**Supplementary information for**

**Full title:** Alkylresorcinol detection and identification in archaeological pottery by ultra high performance liquid chromatography-quadrupole/Orbitrap mass spectrometry

**Short title:**  Alkylresorcinol detection and identification in pottery by UHPLC-Q/Orbitrap MS

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**Supplementary Discussion**

**Comparison of the three instruments**

Overall, UHPLC-Q/Orbitrap MS was the most sensitive instrument among the three techniques, able to detect the major AR homologues (AR 19:0, AR 21:0, and AR 23:0) at a 1:10,000 dilution of common wheat extracts using the developed method. UHPLC-Q/TOFMS and GC/MS detected the same ARs of the same cereal extracts at 1:1000 and 1:100 dilutions, respectively (Supplementary Figure 1). Further comparison among the three techniques is presented in Supplementary Table 3.

UHPLC-Q/Orbitrap MS instrument detected more types and numbers of AR homologues (five saturated ARs, two unsaturated ARs, and four 5-(2-oxo)ARs) in a common wheat extract at 1:100 dilution than the other two instruments. UHPLC-Q/TOFMS detected five saturated ARs, and three 5-(2-oxo)ARs, while the conventional GC/MS in SIM mode didn’t detect any other type of AR homologues other than the four saturated ones (Supplementary Table 3).

**Supplementary Tables**

**Supplementary Table 1.** Modern cereal samples analysed in this study.

|  |  |
| --- | --- |
| Species | Ploidy level\* |
| Common wheat (*Triticum aestivum* spp. *aestivum*) | hexaploid (6x) |
| Khorasan wheat (*Triticum turgidum* spp. *turanicum*) | tetraploid (4x)  |
| Emmer (*Triticum turgidum* spp. *dicoccum*) |
| Einkorn (*Triticum monococcum* spp. *monococcum*) | diploid (2x) |
| Barley (*Hordeum vulgare* L.) | - |

\*Ploidy level = number of sets of chromosomes.

**Supplementary Table 2.** Archaeological samples from the site of Must Farm[8](https://paperpile.com/c/YjTOZI/tSmN).

|  |  |  |  |
| --- | --- | --- | --- |
| Sample ID | Structure | Typological classification | Location of the sample |
| MUS 76 | M1 | - | - |
| MUS 1192 | 1 | Fineware bowl | Body under the rim |
| MUS 1281 | MI | Coarseware jar | Bottom |
| MUS 1379A | - | Coarseware jar | Rim |
| MUS 1924 | 1 | Coarseware bowl | Under the rim |
| MUS 2828 | 1 | Coarseware bowl | Rim and under the rim |
| MUS 2830 | 1 | Fineware bowl | Body under the rim |
| MUS 2835 | 1 | Fineware bowl | Rim and under the rim |

\*M: midden

**Supplementary Table 3.** Comparison of the three instruments – GC/MS vs UHPLC-Q/TOFMS vs UHPLC-Q/Orbitrap MS. The sample analysed was common wheat extract at 1:100 dilution.

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | GC/MS | UHPLC-Q/TOFMS | UHPLC-Q/Orbitrap MS |
| detected saturated AR at 1:100 dilution | AR 17:0, AR 19:0, AR 21:0, AR 23:0 | AR 17:0, AR 19:0, AR 21:0, AR 23:0, AR 25:0 | AR 17:0, AR 19:0, AR 21:0, AR 23:0, AR 25:0 |
| detected unsaturated AR at 1:100 dilution | nd | nd | AR 19:1 and AR 21:1 |
| detected 5-(2-oxo)ARs at 1:100 dilution | nd | AR 21:0 oxo, AR 23:0 oxo, AR 25:0 oxo | AR 21:0 oxo, AR 23:1 oxo, AR 23:0 oxo, AR 25:0 oxo |
| dilution in which major ARs\* are detectable | 1:100 | 1:1000 | 1:10,000 |
| derivatisation step | required | not required | not required |
| analysis time (min/sample) | 98 | 17 | 17 |
| cost (£ per sample)\*\* | 7 | 22 | 22 |

\*major ARs include AR 19:0, AR 21:0, and AR 23:0

\*\*cost depends on the laboratory/university

**Supplementary Table 4.** Distribution of AR homologues in purified Must Farm archaeological pot samples.

|  |  |
| --- | --- |
| Sample ID | AR homologue content (%) |
| 17:0 | 19:0 | 21:0 | 23:0 | 25:0 |
| MUS 76 | nd\* | nd\* | 100 | nd\* | nd\* |
| MUS 1192 | nd\* | nd\* | 81 | 19 | nd\* |
| MUS 1281 | nd\* | nd\* | 85 | 15 | nd\* |
| MUS 1379A | nd\* | nd\* | 100 | nd\* | nd\* |
| MUS 1924 | nd\* | nd\* | 100 | <LOD | nd\* |
| MUS 2828 | nd\* | nd\* | 100 | nd\* | nd\* |
| MUS 2830 | nd\* | nd\* | nd\* | nd\* | nd\* |
| MUS 2835 | nd\* | <LOD | 59 | 30 | 11 |

\*nd: not detected

**Supplementary Figures**



**Supplementary Figure 1.** Bar charts showing the relative abundance of saturated AR homologues in modern common wheat extracts diluted at different concentrations. The ARs were detected using GC/MS, UHPLC-Q/TOFMS, and UHPLC-Q/Orbitrap MS.



**Supplementary Figure 2.** Calibration curve and regression equation of AR 22:0 standard solutions analysed by the UHPLC-Q/Orbitrap MS method.



**Supplementary Figure 3.** Structures of some AR homologues detected in modern cereal species using UHPLC-Q/Orbitrap MS. Saturated (A) and unsaturated (B) ARs.



**Supplementary Figure 4.** Relative distribution of AR homologues in different cereal species using GC/[1–5](https://paperpile.com/c/YjTOZI/cLWt%2ByAhj%2BbL2Z%2BiDh7%2BNkLM) (A) and LC/MS-based[6,7](https://paperpile.com/c/YjTOZI/gc8C%2BfuKG) protocols (B). The distribution patterns of AR homologues in the five different cereal species remain unchanged whether LC/ or GC/MS-based methods were used.

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