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Molecular Criteria for Discriminating Adipose Fat and Milk from Different Species by NanoESI MS and MS/MS of Their Triacylglycerols: Application to Archaeological Remains

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A new multistep analytical methodology is described in this paper for the precise identification of triacylglycerols, which are biomarkers of dairy products and subcutaneous fats, that may be chemically identified in archaeological pottery. It consists of the analysis of the total lipid extract from different kinds of fats by high-temperature gas chromatography, performed in order to select the ceramic vessels in which animal fats are well preserved, followed by nanoelectrospray QqTOF mass spectrometry that allows for distinguishing the specific origins of the lipids detected (namely, cow, sheep, or goat). The analysis of model samples, cow and goat dairy products and cow and sheep adipose fats, was successfully achieved. The fatty acid composition of each triacylglycerol was identified, which allowed for the discrimination of subcutaneous fats and dairy fats and distinguishing between cow and goat milk. This methodology was then applied to archaeological samples, and the presence of goat milk, cow milk, and possibly sheep subcutaneous fat was assessed based on the discriminating criteria found on modern fats.

Among natural substances possibly preserved in archaeological ceramic vessels, animal fats, from either subcutaneous matter or dairy products, have been shown to be particularly well preserved in several pre- and protohistoric sites.[1−7] Determining the specific origin of such products but also distinguishing between subcutaneous fats and dairy derivatives are of particular importance in the field of archaeology in order to understand the evolution of neolithic economies.[8,9]

Until the 90s, one way for detecting milking exploitations during prehistory was based on the study of specific ceramic pots, either holed or with particular shapes, systematically interpreted as milk strainers and milk jugs.[8−10] However, the recent identification of beeswax in a so-called "strainer" from the neolithic site of Dikili-Tash (Greece, 6th millenium B.C.) gave evidence that holed pottery may have had other functions than the processing of dairy products, as that kind of pottery may also have been used as a honey or beeswax sieve.[11]

The study of faunal remains is another way of identifying milking exploitations. Indeed, dairy specialization may be emphasized when an important neonatal calf cull indicates postlactation slaughtering.[12] Dairying is nevertheless difficult to assess by this method when animals are exploited for both their milk and meat.

A new way for investigating the question of milk exploitation during prehistory relies on the analysis of lipids extracted from pottery, either from potsherds or carbonized surface residues.[1,13−16] These analyses are based on the detection and identification by high-temperature gas chromatography (HT GC) and HT GC coupled to mass spectrometry (HT GC/MS) of lipids, characteristic of the natural origin of the products processed in the vessels. Among others, waxes, resins, tars, and different kinds of fats can be readily distinguished.[1,3,17−21] The distinction between animal

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(1) Evershed, R. P.; Mottram, H. R.; Dudd, S. N.; Charters, S.; Stott, A. W.; Lawrence, G. J. *Naturwissenschaften* **1997**, *84*, 402−406.
(2) Craig, O.; Mulvillet, J.; Pearson, M. P.; Sokol, R.; Gelsthorpe, K.; Stacey, R.; Collins, M. *Nature (London, U.K.)* **2000**, *408*, 312.
(3) Evershed, R. P.; Dudd, S. N.; Copley, M. S.; Berstan, R.; Stott, A. W.; Mottram, H. R.; Buckley, S. A.; Crossman, Z. *Acc. Chem. Res.* **2002**, *35*, 660−668.
(4) Copley, M. S.; Berstan, R.; Dudd, S. N.; Straker, V.; Payne, S.; Evershed, R. P. *J. Archaeol. Sci.* **2005**, *32*, 485−503.
(5) Copley, M. S.; Berstan, R.; Straker, V.; Payne, S.; Evershed, R. P. *J. Archaeol. Sci.* **2005**, *32*, 505−521.
(6) Copley, M. S.; Berstan, R.; Mukherjee, A. J.; Dudd, S. N.; Straker, V.; Payne, S.; Evershed, R. P. *J. Archaeol. Sci.* **2005**, *32*, 523−546.
(7) Regert, M.; Garnier, N.; Decavallas, O.; Cren-Olive, C.; Rolando, C. *Meas. Sci. Technol.* **2003**, *14*, 1620−1630.
(8) Sherratt, A. *World Archaeology* **1983**, *15*, 90−104.
(9) Bogucki, P. I. *Expedition* **1986**, *28*, 51−58.
(10) Bogucki, P. I. *Oxford J. Archaeology* **1984**, *3*, 15−29.
(11) Regert, M.; Colinart, S.; Degrand, L.; Decavallas, O. *Archaeometry* **2001**, *43*, 549−569.
(12) Balasse, M.; Bocherens, H.; Tresset, A.; Mariotti, A.; Vigne, J.-D. *C. R. Acad. Sci., Ser. IIa: Sci. Terre Planetes* **1997**, *325*, 1005−1010.
(13) Dudd, S. N.; Evershed, R. P. *J. Archaeol. Sci.* **1999**, *26*, 1473−1482.
(14) Regert, M.; Dudd, S. N.; van Bergen, P. F.; Pétrequin, P.; Evershed, R. P. *BAR* **2001**, *S939*, 78−90.
(15) Evershed, R. P.; Heron, C.; Charters, S.; Goad, L. J. *Proc. Br. Acad.* **1992**, *77*, 187−208.
(16) Heron, C.; Evershed, R. P. *Archaeol. MethodTheory* **1993**, *5*, 247−279.
(17) Evershed, R. P.; Heron, C.; Goad, L. J. *Antiquity* **1991**, *65*, 540−544.

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dairy and subcutaneous fats relies on a precise identification of the triacylglycerol (TAG) content, as they are the most characteristic biomarkers of animal fats. Although the triacylglycerol distribution of fresh milk is very characteristic, its degraded profile sometimes becomes indistinguishable from that of ruminant adipose fat through diagenetic degradation.[22,23] A complementary method based on the analysis of stable carbon isotope ratio $^{13}C/^{12}C$ by GC/C-IRMS (gas chromatography/combustion-isotope ratio mass spectrometry) was also developed.[1,3,13,22] It allows ascertaining more soundly the distinction between ruminant subcutaneous fats and dairy products. Nevertheless this approach does not always allow for distinguishing adipose fats from different species, and it does not provide any criteria for identifying the specific origin of dairy products (cow, goat, or sheep). Recently, there has been several interesting attempts to characterize contemporary dairy and subcutaneous fats by their TAG composition, using LC/APCI MS (liquid chromatography/atmospheric pressure chemical ionization mass spectrometry).[24,25] Although the results obtained were very conclusive in terms of modern products, they were rarely applied to archaeological samples. Moreover, such analyses require many separation steps in order to assess the complete structure of milk TAGs; thus, they may be difficult to apply to archaeological samples. To our knowledge, there is a single example of the application of LC/APCI MS to an archaeological sample that showed a simple TAG distribution characteristic of adipose ruminant fat.[26] However, no comparison of adipose and dairy TAGs or of their specific provenance has ever been studied for that kind of degraded complex sample.

To precisely identify archaeological animal fats, from their nature to their origin, we developed a new methodology consisting of a general screening of the preserved lipids by HT GC and HT GC/MS followed by the analysis of selected samples by nanoESI MS/MS in order to more precisely assess the triacylglycerol distribution. Because ESI MS/MS had to be carried out on TAGs as pure as possible, the selected samples were fractionated to separate TAGs from well-ionized compounds. The analyzed solutions were also doped with lithium chloride in order to palliate the weak ionization yield of triacylglycerols.[27] Before analyzing precious archaeological lipid extracts, sample treatment and ESI MS analyses were developed on reference contemporary milk and adipose samples from different species. Besides a very sensitive detection of the TAGs, the distribution, the chain-length, and the degree of unsaturation of the fatty acid moieties involved in TAGs were elucidated. Eventually, these data provided new criteria for the discrimination of fatty products from different animal species, for both modern and archaeological samples. Particularly, the differentiation of cow and goat milk fats and of cow and sheep adipose fats was assessed for a number of TAGs that were then used to identify archaeological samples from two neolithic sites, namely, Chalain 4 (CH 4) and Clairvaux XIV (CL XIV), located in the French Jura region.

EXPERIMENTAL SECTION

Solvents and Reagents. All organic solvents were analytical grade purchased from VWR (VWR-France, Fontenay-sous-Bois, France). Lithium chloride (>98%), silver nitrate, and *N*,*O*-bis-(trimethylsilyl)trifluroacetamide (BSTFA) were purchased from Sigma (Lyon, France).

Commercial Standards. All commercial standards, *n*-tetratriacontane ($C_{34}H_{70}$, C_{34}), myristic acid ($C_{14}H_{28}O_2$, $C_{14:0}$), palmitic acid ($C_{16}H_{32}O_2$, $C_{16:0}$), stearic acid ($C_{18}H_{36}O_2$, $C_{18:0}$), and oleic acid ($C_{18}H_{34}O_2$, $C_{18:1}$), dipalmitin ($C_{35}H_{68}O_5$, $D_{32:0}$) and distearin ($C_{39}H_{76}O_5$, $D_{36:0}$), trimyristin ($C_{45}H_{86}O_6$, $T_{42:0}$), triplamitin ($C_{51}H_{98}O_6$, $T_{48:0}$), and tristearin ($C_{57}H_{110}O_6$, $T_{54:0}$), cholesterol ($C_{27}H_{46}O$), *n*-1-docosanol ($C_{22}H_{46}O$, $C_{22}OH$), and *n*-1-octacosanol ($C_{28}H_{58}O$, $C_{28}OH$) were purchased from Sigma (Lyon, France).

Reference Samples. Modern potsherds were handmade, using the same techniques as those used at the archaeological settlements of Chalain during the neolithic period.[28] The potsherds were impregnated at the laboratory for 2 days with whole cow and goat milk purchased from organic food shops. They were then dried at room temperature until they reached a constant weight. Cow and sheep adipose fats were bought from organic food stores and prepared directly.

Archaeological Samples. Five vessels from the late neolithic site of Chalain 4 (CH 4, ~3000 B.C., Jura, France; 22 samples) and 17 from the middle neolithic site of Clairvaux XIV (CL XIV, ~4000 B.C., Jura, France; 22 samples) were analyzed by HT GC and HT GC/MS. Samples consisted of potsherds and the corresponding charred surface residues when present, in order to determine whether differences between their molecular compositions could be detected. Among these archaeological samples, four were chosen for ESI MS/MS depending on their high degree of preservation and their complex triacylglycerol distribution. Table 1 details the references and characteristics of the samples investigated in this research.

Lipid Extraction. Both archaeological and modern samples were treated following the same procedure. The protocol described below was adapted from Evershed and collaborators.[29] Potsherds (~2 g) and carbonized surface residues (~200 mg) were first cleaned using a sterile scalpel blade and then crushed with a glass mortar and pestle. Lipids were extracted with a solution of chloroform/methanol ($CHCl_3/CH_3OH$, 2:1 v/v, 10 mL) after addition of an internal standard (*n*-tetratriacontane C_{34}; 20 μL, 1 mg/mL in *n*-hexane). After sonication (2 × 15 min), the samples were centrifuged (20 min, 3000 rpm) and the clear solution was evaporated to dryness under a gentle stream of nitrogen (40 °C). The total lipid extract was then dissolved in 500 μL of chloroform/methanol (2:1 v/v) and stored at 4 °C.

Derivatization of the Total Lipid Extract for HT GC and HT GC/MS Analysis. An aliquot of each total lipid extract (10−100 μL) was evaporated to dryness under a gentle stream of

(18) Heron, C.; Nemcek, N.; Bonfield, K. M.; Dixon, D.; Ottaway, B. *Naturwissenschaften* **1994**, *81*, 266−269.
(19) Regert, M. *J. Sep. Sci.* **2004**, *27*, 244−254.
(20) Regert, M.; Alexandre, V.; Thomas, N.; Lattuati-Derieux, A. *J. Chromatogr., A* **2006**, *1101*, 245−253.
(21) Mathe, C.; Culioli, G.; Archier, P.; Vieillescazes, C. *J. Chromatogr., A* **2004**, *1023*, 277−285.
(22) Dudd, S. N.; Evershed, R. P. *Science* **1998**, *282*, 1478−1481.
(23) Dudd, S. N.; Regert, M.; Evershed, R. P. *Org. Geochem.* **1998**, *29*, 1345−1354.
(24) Mottram, H. R.; Crossman, Z.; Evershed, R. P. *The Analyst* **2001**, *126*, 1018−1024.
(25) Mottram, H. R.; Evershed, R. P. *J. Chromatogr., A* **2001**, *926*, 239−253.
(26) Kimpe, K.; Jacobs, P. A.; Waelkens, M. *J. Chromatogr., A* **2002**, *968*, 151−160.
(27) Hsu, F.-F.; Turk, J. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 587−599.
(28) Martineau, R., Université de Franche-Comté, Besançon, 2003.
(29) Charters, S.; Evershed, R. P.; Blinkhorn, P. W.; Denham, V. *Archaeometry* **1995**, *37*, 113−127.

Table 1. Reference and Data Obtained by GC, GC/MS, and ESI MS/MS for the Archaeological Samples Analyzed

	site	vessel reference	sample name	Figure 1 equiv number	type of sample	lipid concn (μg/g of sample)	TAG %	HTGC interpretation	ESI MS/MS interpretation
large distribution of TAGs (T40−T54)	CH 4	**4069**	MR0463T	8	potsherd, rim	110.3	1.3	dairy products	
			MR0463R	7	residue, rim	5225.8	14.0		
			MR0464T	none	potsherd, body	0.0	0.0		
			MR0465R	**9**	**residue, body**	**6649.7**	**21.0**	→	**goat milk fat**
			MR0466R	10	residue, rim	4770.3	17.8		
	CL XIV	48	MR1039T	none	potsherd	18.7	0.0		
			MR1040R	1	residue	1407.5	18.6		
		60	T1041A	6	potsherd	125.8	23.2		
		130	MR1049T	2	potsherd 1	167.5	11.7		
		150	**T1050A**	**3**	**potsherd**	**175.4**	**16.1**	→	**cow milk fat**
		195	T1058A	4	potsherd	112.2	18.7		
		214	MR1061TB	none	potsherd	243.6	0.0		
			MR1062RC	5	residue	3018.5	7.9		
medium distribution of TAGs (T44−T54)	CL XIV	87	T1047A	11	potsherd	207.6	12.8	adipose fat or much degraded dairy fat	
		168	**T1052A**	**12**	**potsherd**	**51.4**	**22.2**	⟹	**subcutaneous fat ?**
		388	T1096A	14	potsherd	208.6	33.4		
		421	**T1101A**	**13**	**potsherd**	**156.7**	**11.4**	→	**subcutaneous fat; probably sheep**
	CH 4	4181	MR0519T	none	potsherd, bottom	33.7	0.4		
			MR0519R	none	residue, bottom	91.0	0.0		
			MR0520T	15	potsherd, rim	1273.9	20.9		
			MR0520R	16	residue, rim	4009.7	35.4		
			MR0521T	17	potsherd, body	107.4	10.4		
			MR0521R	18	residue, body	5138.5	17.6		
		4018	MR0508T	19	potsherd, rim	118.0	3.3		
			MR0509R	21	residue, body	3822.7	13.5		
			MR0509T	20	potsherd, body	68.1	3.2		
			MR0510T	none	potsherd, bottom	8.6	0.0		
		4014	MR0524T	29	potsherd, rim	209.3	0.6		
			MR0525T	30	potsherd, body	279.3	1.2		
			MR0526T	31	potsherd, bottom	290.7	0.8		
narrow distribution of TAGs (T46−T54)	CL XIV	12	T1035A	22	potsherd	234.2	24.4	adipose fat	
		151	T1051A	23	potsherd	416.7	3.6		
		313	MR1079A	none	potsherd	29.7	0.0		
			MR1080A	24	residue	618.6	14.3		
	CH 4	4171	MR0514R	26	outer residue, rim	1566.0	3.2		
			MR0514T	25	potsherd, rim	524.4	0.8		
			MR0515T	27	potsherd, body	28.2	10.0		
			MR0516T	28	potsherd, bottom	55.5	2.6		
non interpretable distribution of TAGs	CL XIV	184	T1055A	none	potsherd	235.6	3.1	undetermined animal fat	
			R1056A	none	residue	533.5	0.0		
		365	T1090A	none	potsherd	1370.8	1.0		
		416	T1100A	none	potsherd	188.6	0.0		
		432	MR1104A	none	potsherd	0.0	0.0		
			MR1105A	none	residue	236.2	3.7		

nitrogen (40 °C). Silylation was achieved by adding 40 μL of BSTFA. The sample was then heated for 30 min at 80 °C. It was then evaporated to dryness under nitrogen (40 °C) and recovered in 20−100 μL of cyclohexane. An amount of 1 μL of the sample was injected into the chromatograph.

Fractionation of Archaeological Samples. Because of the inherent state of degradation of the archaeological samples, triacylglycerols needed to be purified from their degradation products and from the internal standard, *n*-tetratriacontane C_{34}, in order to be analyzed by ESI MS/MS. The samples chosen were a carbonized surface residue MR0465R from CH 4 and three potsherds T1050A, T1052A, and T1101A from CL XIV. Modern samples did not need any purification as triacylglycerols account for 98% of the lipid fraction and were not degraded, and no internal standard had been added. An amount of 100 μL of each total lipid extract were fractionated on silica solid-phase extraction (SPE) cartridges (500 mg, 3 mL, Alltech-France, Templemars, France). The SiO_2 cartridges were rinsed with 2 mL of *n*-hexane, 2 mL of CH_2Cl_2/CH_3OH (2:1 v/v), and then 4 mL of *n*-hexane. The sample was placed on top of the column. Fraction A containing the hydrocarbons was eluted with 1 mL of *n*-hexane, fraction B containing the di- and triacylglycerols was eluted with 1 mL of CH_2Cl_2, fraction C containing the sterols and alcohols with 1.5 mL of CH_2Cl_2/CH_3OH (2:1 v/v), and fraction D containing the fatty acids was eluted with 4 mL of CH_2Cl_2/CH_3OH (1:1 v/v).

The different fractions were evaporated to dryness, derivatized, and analyzed by HT GC to confirm their content. Fraction B, that contained the di- and triacyglycerols, was then prepared and analyzed by infusion nanoESI MS and MS/MS, in order to determine and identify the exact triglyceride composition.

HT GC and HT GC/MS. Gas chromatographic analysis of the silylated total and fractionated lipid extracts were performed

on a HP 6890 high-temperature gas chromatograph equipped with a FID detector (Hewlett-Packard, Palo Alto, CA). Samples were introduced by on-column injection into a 15 m × 0.32 mm fused-silica capillary column, coated with CP Sil 5CB stationary phase with a 0.1 μm film thickness (Varian, Palo Alto, CA) connected to a 1 m noncoated precolumn. Helium was used as the carrier gas with a programmed flow as follows: 2 mL min^{-1} (17 min) to 4 mL min^{-1} (4 min) at 1 mL min^{-2}; then 4−6 mL min^{-1} at 1 mL min^{-2} and hold at 6 mL min^{-1} for 16 min. The temperature program consisted of a 1 min isothermal hold at 50 °C followed by ramping from 50 to 350 °C at 10 °C min^{-1}. It was then kept at 350 °C for 10 min. The injector was used in the track-oven mode.

HT GC/MS analyses were performed on a Thermo Finnigan GCQ (Thermo Electron Corporation, Waltham, MA) mass spectrometer device equipped with an ion-trap analyzer linked to a HP 5890 gas chromatograph. Injection was achieved through a splitless injector, held at 325 °C. The samples were separated using a column similar to that used in HT GC with an i.d. of 0.25 mm, with the same temperature program. Helium pressure was held at 16 psi, and the GC/MS interface temperature was maintained at 350 °C. Mass spectra were recorded in the electron ionization mode at 70 eV, the source being held at 180 °C. In full scan mode, the mass range was scanned from m/z 50 to 800 in 0.6 s.

NanoESI MS and MS/MS. As the lithium ion, Li^+, has been shown to be the most efficient doping reagent, triacylglycerols were ionized as lithium adducts, $[M + Li]^+$.[27] The lithium solution was composed of 2% lithium chloride in methanol. An amount of 5 μL of the archaeological TAG fraction or the model lipid extract and 2 μL of the lithium solution were mixed and diluted to 100 μL in methanol.

Infusion nanoESI MS and nanoESI MS/MS analyses were performed on a Q-Star Pulsar mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a Q-q-TOF analyzer fitted with a nanoelectrospray ion source (Protona, Odense, Denmark). Acquisition was controlled by Analyst software. The lithium-doped solution was placed in a metal-coated glass needle (Proxeon Biosystems, Odense, Denmark) and infused in the source by application of a high voltage (800 V). The electrospray interface was operating with a first declustering potential at 50 V, a focalizing potential at 200 V, and a second declustering potential at 15 V. For ESI MS/MS experiments, the lithiated molecular ions were selected in the first quadrupole, accelerated to an energy of 20−50 eV, collided with argon, and analyzed in the TOF analyzer.

RESULTS AND DISCUSSION

General Screening and Selection of the Samples by HT GC and HT GC/MS. The organic substances derived from the content and the use of ancient ceramic vessels are usually preserved as visible charred surface residues or as absorbed matter inside the porous clay matrix. They may correspond to a large variety of materials including animal fats, vegetable oils, and also beeswax, tars, and resins. However, it is impossible to gain any information on either their origin or their degree of preservation by macroscopic or microscopic observations because of their amorphous nature. It is therefore necessary to carry out rapid analyses, with a minimum sample preparation, a good separation capability (HT GC), and an universal detection (FID or MS), in order to get an overview of the main components preserved along with an insight into their concentration and degree of preservation. Biomarkers-orientated analytical methodologies can then be developed, on selected samples.

HT GC with FID detection and HT GC/MS analyses were thus used as a rapid method of screening of the molecular composition of the preserved organic matter and the determination of the lipid concentration.[29−31] The 22 vessels for which we detail the results in this paper are part of a larger series of 63 ceramics from CH 4 and CL XIV. Among all these vessels, vegetable oil, animal fats, beeswax, and birch bark tar were detected and identified by HT GC and HT GC/MS. We focused the present work on ceramic vessels that contained various animal fats (Table 1).

Biomarkers and degradation markers of animal fats (fatty acids, mono-, di-, and triacylglycerols, and cholesterol) were detected in 22 vessels (Figure 1). More precisely, they were detected in 42 samples coming from those 22 vessels and only 2 samples did not have any trace of lipids. In vessels 4014 and 4018 from CH 4, anthropogenic markers, such as long-chain ketones, were also detected and identified by their mass spectra. These compounds have already been described in archaeological ceramic vessels by other authors. They are formed by the condensation of two fatty acids during the heating of animal fats and are thus probably characteristic of the heating process usually involved in culinary activities.[32]

All the samples from a single vessel showed the same chromatographic pattern, more or less degraded, which means that the vessels were systematically used with the same content.[33] In a single case (Table 1, CH 4, vessel 4018, sample MR0508T; Figure 2, number 19), the chromatographic pattern of one sample was quite different from the others of the same vessel, especially considering its TAG distribution (T_{48}−T_{54} instead of T_{44}−T_{54}). This may be due to the preferential alteration of samples issued from the rim that were more exposed to atmosphere during the use of the vessel. In the case of four vessels from CL XIV (vessels 184, 365, 416, and 432, Table 1), only fatty acids with traces of triacylglycerols were detected. This did not allow drawing any hypothesis on the exact nature of the animal fats processed in these vessels. The total lipid extracts of the charred surface residues from the ceramic vessels from CH 4 were more concentrated than those from CL XIV (average lipid concentration of 3909 μg per g of residue for CH 4 versus 1163 μg per g of residue for CL XIV). As seen in Table 1, carbonized surface residues had a lipid concentration up to more than 1 mg per g of residue, whereas potsherds were less concentrated, from 20 to 500 μg per g of potsherd in most cases. Those concentrations are higher than the acceptable limit currently admitted for interpretation.[16,31] Interestingly, in 20 samples, TAGs represented more than 10% of the total lipids, reaching 35% in one case (CH 4, vessel 4181, sample MR0520R, Table 1). As TAGs are known to be the most easily degraded lipids, one can notice that the samples were exceptionally well-preserved, after more than 5000 years underground. This is due to the highly favorable lacustrian archaeological context,

(30) Charters, S.; Evershed, R. P.; Goad, L. J.; Leyden, A.; Blinkhorn, P. W.; Denham, V. *Archaeometry* **1993**, *35*, 211−223.
(31) Heron, C.; Evershed, R. P.; Goad, L. J. *J. Archaeol. Sci.* **1991**, *18*, 641−659.
(32) Evershed, R. P.; Stott, A. W.; Raven, A.; Dudd, S. N.; Charters, S.; Leyden, A. *Tetrahedron Lett.* **1995**, *36*, 8875−8878.
(33) Regert, M.; Dudd, S. N.; Pétrequin, P.; Evershed, R. P. *Rev. Archéométrie* **1999**, *23*, 91−99.

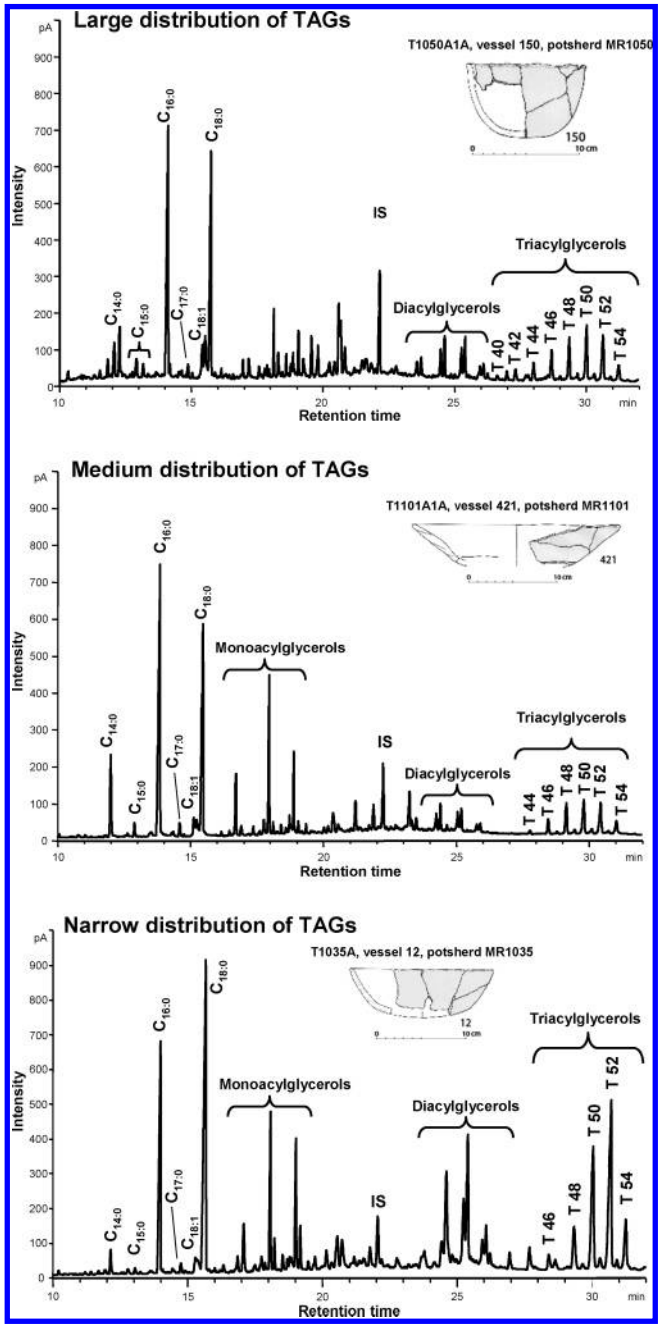

Figure 1. Gas chromatograms obtained from the total lipid extracts of three vessels from the archaeological site CL XIV.

which consists of a humid anaerobic sediment. It explains why the degradation processes due to bacteria and oxygen were greatly slowed.[14]

Depending on their TAG distribution, the samples were classified into three main categories. These categories were realized using a simple statistical treatment that allowed plotting the dispersion factor (DF) versus the average carbon number (M) contained in the fatty acid moieties of each TAG (Figure 2). This latter value was calculated as follows:

$$M = \frac{\sum(P_i C_i)}{\sum P_i}$$

where P_i corresponds to the relative percentage of each TAG and C_i to the number of atoms of fatty acid moieties in each TAG (CN). $\sum P_i$ corresponds to 100 since the sum of the area of each TAG has been brought back to 100%. The dispersion factor was obtained by the following formula:

$$\mathrm{DF} = \frac{\sqrt{\sum[(C_i - M)^2 C_i P_i]}}{\sum P_i}$$

On the graph obtained, the lower is the average CN and the higher is the DF, the larger is the TAG distribution.

Figure 2 shows that, except three samples (8 corresponding to MR0463T, vessel 4069; 14 corresponding to T1096A, vessel 388; 29 corresponding to MR0524T, vessel 4014), all the samples could be attributed to one of the three different classes that appears on the graph. The first class, comprising seven vessels, one from CH 4 and six from CL XIV (Table 1), contains samples that present a large distribution of TAGs, from T_{40} to T_{54} (Figures 1 and 2, vessel 150). The second class, with seven vessels, is characterized by a medium distribution of TAGs, from T_{44} to T_{54} (Figure 1, vessel 421 and Figure 2, vessel 168), and the last class, with four vessels, comprises samples with a narrow distribution of TAGs, from T_{46} or T_{48} to T_{54} (Figure 1, vessel 12 and Figure 2, vessel 151). By comparing these data with those from literature, it is possible to attribute the largest and the narrowest TAG distribution to, respectively, degraded dairy products and subcutaneous animal fats.[3,7,13,22,23] Indeed, dairy products are known to be characterized by a very large TAG distribution, from T_{28} to T_{54}, with many isomers not well-separated by HT GC, while subcutaneous ruminant fat is characterized by a narrow distribution, from T_{46} to T_{54}, with less-shouldered peaks. This is due to the far more simple composition of adipose TAGs compared to those from dairy fat.[25,34−36] As asserted in the literature, triacylglycerols are highly sensitive to degradation, especially hydrolysis in the present archaeological context. Short-chain TAGs were proven to be preferentially hydrolyzed, thus making the profile of degraded milk fat resembling that of ruminant adipose fat.[23] Consequently, if the broad-TAG-distribution samples could be readily identified as degraded dairy products, the medium class is more subjected to questioning. Indeed, those samples could be either well preserved adipose fat or degraded dairy fat. And last, the third-class distribution is more probably characteristic of adipose degraded fat, especially when the amount of T_{50} and T_{52} is largely higher than the amount of the other TAGs and close to the amount of fatty acids (CL XIV, vessel 12, Figure 1). On the contrary, if the amount of T_{50} and T_{52} is close to that of the other TAGs, while fatty acids are in a larger amount, the distribution again is only characteristic of much degraded animal fat (CL XIV, vessel 151, Figure 2).

HT GC remains an interesting and necessary step of analysis, as it allows distinguishing animal fats from other commodities such as resins, beeswax, and so on. Among animal fats, it is efficient to discriminate different categories of TAG distribution

(34) Jensen, R. G. *J. Dairy Sci.* **2002**, *85*, 295−350.
(35) Ruiz-Sala, P.; Hierro, M. T. G.; Martinez-Castro, I.; Santa-Maria, G. *JAOCS, J. Am. Oil Chem. Soc.* **1996**, *73*, 283−293.
(36) Gresti, J.; Bugaut, M.; Maniongui, C.; Bezard, J. *J. Dairy Sci.* **1993**, *76*, 1850−1869.

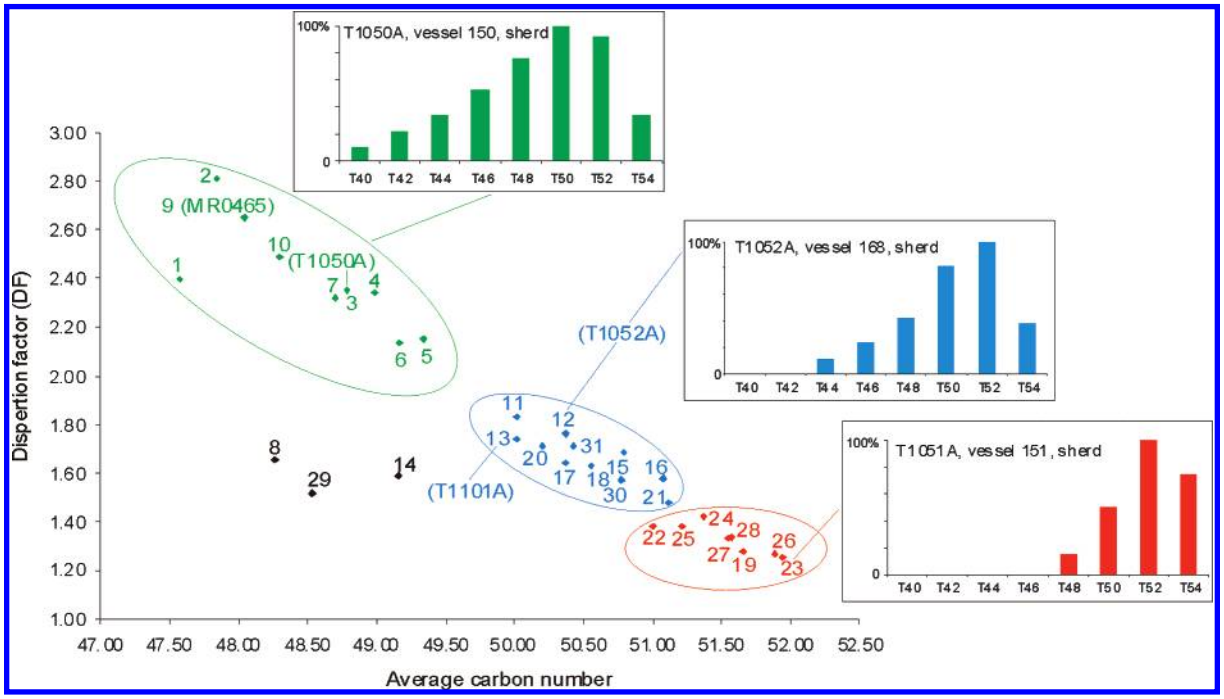

Figure 2. Distribution of the archaeological samples based on HT GC data of the TAGs distribution.

that may be related to various fatty materials from meat, subcutaneous animal fat, or milk and its derivatives, butter, yogurt, cheese, as already discussed by other authors.[1,3,7,22,23] However, HT GC/MS did not permit the identification of TAG structure, because of the poorly informative spectra obtained by electron ionization mass spectrometry. The only fragmentation observed is due to the loss of acetate radical RCOO•, for the main fatty acid moieties. Furthermore, the specific origins of fats could not be assessed by HT GC or HT GC/MS.

Consequently, in order to focus on the structural characterization of TAGs, we proceeded to the analysis of model and archaeological samples by nanoESI MS and MS/MS.

NanoESI MS and MS/MS. Before application of nanoESI MS and nanoESI MS/MS to archaeological samples, it was necessary to assess their efficiency for the analysis of triacylglycerol constituents of contemporary reference samples. Moreover, by analyzing subcutaneous and dairy fats from different animals, cow, goat, and sheep, we wanted to know if we could find new criteria for the discrimination of these substances.

We began by the nanoESI MS analysis of cow and goat milks, impregnated on model potsherds, in parallel to the analysis of cow and sheep subcutaneous fats. The TAG distributions obtained for both milk samples ranged from T_{24} to T_{54} whereas for both subcutaneous fats it ranged from T_{44} to T_{54}. Figure 3a presents the distribution obtained for cow milk, with only the highest-peak isomer indicated for each CN. The comparison of the nanoESI MS TAG distribution with that from HT GC analysis (Figure 3b) clearly showed that nanoESI MS was much more sensitive, as it allowed the detection of TAG with a small CN (compounds T_{24} and T_{26}) that were never detected in any sample before to our knowledge.[34] NanoESI MS also appeared to be more sensitive than APCI MS (atmospheric pressure chemical ionization mass spectrometry), which does not seem to detect TAG under T_{28}.[25] Another advantage of HT GC and HT GC/MS was the possibility to distinguish for each series of TAG with the same CN those containing saturated acids from those with unsaturated acids. The number of unsaturations in each series of TAG could then be determined as well as the "unsaturation" distribution.

The use of lithium salts, to enhance the ionization yield, led to a very high stability of the TAG−Li adducts, thus precluding any in-source random fragmentation.[27] Other ionization techniques, such as chemical ionization, electron impact, and APCI could not avoid those in-source fragmentations that preclude any identification of both small TAGs and diacylglycerols. Last, this technique required only a minute amount of sample, as the injection flow is about 200 nL/min, and the concentration required can be as low as 1 nmol/μL.

The possibility of doing tandem mass spectrometry experiments with the nanoESI Q-q-TOF mass spectrometer was also exploited in order to gain insight into the structure of TAGs. Because of the presence of lithium adducts, the fragmentation was charge-remote. The notations used here were derived from Hsu and Turk,[27] who described the fragmentations obtained for lithium adducts by ESI MS/MS. One must note that with the mass spectrometer used for the experiments, the fragmentation energies used were gentler than those described by Hsu and Turk and thus did not lead to the same secondary fragmentations.

The spectra can be described by the losses of the fatty acid moieties, $[M + Li - RCOOH]^+$ and $[M + Li - RCOOLi]^+$ and by the formation of the corresponding acylium ions RCO^+ from a parent TAG. All the fatty acids linked to the glycerol backbone were thus identified, giving for each TAG an overview of its composition. The complexity of the spectra obtained was due to the initial complexity of the TAG mixture. Indeed, for a given molecular peak of lithiated TAGs, corresponding to a given CN and number of double bonds, one can note that several fatty acids may be lost depending on the initial complexity of those TAG isomers. For example, the group of saturated TAGs $T_{44:0}$ from cow milk lost all the even-numbered fatty acids from $C_{8:0}$ to $C_{20:0}$, which meant that more than eight possible structures could exist for

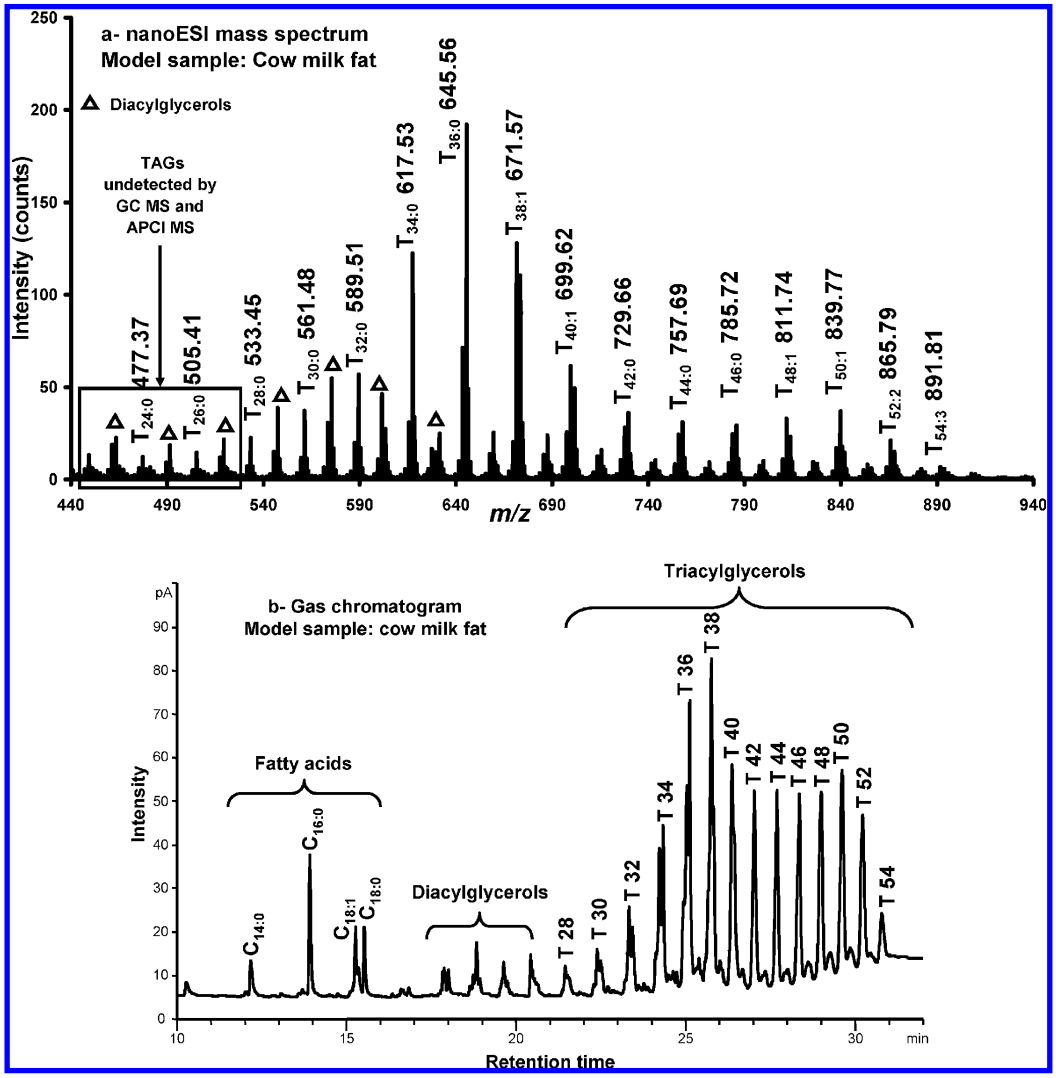

Figure 3. (a) NanoESI mass spectrum and (b) gas chromatogram of the total lipid extract from cow milk. For each group of TAGs with the same carbon number (CN), a single peak, the highest one, is assigned on the mass spectrum with the indication of the *m*/*z* and the name of the TAG.

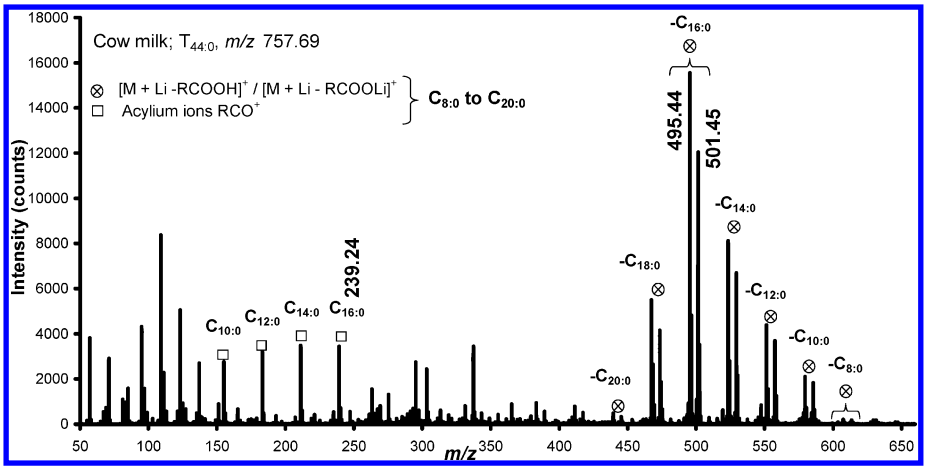

Figure 4. NanoESI MS/MS spectrum of the group of triacylglycerols $T_{44:0}$ from cow milk.

this group (Figure 4). To obtain a valuable way of discriminating cow and goat milk samples, dairy and subcutaneous samples, and cow and sheep subcutaneous fats, we studied in detail, for each group of TAGs defined by the same CN and number of unsaturation, the loss of lithiated fatty acid moieties (see Supporting Information 1).

Thus, we drew graphs that plot the relative intensity of the peak corresponding to the loss of a specific fatty acid ($[M + Li - RCOOLi]^+$) on the MS/MS spectrum against its number of carbon atoms for each series of TAGs with the same CN and unsaturation number (Figure 5: example of $T_{44:0}$ for the four model samples). Indeed, as demonstrated by Hsu and Turk, the intensities linked

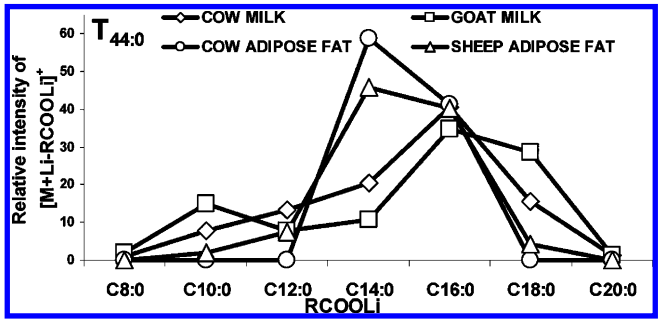

Figure 5. Graph plotting the relative intensity of the RCOOLi losses against the corresponding fatty acid for the group of TAGs $T_{44:0}$ (CN = 44, no unsaturation), for cow subcutaneous and milk fat, goat milk, and sheep subcutaneous fat.

with the loss of a fatty acid reported on the *y*-axis of graphs presented in Figure 5 are closely related to the structure of the isomers of TAGs.

First of all, we have compared the results obtained for the saturated TAGs extracted from cow substances from subcutaneous fats and milk (See Supporting Information 2). In subcutaneous fats, triacylglycerols were present in a narrow range, from T_{44} to T_{52}, whereas a larger distribution, from T_{24} to T_{52}, was observed in cow milk, as already discussed.[23,34,36] Concerning the saturated TAGs common to both substances, the distribution of fatty acids for TAGs with a carbon number of 48 was the same whatever the origin considered (adipose fat or milk) (See Supporting Information 2). However, the distribution of fatty acids involved in T_{44} and T_{46} was very different. Indeed, whereas T_{44} and T_{46} from adipose cow fat only contain two to four fatty acids ($C_{14:0}$ and $C_{16:0}$ for T_{44} and $C_{12:0}$, $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$ for T_{46}), they contain fatty acids ranging from $C_{8:0}$ (T_{44}) or $C_{10:0}$ (T_{46}) to $C_{20:0}$ in cow milk. This is important to note with the perspective of identifying archaeological samples. Indeed, it has been shown that low molecular weight TAGs were more sensitive to degradation processes than the others. Consequently, the differences observed on the structure of medium to long-chain TAGs, which are usually well preserved, could be further exploited for a better discrimination of cow adipose fat and milk.

Concerning the results on adipose fats from different species (sheep and cow), they both contain TAGs ranging from T_{44} to T_{52}. The distribution of fatty acids for $T_{48:0}$, $T_{50:0}$, and $T_{52:0}$ perfectly fits (See Supporting Information 4, example of $T_{48:0}$ and $T_{50:0}$). Clear differences may be noted for $T_{44:0}$ and $T_{46:0}$ concerning the fatty acids involved in each series of TAGs ($C_{10:0}$–$C_{18:0}$ for sheep versus

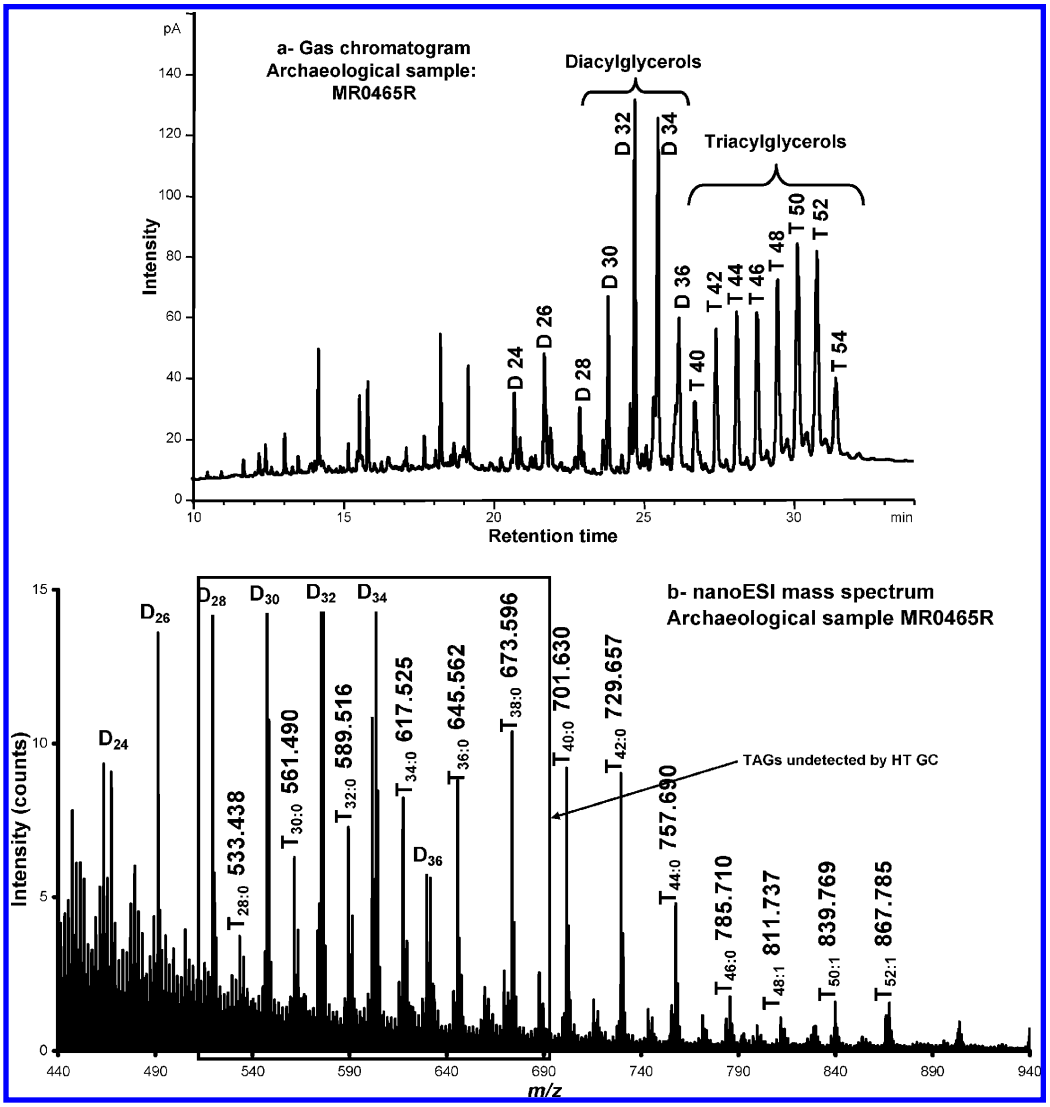

Figure 6. (a) Gas chromatogram and (b) nanoESI mass spectrum of the TAG fraction from the archaeological sample MR0465R.

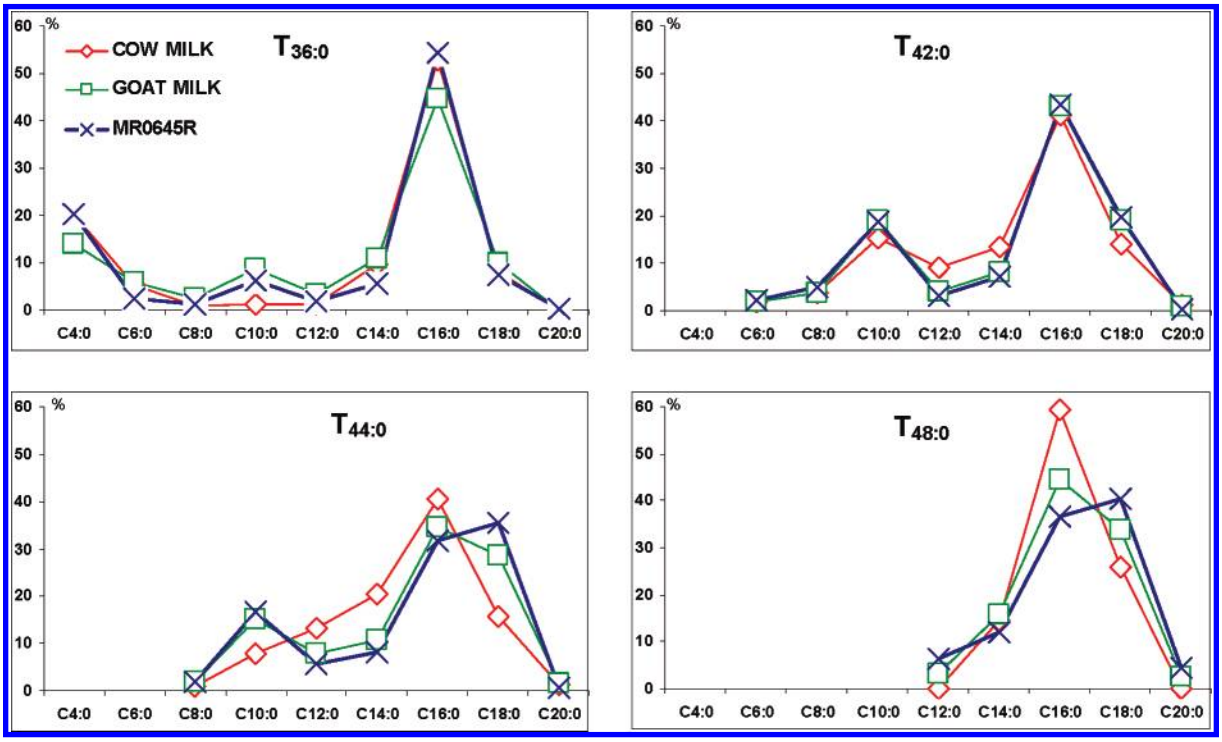

Figure 7. Graphs plotting the relative intensity of the fatty acid RCOOLi losses against their CN for $T_{36:0}$, $T_{42:0}$, $T_{44:0}$, and $T_{48:0}$ from the archaeological sample MR0465R (blue) compared with those from cow milk (red) and goat milk (green).

$C_{14:0}$ and $C_{16:0}$ for cow for $T_{44:0}$, for example), revealing some interesting differences in the fatty acid distributions for TAGs that could be used for distinguishing adipose fat from cow and sheep.

Last, in the case of comparison of milks from different species (cow and goat), the situation was much more complex due to the numerous fatty acids present in dairy products (See Supporting Information 3). Nevertheless some general information can be discussed. In most cases, the same fatty acids are involved in every series of TAGs with the same CN. This is true for $T_{28:0}$−$T_{38:0}$, with fatty acids ranging from $C_{4:0}$ to $C_{18:0}$, but also for $T_{42:0}$, $T_{44:0}$, and $T_{46:0}$, with fatty acids, respectively, ranging from $C_{6:0}$, $C_{8:0}$, and $C_{10:0}$ to $C_{20:0}$, and for $T_{50:0}$ that presents a distribution of fatty acids from $C_{14:0}$ to $C_{20:0}$. For a few TAGs, one or two fatty acids are present in one type of milk whereas they are absent from the other ($C_{18:0}$ present in goat $T_{24:0}$ but not in cow, $C_{20:0}$ present in goat $T_{26:0}$ but not in cow, $C_{20:0}$ present in $T_{40:0}$ from cow but not from goat, and $C_{12:0}$ and $C_{20:0}$ present in $T_{48:0}$ from goat but not in cow, see Supporting Information for details). The amount of $C_{10:0}$ in TAGs from goat milk was noticed to be systematically higher than that from cow milk, this being coherent with the literature.[34,35] The difference is particularly noticeable for $T_{24:0}$, $T_{26:0}$, $T_{28:0}$, $T_{30:0}$, $T_{32:0}$, and $T_{34:0}$. One must also note that the relative amount of $C_{12:0}$ and $C_{14:0}$ is lower in goat milk than in cow milk, particularly for $T_{44:0}$. To summarize the results obtained for milk samples, it appears that some noticeable differences exist in the composition of fatty acids for several TAGs, particularly $T_{24:0}$, $T_{26:0}$, $T_{28:0}$, $T_{30:0}$, $T_{32:0}$, $T_{34:0}$, $T_{40:0}$, $T_{44:0}$, and $T_{48:0}$. For all those samples, the differences noticed for unsaturated TAGs were not used as discriminative means, as they were not very characteristic. Moreover in archaeological degraded samples, unsaturated TAGs are barely preserved.

Thus, it appeared from all these results that the differentiation between subcutaneous and dairy fats, and between species, based on the identification of the fatty acid composition of each series of triacylglycerols with the same CN was interestingly achieved by the use of nanoESI MS/MS. This methodology was then applied to archaeological samples.

Archaeological Samples. The analysis of archaeological samples and the transposition of the discrimination means already shown for model samples were successfully implemented. We chose four samples, one from Chalain 4, MR0465R, and three from Clairvaux XIV, namely, T1050A, T1052A, and T1101A. From the HT GC and HT GC/MS analyses, two samples (Figures 1 and 2 for T1050A and Figure 6a for MR0465R) were shown to have large TAG distributions from T_{40} to T_{54}, whereas the other two presented narrower distributions, from T_{44-46} to T_{54} (Figures 1 and 2 for T1101A). The identification of the original products was thus quite straightforward for MR0465R and T1050A as degraded dairy products, as seen in Figure 2. No undoubted identification was possible from the gas chromatograms for the others, as the distributions were exactly in the nonconclusive range (Figure 2, second class). They could be either much-degraded dairy products or well-preserved subcutaneous fats. The specific origin of all four samples could not be deduced from the data obtained. For each sample, the fraction containing the triacylglycerols was isolated and analyzed by nanoESI MS and MS/MS. The nanoESI MS spectrum of MR0465R is presented in Figure 6b in parallel to its gas chromatogram (Figure 6a), as an example of the gain in sensitivity with nanoESI MS. Indeed, TAGs down to $T_{28:0}$ were detected, whereas GC could not detect TAGs under T_{40}. It is the first time to our knowledge that such a small TAG is detected in an archaeological sample. The distributions obtained by nanoESI MS for the other samples were much closer to those observed in GC: T_{40}−T_{52} for T1050A instead of T_{40}−T_{54}; T_{42}−T_{52} for T1052A instead of T_{44}−T_{54}, and T_{44}−T_{52} for T1101A instead of T_{44}−T_{54}. Only one ($T_{50:1}$ for T1101A and T1052A) to three ($T_{48:1}$, $T_{50:1}$, and $T_{52:1}$ for T1050A) unsaturated TAGs were detected for those

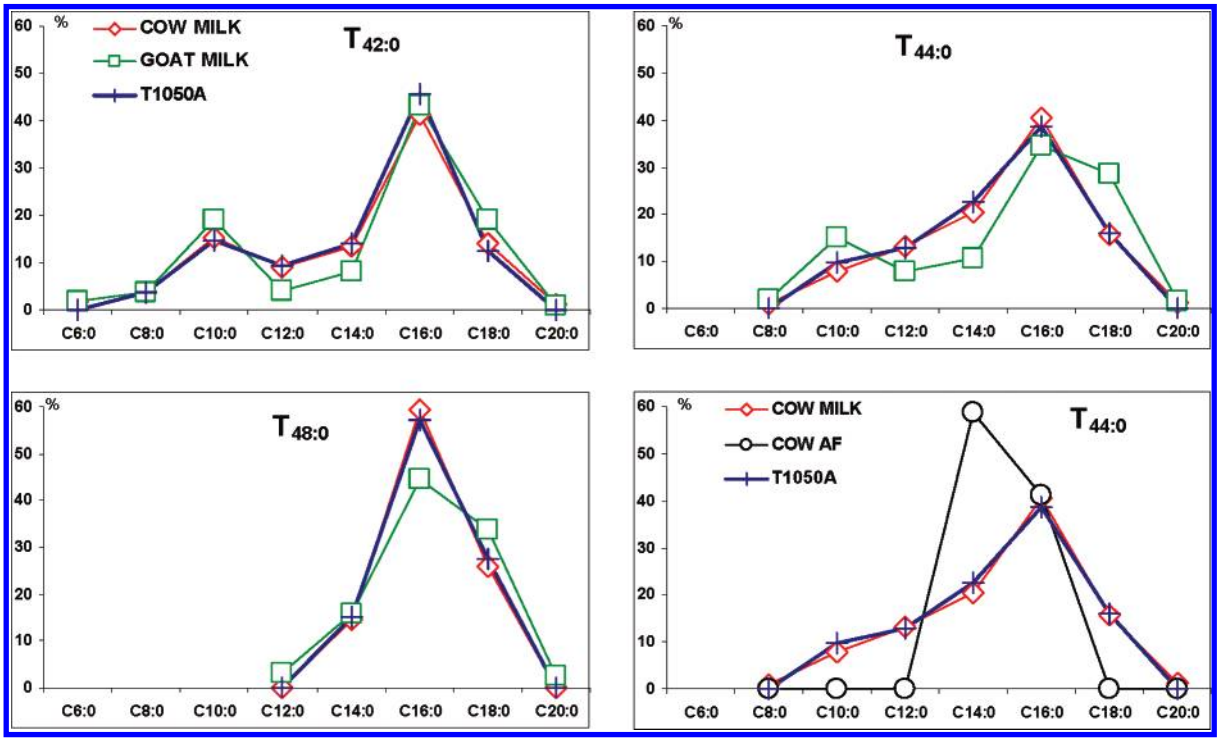

Figure 8. Graphs plotting the relative intensity of the fatty acid RCOOLi losses against their CN for the archaeological sample T1050A, compared with cow and goat dairy fat ($T_{42:0}$, $T_{44:0}$, and $T_{48:0}$) and cow subcutaneous and dairy fat ($T_{44:0}$).

samples. In none of the samples was T_{54} detected by nanoESI MS, probably because of its presence in very low amount and its poor ionization yield.

For sample MR0465R, among the 16 TAG species detected and fragmented, 5 were unsaturated, with even 2 double bonds for $T_{52:2}$. The fatty acid distributions were rather large too. For example, $T_{32:0}$ had even-numbered fatty acids from $C_{4:0}$ to $C_{20:0}$, which is surprising for an archaeological sample, as short-chain TAGs are preferentially hydrolyzed, and short-chain fatty acids are lost. This demonstrated, if needed, the exceptional state of preservation of the organic matter on this archaeological site. Given its TAG distribution and the presence of small fatty acids such as $C_{4:0}$ and $C_{6:0}$, this sample was readily identified as dairy product. The question of its specific origin was then raised. Comparison of each group of TAG and its fatty acid (FA) distribution with that of cow and goat milks, it appeared that it is most probably derived from goat. Indeed, four TAGs have FA distributions closer to that of goat milk than that of cow milk, namely, $T_{36:0}$, $T_{42:0}$, $T_{44:0}$, and $T_{48:0}$ (Figure 7). The intensity of the $C_{10:0}$ loss for $T_{36:0}$ and $T_{44:0}$ was also higher than that of cow milk, very close to that of goat milk. As described for the model samples, the data obtained for unsaturated TAGs were not conclusive and could not be used as discriminative criteria.

We could note some general trends for the Clairvaux XIV samples when monitoring the fragmentation experiments. The fatty acids distributions were small, from $C_{8:0}$ (T1050A), $C_{10:0}$ (TT1052A), and $C_{12:0}$ (T1101A)−$C_{18:0}$. Those samples came from an archaeological site that is a millennium older than Chalain 4, and the conservation conditions must have been less favorable. The identification of those samples was not easy. For T1050A, two TAGs were characteristic of milk fat versus adipose fat, namely, $T_{42:0}$ and $T_{44:0}$. Indeed, $T_{42:0}$ does not exist in subcutaneous fats, and $T_{44:0}$ had fatty acids from $C_{10:0}$ to $C_{18:0}$, whereas subcutaneous $T_{44:0}$ has only $C_{16:0}$ and $C_{18:0}$ (Figure 8). Moreover, the comparison of TAGs $T_{42:0}$, $T_{44:0}$, and $T_{48:0}$ with goat milk versus cow milk is in favor of a cow origin as their patterns closely matched those of cow milk (Figure 8). The last three saturated TAGs were more characteristic in their fatty acid composition of cow adipose fat. This was most probably a consequence of the aging of triacylglycerols. Among the five saturated TAGs detected and identified from sample T1101A, one was characteristic of sheep subcutaneous fat, namely, $T_{44:0}$. Two were characteristic of subcutaneous fat versus milk fat, namely, $T_{46:0}$ and $T_{48:0}$. The last two TAGs $T_{50:0}$ and $T_{52:0}$ are not present in model milk samples and could thus not be used as discriminative means. They could be characteristic of subcutaneous fat or of degraded milk fat. Given the information obtained, it can be concluded that this sample is subcutaneous fat, probably from sheep. The last sample T1052A was not identified further than degraded animal fat. Indeed, the FA composition and distributions for its five saturated TAGs were very close to that of adipose fat but with no special feature that could allow a more precise determination.

CONCLUSION

The complexity, the state of degradation, and the low amount of preserved matter in archaeological samples usually necessitate the combination of several complementary analytical techniques for unravelling their molecular composition and then for determining their natural origin.[7,37−40] In the case of organic compounds preserved in ceramic potsherds, most of the studies were based

(37) Regert, M.; Langlois, J.; Laval, E.; Le Hô, A.-S.; Pagès-Camagna, S. *Anal. Chim. Acta* **2006**, *577*, 140−152.
(38) Regert, M.; Langlois, J.; Colinart, S. *J. Chromatogr., A* **2005**, *1091*, 124−136.
(39) Garnier, N.; Cren-Olive, C.; Rolando, C.; Regert, M. *Anal. Chem.* **2002**, *74*, 4868−4877.
(40) Regert, M. *BAR* **2007**, *S1650*, 61−76.

either on GC/EI-MS analysis or on the use of soft ionization modes such as APCI and ESI, but such techniques were rarely used together in the field of archaeology.[39]

Combined preliminary analysis by HT GC and HT GC/MS on a large series of samples, in order to obtain information on their composition and their state of alteration, and ESI MS followed by ESI MS/MS, to obtain precise structural information on TAG biomarkers, was performed here for the first time. This methodology allowed detecting the presence of animal fats in a series of 22 vessels from 2 neolithic sites out of a total of 63 analyzed by HT GC. Among them, four samples were investigated by ESI MS and ESI MS/MS after purification of the TAG fraction and their lithiation. This technique was revealed to be very sensitive, which considerably enhanced the probability to detect molecular compounds present in low amount in archaeological samples. Particularly, TAGs with 28 and 30 carbon atoms, never mentioned until now in any archaeological sample, could be detected and identified. Furthermore, by comparing the results obtained on archaeological and model samples (cow milk and adipose fat, sheep adipose fat, goat milk), it was possible to draw new molecular criteria for distinguishing these substances. Indeed, by a detailed study of the ESI MS/MS data obtained, it was possible, for a number of series of TAGs (same CN, same number of unsaturations), to reveal some specific differences in their fatty acid distribution. By this way, goat milk was suspected in a vessel from the site of Chalain 4, cow milk in a vessel from Clairvaux XIV, and sheep adipose fat in another vessel from Clairvaux XIV.

The possibility to discriminate the specific origin of different animal fats with molecular criteria and the implementation of this methodology to archaeological samples are of prime importance to understand more precisely the ways of exploitation of domesticated animals over the neolithic period. Such results, that now have to be extended to other model samples, to artificially aged materials and to various archaeological potsherds, open new avenues for a better understanding of the structural characteristics of TAGs preserved through time. It should also allow gaining insight into the strategies of procurement and consumption of animal fats during prehistory. From a more general point of view, being able to fully characterize the structure of TAGs in fatty substances may have further applications in the field of adulteration of dairy products and animal fats. This could also permit a better understanding of the quality and the composition of animal fats in relation with the diet and the species or subspecies of the animals from which they are issued.

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SUPPORTING INFORMATION AVAILABLE

Relative intensities of RCOOLi losses for each series of TAGs from the four model samples and plots of intensity of fatty acid RCOOLi losses vs CN. This material is available free of charge via the Internet at http://pubs.acs.org.

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