; b) sediment control for Juglet TM-116.D. (HB - 4-hydroxybenzaldehyde; C_{16ol} - 1-hexadexanol; C_{16}- palmitic acid; C_{18ol}- 1-octadecanol; $C_{18:01}$- oleic acid; C_{18}- stearic acid; MAG_{16} – 1-palmitoyl-glycerol; MAG_{18} - 1-stearoyl-glycerol; I – Impurity; A-alkane; Internal standardy-C_{34} - tetratriacontane and C_{36} - hexatriacontane. Peaks not annotated are unidentified).



S4: Total ion chromatogram of the lipid extracts analysed after trimethylsilylation from Juglet TM-140.D showing the absence of vanillin and other compounds. (C_{16} - palmitic acid; C_{18ol} - 1-octadecanol; MAG_{16} – 1-palmitoyl-glycerol; MAG_{18} - 1-stearoyl-glycerol; S - squalene; CH - cholesterol; VE - vitamine E; I – Impurity; Internal Standards- C_{34} - tetratriacontane and C_{36} – hexatriacontane. Peaks not annotated are unidentified).