Received: 1 March 2024 | Revised: 26 April 2024 | Accepted: 27 April 2024

DOI: 10.1002/rcm.9771

RESEARCH ARTICLE

Rapid Communications in Mass Spectrometry WILEY

Alkylresorcinol detection and identification in archaeological pottery using ultra-high-performance liquid chromatography-quadrupole/Orbitrap mass spectrometry

Jonica Ella Doliente[1,2,3] | Swen Langer[4,5] | Marc R. Dickinson[1] | Miriam Cubas[6] | André C. Colonese[3] | Kirsty Penkman[1] | Oliver E. Craig[2]

[1]Department of Chemistry, University of York, York, UK

[2]BioArCh, Department of Archaeology, University of York, York, UK

[3]Department of Prehistory, Institute of Environmental Science and Technology (ICTA), Universitat Autònoma de Barcelona, Barcelona, Spain

[4]Department of Biology, Bioscience Technology Facility, University of York, York, UK

[5]Centre of Excellence in Mass Spectrometry, University of York, York, UK

[6]Department of History and Philosophy, University of Alcalá, Madrid, Spain

Correspondence
Jonica Ella Doliente, Department of Chemistry, University of York, York YO10 5DD, UK.
Email: jonica.doliente@york.ac.uk

Funding information
This project is part of a Marie Sklodowska-Curie innovative training network (ITN)—ChemArch: The Chemistry and Molecular Biology of Prehistoric Artefacts, funded by the European Union's Horizon 2020 Research and Innovation programme under the H2020 Marie Skłodowska-Curie Actions grant agreement number 956351.

Rationale: Alkylresorcinols (AR) are cereal-specific biomarkers and have recently been found in archaeological pots. However, their low concentrations and high susceptibility to degradation make them difficult to detect using conventional gas chromatography mass spectrometry (GC/MS). Here we describe the development of a more sensitive liquid chromatography mass spectrometry (LC/MS) method to detect these compounds.

Method: A method based on the use of ultra-high-performance liquid chromatography (UHPLC) coupled to an Orbitrap mass analyser was established and validated for the detection of low-concentration ARs in pottery. During the preliminary experiments, UHPLC-Q/Orbitrap MS (ultra-high-performance liquid chromatography-quadrupole/Orbitrap mass spectrometry) was demonstrated to be more sensitive, and a wide range of AR homologues in cereal extracts were detected, unlike UHPLC-QTOFMS (ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry) and GC/MS. The developed method was utilised to profile AR homologue distribution in modern cereal samples and reanalyse AR-containing pots from the archaeological site of Must Farm.

Results: A highly sensitive LC/MS method with a limit of detection (LOD) of 0.02 μg/g and a limit of quantification (LOQ) of 0.06 μg/g was used to profile ARs in five modern cereal grains. The obtained LOD is 250 times lower than that obtained using the conventional GC/MS approach. AR 21:0 was the most abundant homologue in all four *Triticum* spp.—einkorn, emmer, Khorasan wheat and common wheat. Meanwhile, AR 25:0 was the predominant homologue in barley, potentially enabling differentiation between wheat and barley. The developed LC/MS-based method was successfully used to analyse ARs extracted from Must Farm potsherds and identified the cereal species most likely processed in the pots—emmer wheat.

Conclusion: The described method offers an alternative and more sensitive approach for detecting and identifying ARs in ancient pottery. It has been successfully utilised to detect AR homologues in archaeological samples and discriminate which cereal species—wheat and barley—were processed in the pots.

1 | INTRODUCTION

Cultivation of cereals (e.g., wheat and barley) is thought to have been a major driver of population growth and sedentism during the spread of the Neolithic in Europe,[1] but their relative economic importance and social significance are debated.[1–3] Apart from the direct recovery of botanical and faunal remains in archaeological deposits, chemical analysis of residues from ceramic cooking vessels is an essential line of evidence for investigating ancient food production systems. Such studies have tended to focus on animal products,[4–8] although the chemical identification of cereal remains associated with ceramic artefacts remains a challenge due to their relatively low quantities of chemically stable lipid compounds.[9]

Alkylresorcinols (ARs) have been recently recognised as biomarkers for the presence of cereals in archaeological pottery from cold climates (e.g., the Alps)[10] and in anoxic conditions (e.g., waterlogged environments).[11,12] They are a class of phenolic lipids characterised by a nonpolar odd-numbered alkyl side chain attached to a polar resorcinol ring (Figure 1A).[13] ARs are reported to be present in high concentrations in rye and wheat, and in low concentrations in barley and millet,[13–15] with alkyl chains ranging from 15 to 27 carbon atoms. The shorthand naming convention for AR homologues is based on the length of the alkyl chain (e.g., the homologue with a 17-carbon alkyl chain is referred to as AR 17:0). Additionally, their survival in ancient pots depends highly on burial contexts, with anoxic environments providing the most ideal conditions.[10–12] Detecting these phenolic lipids in archaeological deposits offers a means to determine the spread of cereal exploitation by prehistoric communities,[10,16] especially when botanical remains are poorly preserved or were not adequately collected in archaeological excavations.

Analysis of ARs has typically been carried out using gas chromatography mass spectrometry (GC/MS)–based methods.[10–12,17] Their high susceptibility to degradation during cooking and microbial attack in burial environments[10,17–19] make these phenolic lipids elusive. Several strategies have been developed to increase the probability of detecting ARs using GC/MS. These include optimisation of the extraction process, application of enrichment steps for ARs and utilisation of targeted approaches, such as selected ion monitoring (SIM).[11,20] Gas chromatography-quadrupole time-of-flight mass spectrometry (GC-Q/TOFMS) has also been used to increase the sensitivity of the approach.[11,12,17]

Whereas GC/MS is becoming a conventional method for AR identification in archaeological ceramics,[10,11,17] liquid chromatography mass spectrometry (LC/MS) has been extensively used for decades for AR profiling and quantification in modern cereals.[21-24] High-performance liquid chromatography-diode array detector/fluorescence detector-multi-stage mass spectrometry (HPLC-DAD/FLD-MS^n) was used by Ziegler et al.[23] to analyse the total AR concentration of various *Triticum* spp. with different ploidy degrees. Ploidy level refers to the number of chromosome sets in a cell, with prefixes indicating the number of sets in a given organism and the symbol '×' indicating the number of chromosomes in a set (e.g., diploid [2×] has two sets of chromosomes). The study revealed that the relative abundance of AR homologues differed significantly among hexaploid (common wheat), tetraploid (Khorasan wheat and emmer) and diploid (einkorn) species.[23] This information could be valuable in distinguishing between different types of wheat species.[23]

LC-TOF and LC-Orbitrap have been increasingly utilised because of their high selectivity, accurate mass measurement (<5 ppm) and high mass resolution (>20 000 FWHM [full width at half maximum]).[25,26] Some key advantages of LC/MS-based methods include minimal sample preparation, fast scan speed and remarkable sensitivity. The main drawback of these high-resolution methods is the relatively high analytical cost (associated with the overall high facility cost), but where this technique is available, it can enable the detection of ARs in modern specimens with much lower concentrations than the conventional GC/MS approach. The utilisation of these techniques in characterising ARs from archaeological pottery has not been reported before; this study therefore aims to determine the efficacy of an LC/MS approach for archaeological ARs.

Preliminary experiments comparing the performance of high-temperature GC/MS, UHPLC-Q/TOFMS and UHPLC-Q/Orbitrap MS were conducted to assess their sensitivity and selectivity in detecting AR homologues in five modern cereal species. Of the three approaches, UHPLC-Q/Orbitrap MS was found to be the most sensitive. We, therefore, used this instrumentation to develop and validate a more sensitive approach to detect and identify AR homologues in modern cereal species and archaeological pottery. We present an LC/MS approach that can rapidly and accurately quantify

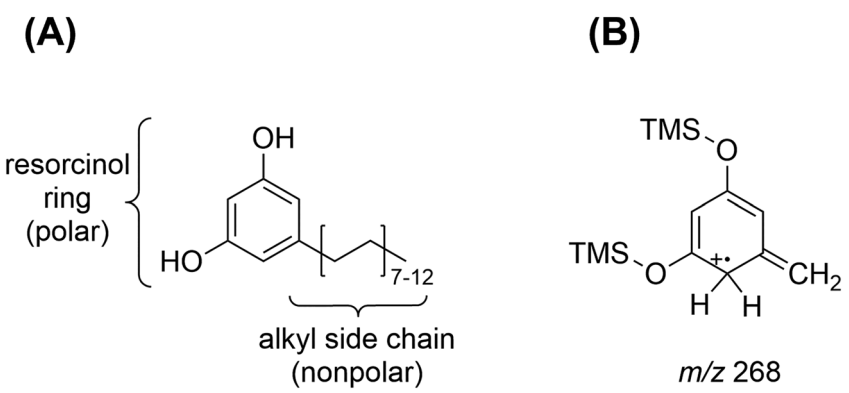


FIGURE 1 (A) General structure of alkylresorcinol and (B) the characteristic base fragment ion of TMS (trimethylsilyl)–derivatised alkylresorcinol at *m/z* 268.

the components detected to establish AR homologue distribution in modern cereal and total archaeological lipid extracts.

2 | MATERIALS AND METHODS

2.1 | Chemicals and materials

The 5-*n*-docosylresorcinol (AR 22:0; purity: >98%) standard was purchased from ReseaChem Life Sciences (Burgdorf, Switzerland). The silylating agent, *N*,*O*-*bis*(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA/TMCS, 99:1, v/v), was obtained from Thermo Fisher Scientific Inc. (Illinois, USA), and the internal standards, *n*-tetratriacontane (C34, >98%) and *n*-hexatriacontane (C36, >98%), were purchased from Scientific Laboratory Supplies Ltd (Nottingham, UK). HPLC-grade isopropyl alcohol was obtained from Sigma-Aldrich (Darmstadt, Germany). HPLC-grade methanol, dichloromethane and hexane were obtained from Fisher Scientific (Leicestershire, UK). HPLC-grade acetic acid was provided by VWR (Leicestershire, UK).

Five cereal species, four of which are varieties of *Triticum* spp. (wheat), were collected and assessed (Table S1). The Khorasan, emmer and einkorn varieties were sourced and stone-milled by Shipton Mill Ltd (Gloucestershire, UK). Meanwhile, dehulled bread wheat, purchased from Bobby's Healthy Shop Karowi Ltd (Devon, UK), and barley grains, produced by the University of Highlands and Islands (Orkney, UK), were crushed in the laboratory to a fine homogenised powder using a dichloromethane-washed mortar and pestle. These cereal species were chosen because they are relevant to our study site—the Late Bronze Age site of Must Farm, Cambridgeshire, UK.[27]

To test the efficacy of this approach on archaeological material, archaeological ceramics were selected from the site of Must Farm. The site's stratigraphy reveals two main events—the construction of structures and the creation of middens, followed by their collapse due to a fire.[27] The Late Bronze Age site showed excellent anoxic preservation due to a wetland setting.[27] Excavations revealed four roundhouses (structures 1, 2, 3 and 5) dating to 1000–800 cal BC. The analysed potsherds were from structure 1, and archaeobotanical analysis of the same roundhouse structure revealed the charred remains of flax, barley and emmer wheat,[27] indicating that this structure was used to store plant resources. Lipid extraction was undertaken on 80 ceramic powdered samples from the Must Farm assemblage.[27] The powdered samples were collected by drilling into the cleaned surface of the interior wall of the sherds using a low-speed electric drill equipped with an abrasive tungsten drill bit. Eight sherds (Table S2) are discussed here in relation to AR detection.

2.2 | Sample preparation

The extraction of lipids from cereals was carried out according to the published methodology.[28] Briefly, cereal powder (~300 mg) was placed in sterilised 8-mL glass culture tubes and spiked with 10 μg of the internal standard (C34 alkane). The powdered sample was extracted thrice under sonication with 5 mL of dichloromethane/methanol (2:1, v/v). After centrifugation, the successive AR-containing supernatants were combined and evaporated under nitrogen flow. The same method,[28] with one modification, was used to extract lipids from the archaeological pots. Instead of using a smaller amount of powdered sample, ~1 g of ceramic powder was used. The dried cereal and Must Farm total lipid extracts (TLE) were stored at −20°C until further sample purification process and chromatographic analyses.

Sample purification was performed to increase sensitivity by concentrating any ARs and removing interferences that may cause coeluting peaks and high background noise. Such interferences include the animal fats that often exist in ancient pots in high concentration, making the detection of low-content ARs more challenging.[11] Moreover, purification through solid-phase extraction (SPE) protects the LC column from contaminants (e.g., ceramic fine powder).[29] Accumulation of these inorganic contaminants leads to an increase in operating pressure, affecting chromatographic selectivity.[30]

The purification protocol involved sample cleanup using silica filters and AR enhancement using commercial SPE columns, as previously described.[11] The protocol was applied with minor modifications. The filter for the sample cleanup was prepared by packing a 3-mL glass column (1 cm i.d.) with 0.5 g of activated silica and conditioned using 5 mL of dichloromethane/methanol (2:1, v/v). The dried TLEs of cereals and archaeological potsherds were redissolved in 2 mL of dichloromethane/methanol (2:1, v/v), and an aliquot (1 mL) was passed through the prepared filter and eluted using 3 × 2 mL of dichloromethane/methanol (2:1, v/v). The solvent was then removed under nitrogen flow.

The extracts were reconstituted in 1 mL of hexane and introduced into a preconditioned SPE column (Isolute NH2, Biotage, 500 mg, 6 mL) after being spiked with 1 μg of AR 22:0 standard. AR 22:0 was utilised because it was not reported in cereals before but was detected in quinoa.[31] The column was preconditioned using 6 mL of hexane. Potential interferences, such as nonpolar lipids, were eluted first using 6 mL of hexane (fraction A) and 6 mL of dichloromethane (fraction B). Then, the targeted ARs were eluted using 6 mL of methanol (fraction C). All fractions were collected and evaporated to dryness using nitrogen flow. Prior to chromatographic analyses, serial dilutions of the purified cereal extracts (fraction C) were prepared: 1:10, 1:100, 1:1000, 1:2000 and 1:10 000.

2.3 | Sample derivatisation and GC/MS conditions

The derivatisation step and GC/MS analysis were previously described.[28,32] The dried, purified and diluted cereal extracts were reconstituted in 50 μL of hexane and derivatised using a silylating agent, BSTFA/TMCS (99:1, v/v). The vial was gently flushed with nitrogen, sealed and heated at 70°C for 60 min. Then, the sample was evaporated to dryness under a nitrogen flow, removing the excess silylating agent. The resulting residue was reconstituted in 90 μL and 10 μg of the second internal standard (C36 alkane), transferred to a 2-mL GC vial with insert and used for GC/MS analysis.

The silylated samples were injected into a Clarus 690 gas chromatograph coupled to a Clarus SQ 8 T mass spectrometer (PerkinElmer) in electron ionisation mode (70 eV). The separation was performed on a DB-5HT (5%-phenyl)-methylpolysiloxane column (30 m × 0.250 mm × 0.10 μm, J&W Scientific, Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was set at 50°C for 2 min and then increased to 375°C at a rate of 10°C/min, where it was held for 15 min. The spectra were obtained in full-scan (*m/z* 50–610) and SIM (*m/z* 73, 268, 464, 492, 520, 548, 576 and 604) modes. The latter mode was applied to target ions of AR homologues.

2.4 | UHPLC-Q/TOFMS conditions

An ACQUITY I-Class UHPLC separation system (Waters, UK) coupled to a maXis HD QTOF mass spectrometer (Bruker, UK) equipped with an electrospray ionisation interface was used for the analysis of the serially diluted extracts. The purified cereal residues were reconstituted in 300 μL of methanol/isopropanol (7:3, v/v) and were analysed in duplicate, modifying the reported method elsewhere.[24] Briefly, ARs were isolated using a reversed-phase C18 ACQUITY HSS T3 column (2.1 × 100 mm, 1.8-μm particle size) (Waters) at 40°C with methanol/water (8:2, v/v) as solvent A and methanol/isopropanol (7:3, v/v) as solvent B. Both solvents were acidified with 0.1% acetic acid. The binary elution gradient was programmed as follows: 0–2 min (0% solvent B), 2–14 min (0%–100% solvent B), 14–16 min (100% solvent B), 16–16.1 min (100%–0% solvent B) and 16.1–17 min (0% solvent B). The flow rate and injection volume were set to 0.5 mL/min and 2 μL, respectively. Data were collected in negative electrospray mode and scanned between *m/z* 40 and 1000. The source settings were as follows: capillary voltage, 3 kV; drying gas heater, 300°C; and drying gas flow, 8 L/min.

2.5 | UHPLC-Q/Orbitrap MS conditions

The same LC separation system described in Section 2.4 was coupled to an Orbitrap Fusion Tribrid mass spectrometer (Thermo, UK). The purified cereal and archaeological residues were reconstituted in 300 μL of methanol/isopropanol (7:3, v/v) and were analysed in duplicate. The mass spectrometer was operated in negative mode at 60 000 resolution (FWHM) for MS1 data and a scan range of 120–1200 *m/z*. The spectra were obtained at a spray voltage of 2500 V and sheath, auxiliary and sweep gas settings of 50, 10 and 1 arbitrary units, respectively. The collected data were analysed using Xcalibur, version 4.0.27, software.

2.6 | Method validation

The method used in the study was validated for linearity, limit of detection (LOD) and limit of quantification (LOQ) following the guidelines of the International Conference of Harmonisation (ICH).[33] According to the ICH guidelines,[33] LOD refers to the lowest amount of analyte in the sample that can be detected and is not necessarily quantifiable as an exact value. LOQ, on the contrary, is the lowest detectable amount of analyte that can be quantified.

Linearity was tested on the standard solutions of AR 22:0, and the calibration curve was obtained by analysing standard solutions at seven concentration levels between 0.0025 and 0.5 ng/μL. A lower set of standard solutions was used to obtain a linear response. It was constructed by plotting the peak area (*y*) versus the seven concentrations of AR 22:0 (*x*) with least square linear regression. LOD and LOQ were calculated based on the standard deviation of the signals and the slope of the calibration curve. The values were obtained using the following equations from the ICH[33]:

$$\mathrm{LOD} = 3.3(\mathrm{SD}/S) \quad (1)$$

$$\mathrm{LOQ} = 10(\mathrm{SD}/S) \quad (2)$$

where SD is the standard deviation of the response and *S* is the slope of the calibration curve.[33]

3 | RESULTS AND DISCUSSION

3.1 | Comparison of the three instruments

The performance of UHPLC-Q/Orbitrap MS, UHPLC-Q/TOFMS and GC/MS was first assessed by detecting the presence of AR homologues in five cereal species to determine the most appropriate instrumentation for AR analysis. The latter used a GC system to separate the lipids of interest. The remaining two used an LC system, and thus, a derivatisation step is not required.

ARs were analysed using GC/MS after they were converted to their respective trimethylsilyl (TMS) ethers. TMS derivatives of AR provide characteristic electron mass spectra with a distinct base fragment at *m/z* 268 (Figure 1B), which is due to the McLafferty rearrangement of the phenolic ring.[20] Moreover, GC/MS analysis was performed in SIM mode, which results in better sensitivity as it lowers the detection limit[34] by monitoring only the targeted ions—*m/z* 73, 268, 464, 492, 520, 548, 576 and 604. These molecular ions correspond to the *bis*-TMS derivatives of saturated AR homologues (Table 1) and, together with the base ion *m/z* 268, confirm the identity of the targeted compounds. The SIM (*m/z* 268) chromatogram of common wheat samples (Figure 2A) shows the presence of saturated ARs. The mass spectra (Figure 2B) further substantiate their identities. Meanwhile, AR homologues were identified using LC/MS-based methods by examining their respective accurate masses (Table 1), retention times and fragmentation patterns. The saturated and unsaturated AR homologues were quantified using AR 22:0 (0.002–200 ng/μL) as an external calibration standard, and the total AR concentration was obtained by calculating the sum of the saturated and unsaturated AR homologues. Figure 3A

TABLE 1 Molecular formulas and accurate masses used to identify underivatised and derivatised ARs.

AR homologue	Molecular formula (underivatised)	$[M-H]^-$ theoretical mass (*m/z*)	Molecular formula (derivatised)	*Bis*-TMS theoretical mass (*m/z*)
AR 17:1	$C_{23}H_{38}O_2$	345.2799	$C_{29}H_{54}O_2Si_2$	490.3663
AR 17:0	$C_{23}H_{40}O_2$	347.2956	$C_{29}H_{56}O_2Si_2$	492.3819
AR 19:1	$C_{25}H_{42}O_2$	373.3112	$C_{31}H_{58}O_2Si_2$	518.3975
AR 19:0	$C_{25}H_{44}O_2$	375.3269	$C_{31}H_{60}O_2Si_2$	520.4132
AR 21:1	$C_{27}H_{46}O_2$	401.3425	$C_{33}H_{62}O_2Si_2$	546.4289
AR 21:0	$C_{27}H_{48}O_2$	403.3582	$C_{33}H_{64}O_2Si_2$	548.4445
AR 22:0[a]	$C_{28}H_{50}O_2$	417.3738	$C_{34}H_{66}O_2Si_2$	562.4602
AR 23:1	$C_{29}H_{50}O_2$	429.3738	$C_{35}H_{66}O_2Si_2$	574.4602
AR 23:0	$C_{29}H_{52}O_2$	431.3895	$C_{35}H_{68}O_2Si_2$	576.4758
AR 25:1	$C_{31}H_{54}O_2$	457.4051	$C_{37}H_{70}O_2Si_2$	602.4915
AR 25:0	$C_{31}H_{56}O_2$	459.4208	$C_{37}H_{72}O_2Si_2$	604.5071

Note: The silylating agent utilised was BSTFA/TCMS (99:1, v/v).
Abbreviations: AR, alkylresorcinol; BSTFA, *N,O-bis*(trimethylsilyl)trifluoroacetamide; TMCS, trimethylchlorosilane; TMS, trimethylsilyl.
[a]Standard used to quantify AR homologues.

shows the UHPLC-Q/Orbitrap MS chromatogram of common wheat extract, and the obtained MS spectra (Figure 3B) established the identification of the homologues.

Preliminary results revealed that LC/MS is a more effective technique for AR detection and identification in modern cereals. Overall, UHPLC-Q/Orbitrap MS was the most sensitive instrument among the three techniques and was able to detect a wider range of AR homologues (Table S3). In particular, it detected more types (saturated, unsaturated and oxidised forms) and numbers of AR homologues even in a more diluted concentration than UHPLC-Q/TOFMS and the conventional GC/MS in SIM mode (see Supplementary Discussion). UHPLC-Q/Orbitrap MS is 10- and 100-fold more sensitive than UHPLC-Q/TOFMS and GC/MS, respectively (Figure S1). Moreover, analysis time using the LC/MS-based instrument is six times less than that using the GC/MS. We note, however, that usage of high-resolution techniques (i.e., TOFMS and Orbitrap MS) costs approximately thrice as much per sample as the conventional GC/MS, although this cost will, of course, depend on the facility. Given these observations, it was therefore decided to develop and validate a protocol based on a UHPLC system coupled to an Orbitrap.

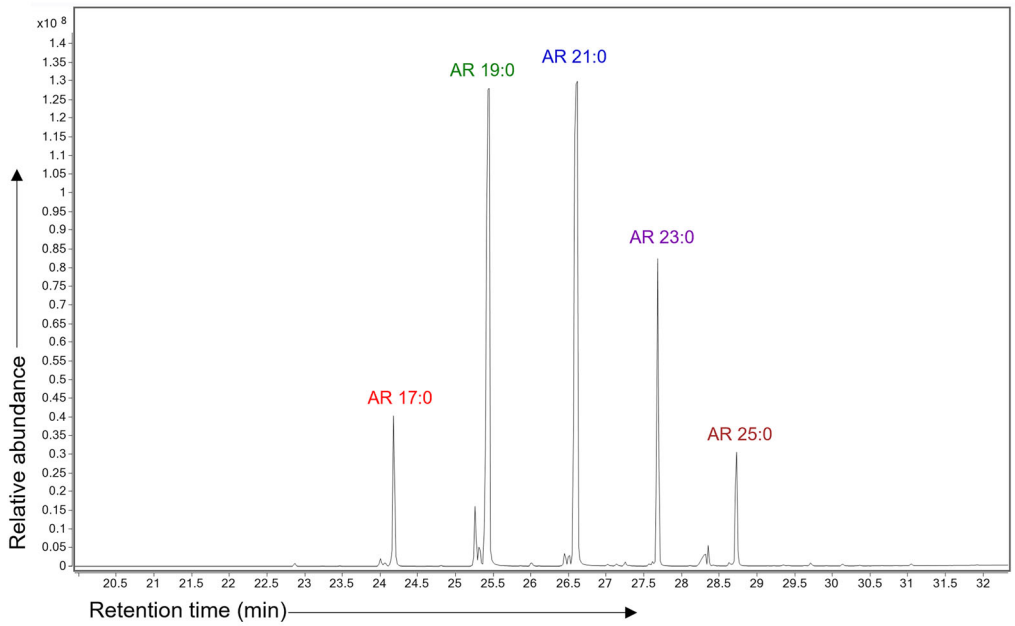
3.2 | Method validation using the UHPLC-Q/Orbitrap MS

The 7-point calibration curve of AR 22:0 standard solutions was linear over the whole concentration range, resulting in a coefficient of determination (R^2) >0.999 (Figure S2). The linear range was 0.002–0.5 μg/g, and the regression equation was $y = 1.36E+07x + 6.19E+04$. The calculated LOD and LOQ values were 0.02 and 0.06 μg/g, respectively. The main validation parameters are summarised in Table 2. The obtained results demonstrate that the LC/MS-based method exhibited greater sensitivity compared to the conventional GC/MS-based method. The LOD of the developed method is 250- and 10-fold lower than the LOD reported using GC/MS and GC-Q/TOFMS, respectively.[11,13,24] This observation is mainly due to Orbitrap's superior sensitivity and high-resolution capabilities.[35]

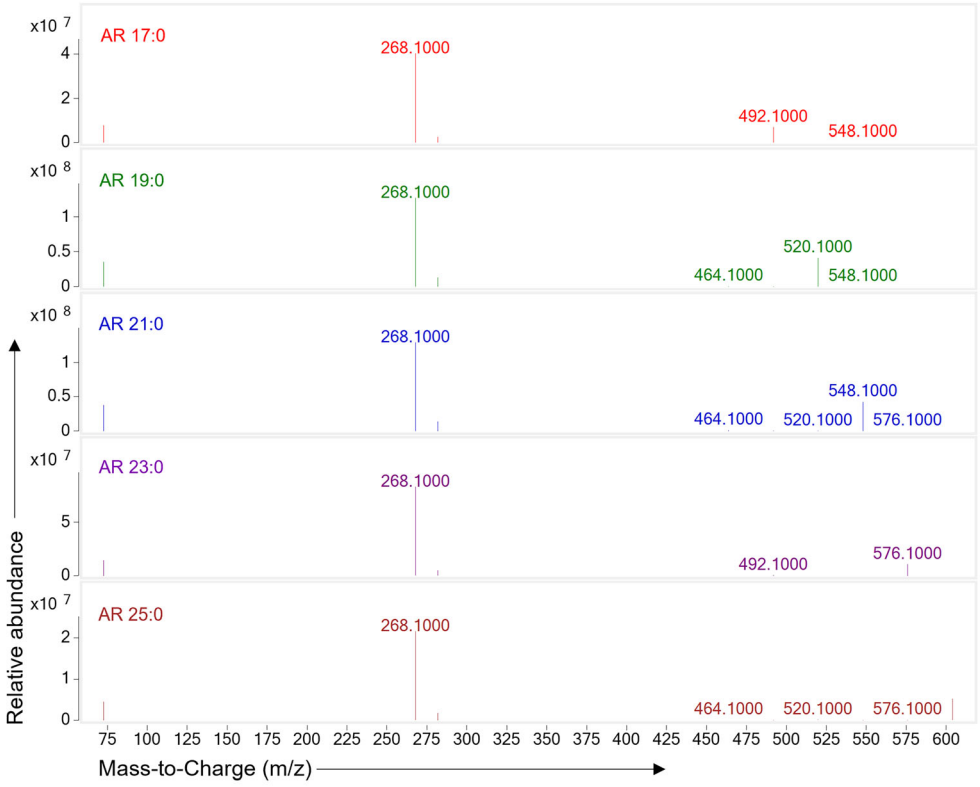
3.3 | AR characterisation in modern cereals

3.3.1 | *Triticum* spp

Ten AR homologues (five saturated and five unsaturated ARs; Figure S3) were identified in wheat extracts, and their relative contributions are summarised in Table 3. The dominant saturated homologues were AR 19:0, AR 21:0 and AR 23:0, with AR 21:0 being the predominant homologue in all four species (Figure 4A), which agreed with previous reports of analyses of modern cereals.[23,24] The percentage of AR 21:0 ranged from 31.5% (common wheat) to 48.3% (Khorasan wheat). AR 23:0 was the second most abundant homologue in the tetraploid (emmer [24.0%] and Khorasan wheat [25.3%]) and diploid (einkorn [23.0%]) species, followed by AR 19:0, AR 25:0 and AR 17:0. In contrast, the hexaploid common wheat contained higher contributions of AR 19:0 (27.8%), followed by AR 23:0, AR 25:0 and AR 17:0. The overall trends of AR homologues in each wheat species are presented in Figure 4. AR 21:0 is the most abundant homologue, and the trends observed in common wheat, emmer and einkorn species agreed with previously reported data[23,24] (analysis of AR compositions in Khorasan wheat has not yet been reported). The relative abundance of AR 17:0 of the common wheat was at most seven times higher (5.9%) than that in the other species (0.8%–1.2%). Such highly variable differences in the relative abundance of AR homologues among the *Triticum* species have been previously reported.[36]



(A)



(B)

FIGURE 2 (A) GC/MS (gas chromatography mass spectrometry) SIM (selected ion monitoring) (*m/z* 268) chromatogram of TMS (trimethylsilyl)–derivatised ARs (alkylresorcinols) detected in purified common wheat samples. (B) The presence of five saturated homologues—AR 17:0, AR 19:0, AR 21:0, AR 23:0 and AR 25:0—was confirmed by their mass spectra. IS, internal standard. [Color figure can be viewed at wileyonlinelibrary.com]

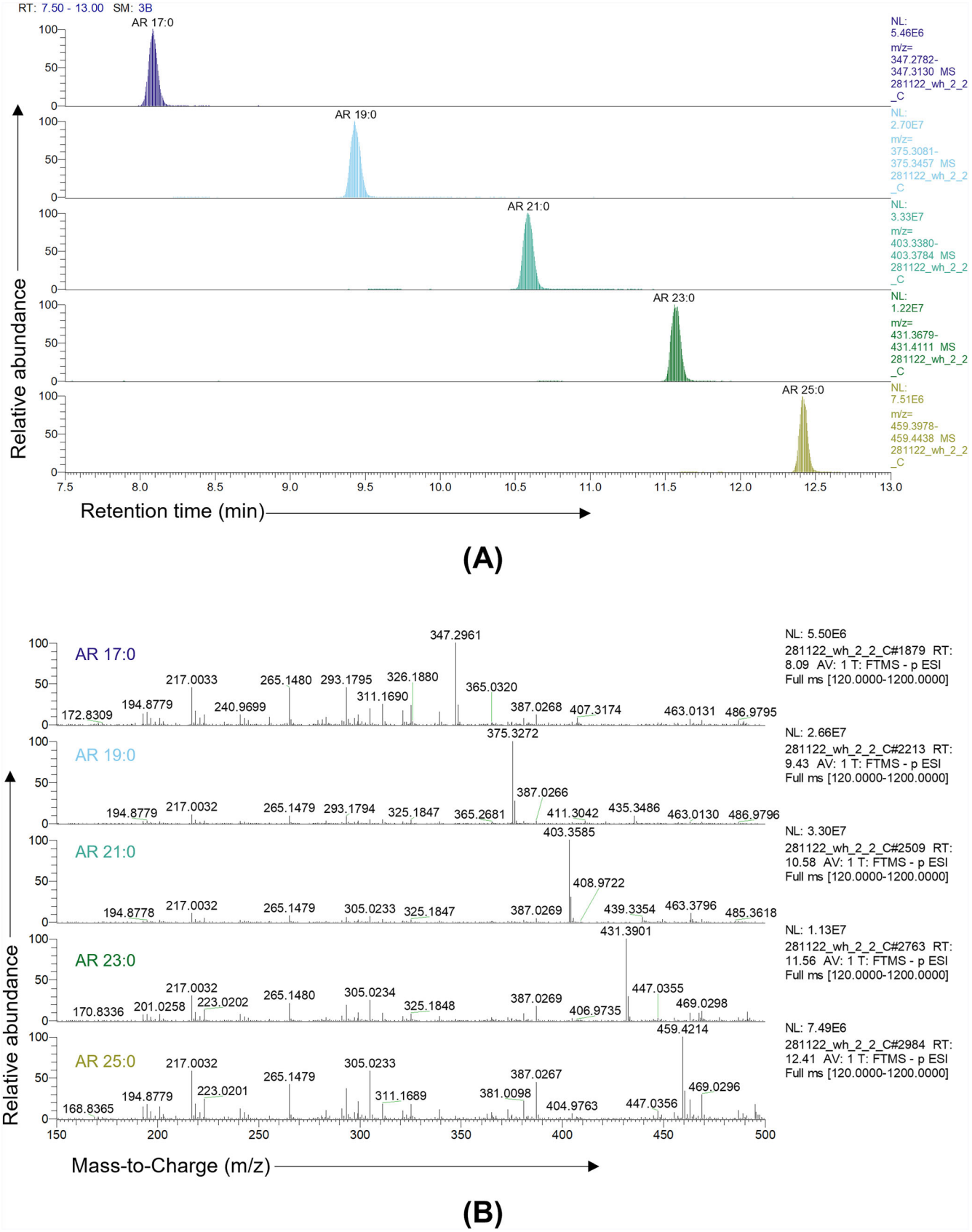


FIGURE 3 (A) UHPLC-Q/Orbitrap MS (ultra-high-performance liquid chromatography-quadrupole/Orbitrap mass spectrometry) chromatogram of AR 17:0, AR 19:0, AR 21:0, AR 23:0 and AR 25:0 detected in purified common wheat samples. (B) The obtained MS spectra confirmed their identities. [Color figure can be viewed at wileyonlinelibrary.com]

To assess the cereal's ploidy level, the ratio of several AR homologues was calculated and discussed (Table 3). In particular, the ratio of AR 17:0 to AR 21:0 discriminates between *Triticum* species,[36] and Pedrazzani et al.[24] reported that this ratio substantially decreased as follows: hexaploid > tetraploid ≥ diploid. The ratios for common wheat (0.19 ± 0.01), Khorasan wheat (0.03 ± 0.00), emmer (0.02 ± 0.00) and einkorn (0.04 ± 0.00) were in agreement with the values and trends reported in the literature.[24,36,37] This proved that the ratio could be used as an authenticity feature to distinguish *Triticum* species. This further demonstrated the differences in AR concentration between diploid, tetraploid and hexaploid wheat species.[23] Meanwhile, the AR 21:0/AR 23:0 ratio has been hypothesised to be a marker of antifungal activity in living crops[38] and is also related to the ploidy level, following the trend: hexaploid > tetraploid > diploid species.[24] In the present study, the hexaploid common wheat (2.60 ± 0.22) showed the highest ratio, followed by Khorasan wheat (1.91 ± 0.01) and emmer (1.69 ± 0.10), which are both tetraploid species. Finally, the diploid einkorn (1.45 ± 0.02) had the lowest ratio. These values agree with those in previous studies,[39] proving the stability of this marker despite the variability in AR 21:0 and AR 23:0 concentrations.

The relative contribution of unsaturated AR homologues was also calculated (Table 3). Common wheat was the only species that yielded five unsaturated ARs, with AR 19:1 (7.2%) having the highest concentration (Figure 4B). The remaining wheat species had very low concentrations, with AR 17:1 <LOD. The small relative contribution of unsaturated ARs compared to their saturated counterparts has also been observed elsewhere.[23,24] The explanation for this observation is unclear, but the genetic background of AR accumulation in wheat could possibly explain the scarcity of unsaturated AR homologues in different *Triticum* species.[23,24]

TABLE 2 Validation parameters of various instruments reported in the literature.

Instrument	LOD (μg/g)	LOQ (μg/g)	References
GC/MS	5	–	13
GC-Q/TOFMS	0.2	–	11
UHPLC-Q/Orbitrap MS	0.02	0.06	This study

Abbreviations: GC/MS, gas chromatography mass spectrometry; GC-Q/TOFMS, gas chromatography-quadrupole time-of-flight mass spectrometry; LOD, limit of detection; LOQ, limit of quantification; UHPLC-Q/Orbitrap MS, ultra-high-performance liquid chromatography-quadrupole/Orbitrap mass spectrometry.

3.3.2 | *Hordeum vulgare* L.

Five of the saturated ARs (AR 17:0, AR 19:0, AR 21:0, AR 23:0 and AR 25:0) and only one of the unsaturated ARs (AR 19:1) were detected in the barley samples (Table 3). Unlike the wheat species, the predominant AR homologue in barley was AR 25:0, followed by AR 21:0, AR 19:0, AR 23:0, AR 19:1 and AR 17:0, which agreed with previous data.[38] Moreover, the observed lower detection of AR homologues is not surprising as they are approximately 10 times less concentrated in barley than in wheat species.[13]

3.3.3 | Saturated AR homologue distribution

Due to the inherently low concentration of unsaturated ARs in cereal species[23,24] and their high susceptibility to oxidation during vessel use and burial,[40] only the distribution patterns of saturated ARs were discussed and analysed. The obtained results corroborated the observation that the distribution of saturated AR homologues is markedly different between the different cereal species (Table 4). For instance, all *Triticum* species (common wheat, spelt, einkorn and emmer) showed AR 21:0 as the dominant homologue.[13,14,23,24,37,43] In contrast, AR 25:0 is the predominant AR in barley,[41,42] and AR 19:0 is the most abundant AR homologue in rye.[14,22,43,44] Thus, these patterns can be utilised to differentiate cereal species from each other in modern samples. Moreover, the distribution patterns remain unchanged irrespective of whether LC- or GC/MS-based (Figure S4) methods were used.[13,14,17,44,45]

TABLE 3 Mean distribution of AR homologues in five different cereal species (mean of duplicates) and SD in parentheses detected and identified using UHPLC-Q/Orbitrap MS.

	AR homologue content (%) (SD)										AR ratio	
Species	17:0	19:0	21:0	23:0	25:0	17:1	19:1	21:1	23:1	25:1	17:0/21:0	21:0/23:0
Einkorn	1.2 (0.0)	15.0 (0.4)	33.5 (0.3)	23.0 (0.5)	13.1 (1.4)	<LOD	1.3 (0.0)	4.2 (0.1)	5.1 (0.0)	3.6 (0.1)	0.04 (0.00)	1.45 (0.02)
Emmer	0.8 (0.0)	13.8 (0.3)	40.5 (0.4)	24.0 (1.2)	15.0 (0.4)	<LOD	1.0 (0.0)	2.8 (0.0)	1.5 (0.0)	0.7 (0.0)	0.02 (0.00)	1.69 (0.10)
Khorasan wheat	1.2 (0.1)	12.7 (0.2)	48.3 (0.9)	25.3 (0.6)	9.8 (1.2)	<LOD	0.9 (0.0)	1.5 (0.0)	0.4 (0.0)	<LOD	0.03 (0.00)	1.91 (0.01)
Common wheat	5.9 (0.3)	27.8 (0.8)	31.5 (0.5)	12.2 (0.8)	8.4 (1.4)	0.9 (0.0)	7.2 (0.3)	4.8 (0.2)	1.0 (0.0)	0.3 (0.0)	0.19 (0.01)	2.60 (0.22)
Barley	0.02 (0.0)	12.7 (0.3)	33.8 (0.1)	9.2 (0.8)	44.2 (1.3)	<LOD	0.09 (0.0)	<LOD	<LOD	<LOD	–	–

Note: The total AR concentration was expressed as the sum of the individual saturated and unsaturated homologues.
Abbreviations: AR, alkylresorcinol; LOD, limit of detection; SD, standard deviation; UHPLC-Q/Orbitrap MS, ultra-high-performance liquid chromatography-quadrupole/Orbitrap mass spectrometry.

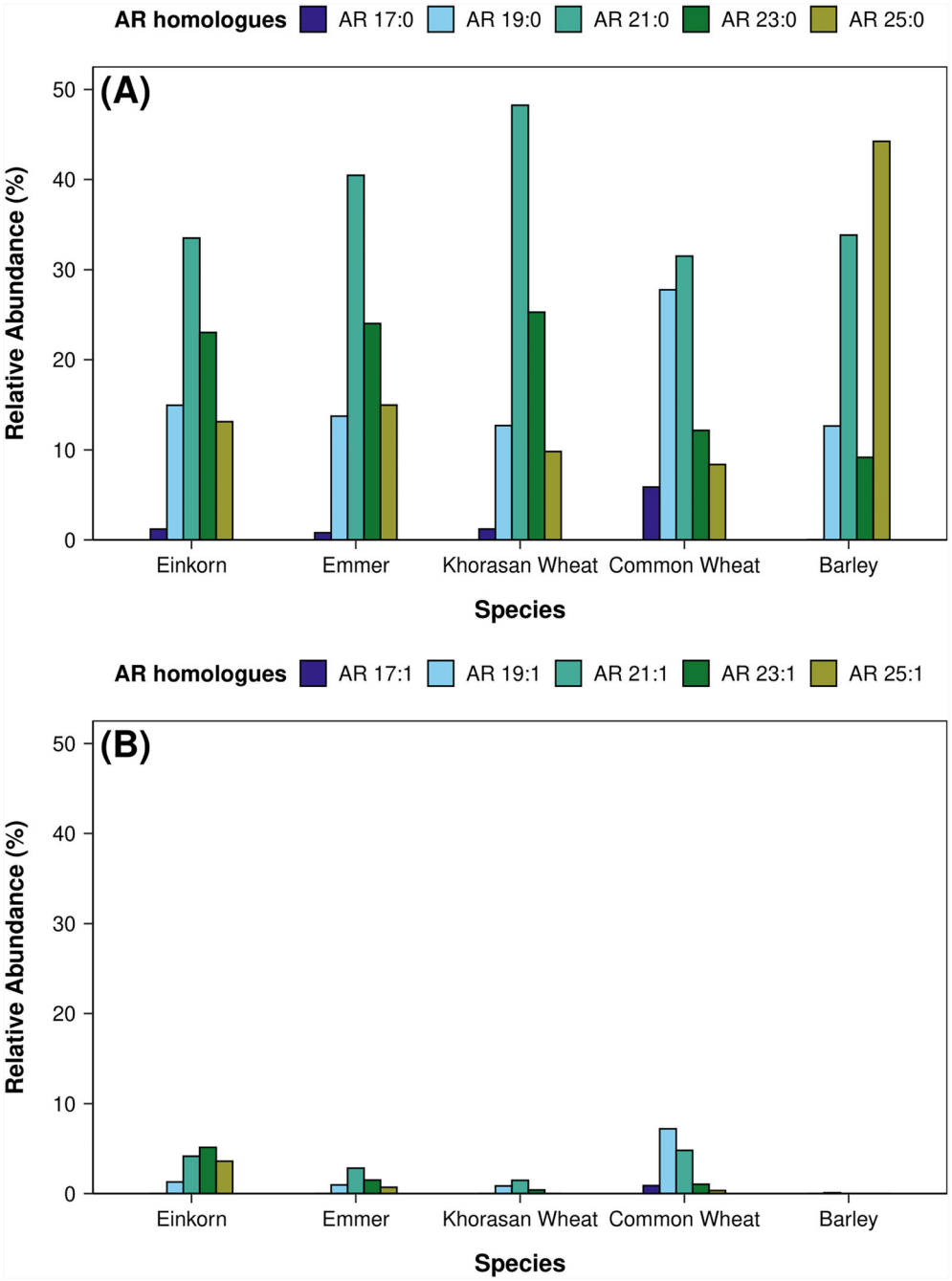


FIGURE 4 (A) Saturated and (B) unsaturated AR (alkylresorcinol) composition of different purified cereal species. The ARs were detected and identified using UHPLC-Q/Orbitrap MS (ultra-high-performance liquid chromatography-quadrupole/Orbitrap mass spectrometry). Saturated AR 21:0 dominated in wheat species, whereas AR 25:0 dominated in barley. [Color figure can be viewed at wileyonlinelibrary.com]

3.4 | Application to archaeological pottery

To explore the application of the developed UHPLC-Q/Orbitrap MS method in an archaeological context, the presence and distribution of AR homologues were determined in pottery from the Must Farm site.

GC/MS analysis of the Must Farm TLEs enabled the detection of multiple compounds with varying contributions (Figure 5A). Detailed information on the organic residue analysis results is published elsewhere.[27] A SIM approach targeting ions of AR homologues was applied in all of the samples to search for the cereal-specific biomarker. Eight extracts showed the possible presence of ARs (Figure 5B). However, identification and distribution profile assessment of AR homologues using the conventional GC/MS was not possible due to the small peak sizes and the presence of several interfering peaks. To remove these interferences and concentrate AR in the extracts, the purification step was applied to all the TLEs. However, when the extracts were analysed using GC/MS in SIM mode, no AR peaks were detected in the purified and derivatised samples, likely due to the very low concentrations of ARs, which this conventional approach was not able to detect. The probable explanation for the peaks detected in the unpurified extracts is that they belong to other interferences coeluting with AR homologues. When these interferences were removed after purification, no peaks were observed, but we know that AR homologues were present in the

purified samples because they were detected and identified using UHPLC-Q/Orbitrap MS (Figure 6; Table S4).

The presence of AR homologues was confirmed using the developed LC/MS-based method (Figure 6) in all but one of the Must Farm extracts (except for MUS 2830), with AR 21:0 being the most dominant homologue. In one sample, the dominant AR 21:0 was followed by AR 23:0 and AR 25:0. Additionally, AR 19:0 was detected in the same sample, but its concentration was too low to quantify (Table S4). Four out of the eight extracts showed only the AR 21:0 homologue, and apart from the dominant homologue, two samples also featured AR 23:0. Meanwhile, AR 17:0 was not detected in any samples. The absence of AR 17:0 and the minimal presence of AR

TABLE 4 Reported distribution of the saturated AR homologues in different cereal species analysed using GC/MS- and LC/MS-based protocols.

Species	Ploidy level	AR homologue relative distribution	References
Common/bread wheat (*Triticum aestivum* spp. *aestivum*)	Hexaploid (6×)	AR 21:0 > AR 19:0 > AR 23:0 > AR 17:0 > AR 25:0	This study[23,24,37]
Spelt (*T. aestivum* spp. *spelta*)			23, 24, 37
Tritordeum (×*Tritordeum martinii*)	Hexaploid (6×)	AR 21:0 > AR 23:0 > AR 19:0 > AR 25:0 > AR 17:0	23, 24, 37
Durum (*Triticum turgidum* spp. *durum*)	Tetraploid (4×)		23
Khorasan wheat (*T. turgidum* spp. *turanicum*)			This study
Emmer (*T. turgidum* spp. *dicoccum*)			This study[23,24,37]
Einkorn (*Triticum monococcum* spp. *monococcum*)	Diploid (2×)		
Barley (*Hordeum vulgare* L.)	–	AR 25:0 > AR 21:0 > AR 23:0 > AR 19:0 > AR 17:0	This study[41,42]
Rye (*Secale cereale* L.)	–	AR 19:0 > AR 21:0 > AR 17:0 > AR 23:0 > AR 25:0	14, 22, 43, 44

Abbreviations: AR, alkylresorcinol; GC/MS, gas chromatography mass spectrometry; LC/MS, liquid chromatography mass spectrometry.

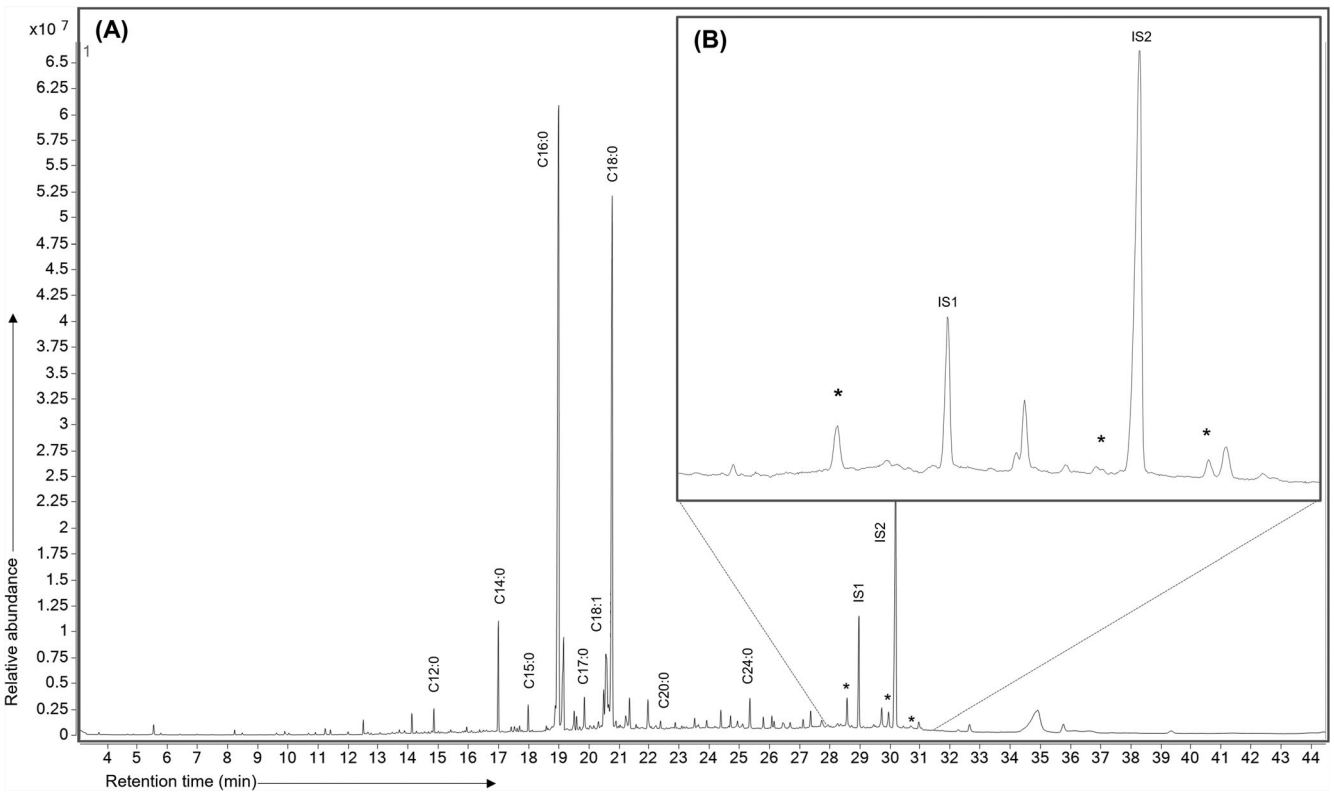


FIGURE 5 (A) GC/MS (gas chromatography mass spectrometry) TIC (total ion chromatogram) and (B) SIM (selected ion monitoring, *m/z* 268) chromatograms of the unpurified and TMS (trimethylsilyl)–derivatised MUS 2835 sample showing some of the detected molecular compounds (C*n*:*x*, fatty acids with carbon length *n* and number of unsaturations *x*) and the possible presence of AR (alkylresorcinol) (*). UHPLC-Q/Orbitrap MS (ultra-high-performance liquid chromatography-quadrupole/Orbitrap mass spectrometry) analysis identified these three peaks as AR 21:0, AR 23:0 and AR 25:0 homologues. Detailed information on other identified lipid compounds can be found elsewhere.[27] IS, internal standard.

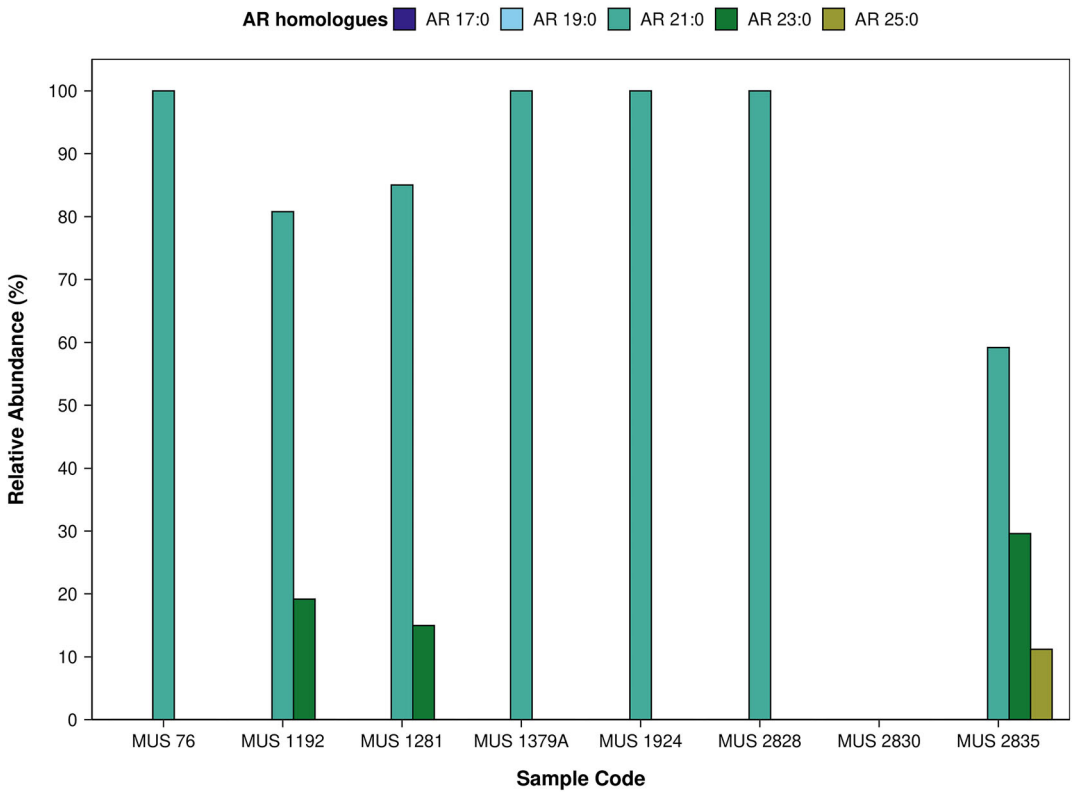


FIGURE 6 Relative distribution of AR (alkylresorcinol) homologues in eight purified Must Farm TLEs (total lipid extracts). The ARs were detected and identified using UHPLC-Q/Orbitrap MS (ultra-high-performance liquid chromatography-quadrupole/Orbitrap mass spectrometry). [Color figure can be viewed at wileyonlinelibrary.com]

19:0 can be attributed to the shorter-chain homologues being more susceptible to degradation when subjected to heat, such as during cooking or fire incidents.[11] However, lipid residue analysis of the samples revealed that the pots were not subjected to protracted heating at high temperatures, suggesting that they were not used as cooking vessels.[27] The absence of AR 17:0 and AR 19:0 then is likely due to their inherently low concentration in the cereal grain to begin with.

Preservation through charring and anoxic waterlogging at the site of Must Farm resulted in exceptional archaeobotanical and carpological records.[27] Charred emmer wheat and barley remains were recovered from the roundhouse structure (structure 1) at the site presumed to store plant resources.[27] As discussed in Section 3.3.3, AR distribution patterns can be used for cereal species discrimination in modern samples.[10,12–14,36] Its usage, however, has to be executed with care when dealing with archaeological samples because the AR homologues' concentration and distribution may change over time.[11,23,46] Previous studies have demonstrated that these distribution patterns are relatively stable and can be used for species identification in archaeological material, at least as old as the 4th millennium BCE.[10,12,36] If so, the pattern observed in this study suggests that a *Triticum* species was processed rather than barley, the other relevant cereal crop at the settlement.[27] This conclusion is based on the dominance of AR 21:0, a characteristic of wheat species, and the presence of AR 25:0, the predominant homologue in barley, in only one sample. Furthermore, the carpological evidence[27] obtained from structure 1 has the potential to significantly narrow down the wheat species from which the detected AR homologues originated. Based on the available evidence, it is reasonable to conclude that emmer wheat is the most likely origin of the detected homologues.

The results obtained here represent that this study is the first to successfully detect and identify a cereal-specific biomarker, typically found in very low concentrations in archaeological pots, using an LC/MS-based method. Additionally, the findings provide significant evidence that AR homologues and their distribution patterns can be remarkably preserved under favourable conditions.

4 | CONCLUSION

A highly sensitive LC/MS-based method for the detection and identification of ARs in modern and archaeological extracts was developed and validated. The LOD achieved is 250-fold lower than that of the conventional GC/MS. The protocol involves the enhancement of ARs and the removal of interfering lipids using an SPE column. The described method was successfully utilised to detect AR homologues in archaeological samples and determine their relative abundance. The observed distribution pattern (preserved by the site's waterlogged conditions) indicates the processing of *Triticum* species,

most likely emmer wheat, based on the excellent archaeological remains at the site of Must Farm.

Although some factors still need to be considered (e.g., modifying AR extraction by introducing an internal AR standard[12] to estimate AR loss during the extraction process and quantify AR homologues), the developed method shows great potential as an alternative approach for targeted lipid biomarker detection in archaeological materials, particularly for samples with low concentrations.

ACKNOWLEDGEMENTS

The authors thank Jane Thomas-Oates for her original involvement, Helen Talbot and Alexandre Lucquin for their assistance with the optimisation experiments, and Mark Knight and the Must Farm Team. Finally, they thank two reviewers whose insightful comments improved this article. This work contributes to EarlyFoods, which has received funding from the Agència de Gestió d'Ajuts Universitaris i de Recerca de Catalunya (SGR-Cat 2021, 00527), and ANIMAL FARM project, which was funded by Proyectos I + D Generación de Conocimiento (PID 2020--115715 GB-I00).

DATA AVAILABILITY STATEMENT

All the relevant data supporting this research are available within the paper and its Supporting Information and Supplementary Data files.

AUTHOR CONTRIBUTIONS

Conceptualisation, *funding acquisition and supervision*: Oliver E. Craig, Kirsty Penkman and André C. Colonese. *Data curation*, *formal analysis*, *investigation and resources*: Jonica Ella Doliente, Swen Langer, Miriam Cubas and Marc R. Dickinson. *Project administration*, *methodology and writing—original draft*: Jonica Ella Doliente, Swen Langer, Oliver E. Craig and Kirsty Penkman. *Visualisation*: Jonica Ella Doliente, Kirsty Penkman, Oliver E. Craig and André C. Colonese. *Writing—review and editing*: Jonica Ella Doliente, Swen Langer, Marc R. Dickinson, Miriam Cubas, André C. Colonese, Kirsty Penkman and Oliver E. Craig.

ORCID

Jonica Ella Doliente https://orcid.org/0000-0001-7043-6916
Miriam Cubas https://orcid.org/0000-0002-2386-8473

REFERENCES

1. Bogaard A. "Garden agriculture" and the nature of early farming in Europe and the Near East. *World Archaeol*. 2005;37(2):177-196. doi:10.1080/00438240500094572
2. Bishop RR. Did late neolithic farming fail or flourish? A Scottish perspective on the evidence for late neolithic arable cultivation in the British Isles. *World Archaeol*. 2015;47(5):834-855. doi:10.1080/00438243.2015.1072477
3. Stevens CJ, Fuller DQ. Did neolithic farming fail? The case for a bronze age agricultural revolution in the British Isles. *Antiquity*. 2012;86(333):707-722. doi:10.1017/S0003598X00047864
4. Copley MS, Berstan R, Dudd SN, et al. Direct chemical evidence for widespread dairying in prehistoric Britain. *Proc Natl Acad Sci USA*. 2003;100(4):1524-1529. doi:10.1073/pnas.0335955100
5. Salque M, Bogucki PI, Pyzel J, et al. Earliest evidence for cheese making in the sixth millennium BC in northern Europe. *Nature*. 2013;493(7433):522-525. doi:10.1038/nature11698
6. Cramp LJE, Evershed RP, Lavento M, et al. Neolithic dairy farming at the extreme of agriculture in northern Europe. *Proc Biol Sci*. 2014;281(1791):20140819. doi:10.1098/rspb.2014.0819
7. Debono Spiteri C, Gillis RE, Roffet-Salque M, et al. Regional asynchronicity in dairy production and processing in early farming communities of the northern Mediterranean. *Proc Natl Acad Sci USA*. 2016;113(48):13594-13599. doi:10.1073/pnas.1607810113
8. Cubas M, Lucquin A, Robson HK, et al. Latitudinal gradient in dairy production with the introduction of farming in Atlantic Europe. *Nat Commun*. 2020;11(1):2036. doi:10.1038/s41467-020-15907-4
9. Dunne J, Mercuri AM, Evershed RP, Bruni S, di Lernia S. Earliest direct evidence of plant processing in prehistoric Saharan pottery. *Nat Plants*. 2016;3(1):16194. doi:10.1038/nplants.2016.194
10. Colonese AC, Hendy J, Lucquin A, et al. New criteria for the molecular identification of cereal grains associated with archaeological artefacts. *Sci Rep*. 2017;7(1):6633. doi:10.1038/s41598-017-06390-x
11. Hammann S, Cramp LJE. Towards the detection of dietary cereal processing through absorbed lipid biomarkers in archaeological pottery. *J Archaeol Sci*. 2018;93:74-81. doi:10.1016/j.jas.2018.02.017
12. Hammann S, Bishop RR, Copper M, et al. Neolithic culinary traditions revealed by cereal, milk and meat lipids in pottery from Scottish crannogs. *Nat Commun*. 2022;13(1):5045. doi:10.1038/s41467-022-32286-0
13. Ross AB, Shepherd MJ, Schüpphaus M, et al. Alkylresorcinols in cereals and cereal products. *J Agric Food Chem*. 2003;51(14):4111-4118. doi:10.1021/jf0340456
14. Chen Y, Ross AB, Aman P, Kamal-Eldin A. Alkylresorcinols as markers of whole grain wheat and rye in cereal products. *J Agric Food Chem*. 2004;52(26):8242-8246. doi:10.1021/jf049726v
15. Andersson AAM, Lampi AM, Nyström L, et al. Phytochemical and dietary fiber components in barley varieties in the HEALTHGRAIN diversity screen. *J Agric Food Chem*. 2008;56(21):9767-9776. doi:10.1021/jf802037f
16. Roffet-Salque M, Dunne J, Altoft DT, et al. From the inside out: Upscaling organic residue analyses of archaeological ceramics. *J Archaeol Sci Rep*. 2017;16:627-640. doi:10.1016/j.jasrep.2016.04.005
17. Hammann S, Korf A, Bull ID, Hayen H, Cramp LJE. Lipid profiling and analytical discrimination of seven cereals using high temperature gas chromatography coupled to high resolution quadrupole time-of-flight mass spectrometry. *Food Chem*. 2019;282:27-35. doi:10.1016/j.foodchem.2018.12.109
18. Evershed RP. Organic residue analysis in archaeology: The archaeological biomarker revolution. *Archaeometry*. 2008;50(6):895-924. doi:10.1111/j.1475-4754.2008.00446.x
19. Evershed RP. Experimental approaches to the interpretation of absorbed organic residues in archaeological ceramics. *World Archaeol*. 2008;40(1):26-47. doi:10.1080/00438240801889373
20. Ross AB, Aman P, Andersson R, Kamal-Eldin A. Chromatographic analysis of alkylresorcinols and their metabolites. *J Chromatogr A*. 2004;1054(1–2):157-164. doi:10.1016/j.chroma.2004.04.020
21. Suzuki Y, Esumi Y, Saito T, Kishimoto Y, Morita T. Identification of 5-n-(2′-oxo)alkylresorcinols from etiolated rice seedlings. *Phytochemistry*. 1998;47(7):1247-1252. doi:10.1016/S0031-9422(97)00720-6
22. Suzuki Y, Esumi Y, Yamaguchi I. Structures of 5-alkylresorcinol-related analogues in rye. *Phytochemistry*. 1999;52(2):281-289. doi:10.1016/S0031-9422(99)00196-X
23. Ziegler JU, Steingass CB, Longin CFH, Würschum T, Carle R, Schweiggert RM. Alkylresorcinol composition allows the differentiation of Triticum spp. having different degrees of ploidy. *J Cereal Sci*. 2015;65:244-251. doi:10.1016/j.jcs.2015.07.013

24. Pedrazzani C, Vanara F, Bhandari DR, et al. 5-n-Alkylresorcinol profiles in different cultivars of einkorn, emmer, spelt, common wheat, and tritordeum. *J Agric Food Chem*. 2021;69(47):14092-14102. doi:10.1021/acs.jafc.1c05451
25. La Nasa J, Ghelardi E, Degano I, Modugno F, Colombini MP. Core shell stationary phases for a novel separation of triglycerides in plant oils by high performance liquid chromatography with electrospray-quadrupole-time of flight mass spectrometer. *J Chromatogr A*. 2013;1308:114-124. doi:10.1016/j.chroma.2013.08.015
26. La Nasa J, Degano I, Brandolini L, Modugno F, Bonaduce I. A novel HPLC-ESI-Q-ToF approach for the determination of fatty acids and acylglycerols in food samples. *Anal Chim Acta*. 2018;1013:98-109. doi:10.1016/j.aca.2017.12.047
27. Cubas M, Dickinson M, von Tersch M, Lucquin A, Craig OE. Chapter 28: Organic residues. In: Knight M, Ballantyne R, Cooper A, Gibson D, Zeki IR, eds. *Must Farm Pile Dwelling Settlement*. McDonald Institute for Archaeological Research:1175-1187.
28. Evershed RP, Heron C, John GL. Analysis of organic residues of archaeological origin by high-temperature gas chromatography and gas chromatography-mass spectrometry. *Analyst*. 1990;115(10):1339-1342. doi:10.1039/AN9901501339
29. Method development guidelines: Solid phase extraction using ISOLUTE® NH2 SPE sorbents for the extraction of aqueous samples. Biotage. 2017. Accessed July 7, 2023. https://www.biotage.com/documents/method-development-guidelines-solid-phase-extraction-using-isolute-nh2-spe-sorbents-for-the-extraction-of-aqueous-samples.pdf
30. Controlling contamination in LC/MS systems. Waters. 2016. Accessed July 17, 2023. https://www.waters.com/webassets/cms/support/docs/715001307rg.pdf
31. Ross AB, Svelander C, Karlsson G, Savolainen OI. Identification and quantification of even and odd chained 5-n alkylresorcinols, branched chain-alkylresorcinols and methylalkylresorcinols in quinoa (Chenopodium quinoa). *Food Chem*. 2017;220:344-351. doi:10.1016/j.foodchem.2016.10.020
32. Lucquin A, Robson HK, Eley Y, et al. The impact of environmental change on the use of early pottery by East Asian hunter-gatherers. *Proc Natl Acad Sci USA*. 2018;115(31):7931-7936. doi:10.1073/pnas.1803782115
33. Validation of analytical procedures: text and methodology. ICH: International Council for Harmonisation. 2005. Accessed July 21, 2023. https://database.ich.org/sites/default/files/Q2%28R1%29Guideline.pdf
34. Hollmann M, Boertz J, Dopp E, Hippler J, Hirner AV. Parallel on-line detection of a methylbismuth species by hyphenated GC/EI-MS/ICP-MS technique as evidence for bismuth methylation by human hepatic cells. *Metallomics*. 2010;2(1):52-56. doi:10.1039/b911945k
35. Orbitrap Fusion Lumos tribrid spectrometer. Thermo Fisher Scientific. 2020. Accessed July 21, 2023. https://assets.thermofisher.com/TFS-Assets/CMD/brochures/BR-64392-LC-MS-Orbitrap-Fusion-Lumos-Tribrid-BR64392-EN.pdf
36. Landberg R, Kamal-Eldin A, Andersson R, Aman P. Alkylresorcinol content and homologue composition in durum wheat (*Triticum durum*) kernels and pasta products. *J Agric Food Chem*. 2006;54(8):3012-3014. doi:10.1021/jf0530805
37. Landberg R, Man P, Kamal-Eldin A. A rapid gas chromatography-mass spectrometry method for quantification of alkylresorcinols in human plasma. *Anal Biochem*. 2009;385(1):7-12. doi:10.1016/j.ab.2008.10.031
38. Ciccoritti R, Pasquini M, Sgrulletta D, Nocente F. Effect of 5-n-alkylresorcinol extracts from durum wheat whole grain on the growth of fusarium head blight (FHB) causal agents. *J Agric Food Chem*. 2015;63(1):43-50. doi:10.1021/jf5054518
39. Righetti L, Rubert J, Galaverna G, et al. Characterization and discrimination of ancient grains: a metabolomics approach. *Int J Mol Sci*. 2016;17(8):1217. doi:10.3390/ijms17081217
40. Evershed RP, Copley MS, Dickson L, Hansel FA. Experimental evidence for the processing of marine animal products and other commodities containing polyunsaturated fatty acids in pottery vessels. *Archaeometry*. 2008;50(1):101-113. doi:10.1111/j.1475-4754.2007.00368.x
41. Zarnowski R, Suzuki Y, Yamaguchi I, Pietr SJ. Alkylresorcinols in barley (*Hordeum vulgare* L. distichon) grains. *Z Naturforsch C*. 2002;57(1–2):57-62. doi:10.1515/znc-2002-1-210
42. Zarnowski R, Suzuki Y. 5-n-Alkylresorcinols from grains of winter barley (*Hordeum vulgare* L.). *J Nature Res*. 2004;59(5-6):315-317. doi:10.1515/znc-2004-5-603
43. Suzuki Y, Esumi Y, Uramoto M, Kono Y, Sakurai A. Structural analyses of carbon chains in 5-alk (en)ylresorcinols of rye and wheat whole flour by tandem mass spectrometry. *Biosci Biotechnol Biochem*. 1997;61(3):480-486. doi:10.1271/bbb.61.480
44. Ross AB, Kamal-Eldin A, Jung C, Shepherd MJ, Åman P. Gas chromatographic analysis of alkylresorcinols in rye (*Secale cereale* L) grains. *J Sci Food Agric*. 2001;81(14):1405-1411. doi:10.1002/jsfa.956
45. Andersson AAM, Kamal-Eldin A, Aman P. Effects of environment and variety on alkylresorcinols in wheat in the HEALTHGRAIN diversity screen. *J Agric Food Chem*. 2010;58(17):9299-9305. doi:10.1021/jf904526d
46. Miller MJ, Whelton HL, Swift JA, et al. Interpreting ancient food practices: stable isotope and molecular analyses of visible and absorbed residues from a year-long cooking experiment. *Sci Rep*. 2020;10(1):13704. doi:10.1038/s41598-020-70109-8

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Doliente JE, Langer S, Dickinson MR, et al. Alkylresorcinol detection and identification in archaeological pottery using ultra-high-performance liquid chromatography-quadrupole/Orbitrap mass spectrometry. *Rapid Commun Mass Spectrom*. 2024;38(15):e9771. doi:10.1002/rcm.9771