BETTER TOGETHER: NMR SPECTROSCOPY AND MASS SPECTROMETRY FOR THE METABOLIC PROFILING OF SOLID MATRICES

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Due to highly dynamic concentration ranges, extensive chemical diversity and different physical properties, the metabolic characterization of complex samples may represent a true analytical challenge [1]. In this context, sophisticated analytical techniques, such as Nuclear Magnetic Resonance (NMR) spectroscopy and Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS), are considered the "gold standard" for the comprehensive characterization of sample mixtures. In particular, their combination represents a powerful tool to expand metabolic coverage and also to facilitate compound identification and quantification [1,2,3].

When working with complex matrices, such as whole cells, biopsies, or food flours, NMR- and LC-MS-based metabolic profiling is usually carried out at the liquid state after metabolite extraction. Besides requiring time-consuming sample manipulation, also relative and absolute quantification may be influenced by diversified extraction efficiencies. To tackle this problem, the application of NMR analysis under High-Resolution Magic Angle Spinning (HR-MAS) conditions and HRMS employing the Atmospheric Solids Analysis Probe (ASAP) may be considered, since they allow the direct analysis of semi-solid and solid samples with minimal to none sample preparation [4,5]. Here, we report the application of both techniques to the analysis of metabolites contained in five different food flour samples (rice, quinoa, chickpeas, faba beans, and lentils). Qualitative and quantitative data collected by extraction and analysis at the liquid state were compared with those obtained under HR-MAS NMR and ASAP HR-MS conditions. Once validated, this direct approach will be very useful for rapid anti-fraud checks of foodstuffs; also, it will increase the versatility and speed of NMR and MS analysis in resolving complex mixtures of organic compounds in a wide range of applications.

References

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BETTER TOGETHER

NMR SPECTROSCOPY AND MASS SPECTROMETRY FOR THE METABOLIC PROFILING OF SOLID MATRICES

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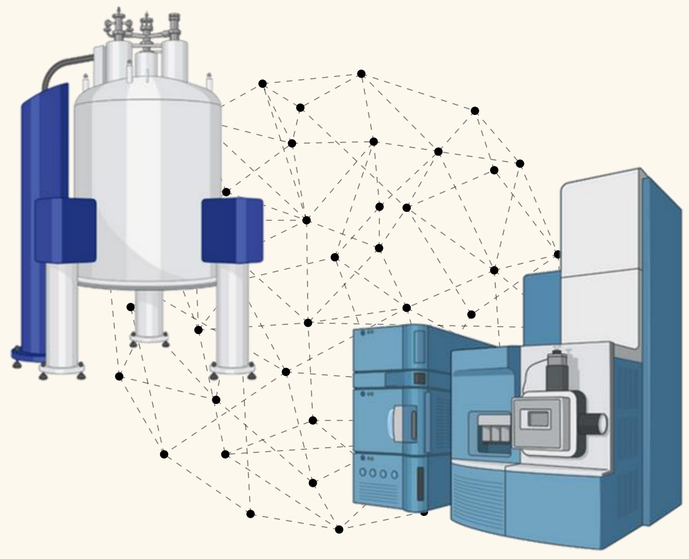
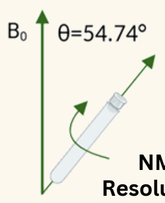


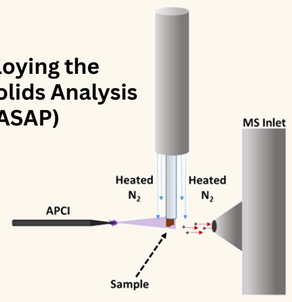
1

Due to highly dynamic concentration ranges, extensive chemical diversity, and different physical properties, the **metabolic characterization of complex samples** may represent a true analytical challenge.[1]

→ The combination of **NMR spectroscopy** and **mass spectrometry** represents a powerful tool **to expand metabolic coverage and facilitate compound identification.**[2,3]



NMR analysis under High-Resolution Magic Angle Spinning (HR-MAS)



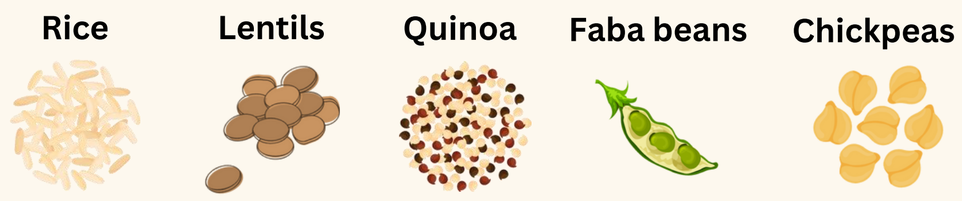
HRMS employing the Atmospheric Solids Analysis Probe (ASAP)

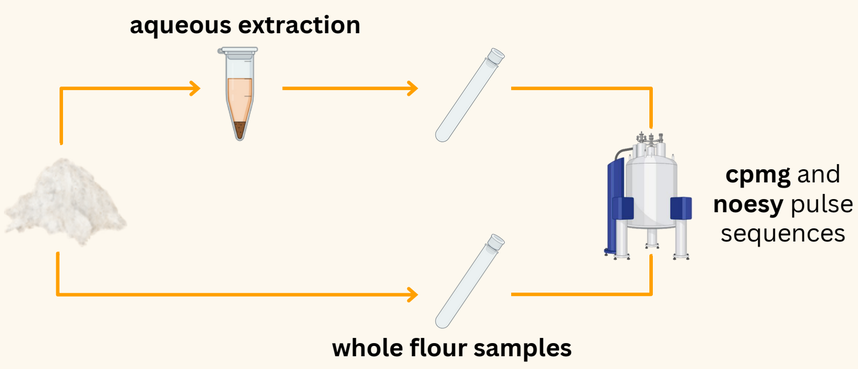
2

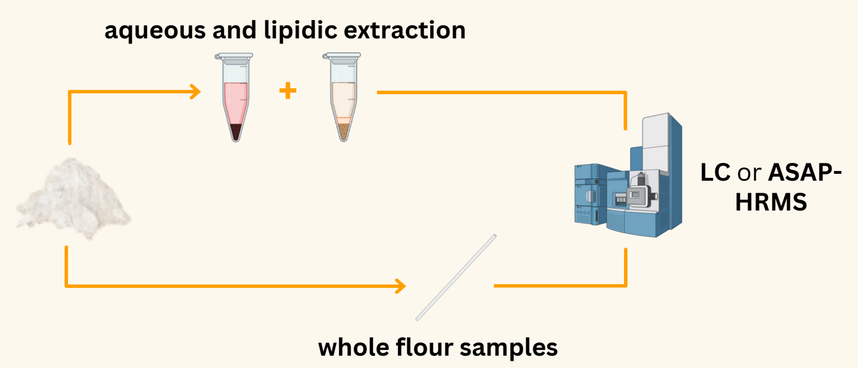
The metabolic profiling is usually carried out **at the liquid state** after metabolite extraction. However, **direct approaches** (HR-MAS and ASAP), which allow the analysis of **solid and semi-solid samples with minimal to none sample manipulation**, are available.[4,5]

3

Application of NMR & MS to the metabolic profiling of five different food flour samples







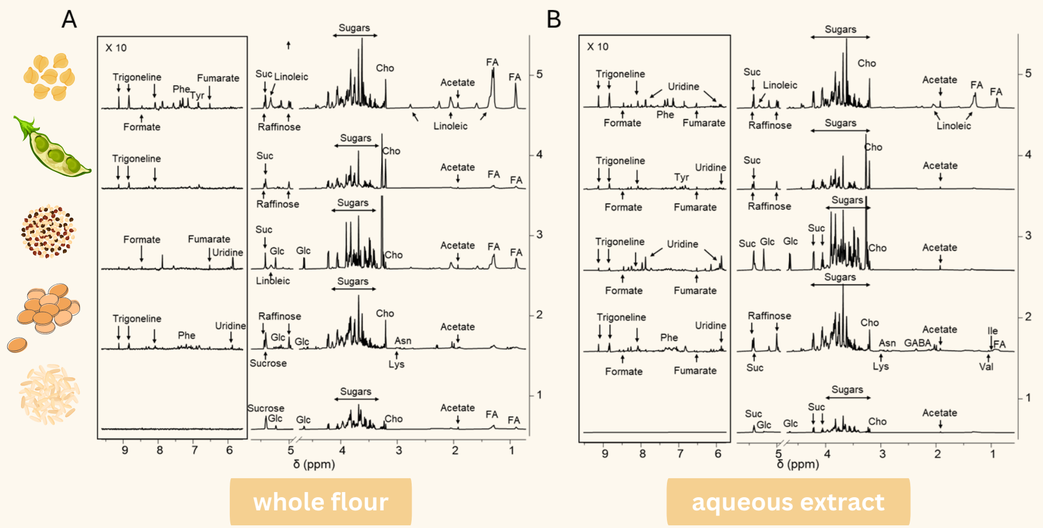


Fig. 1. [1]H-NMR spectra of whole flours (A) and aqueous flour extracts (B) in 10 mM PB in D_2O – acquired with *cpmgpr1d* pulse sequence.

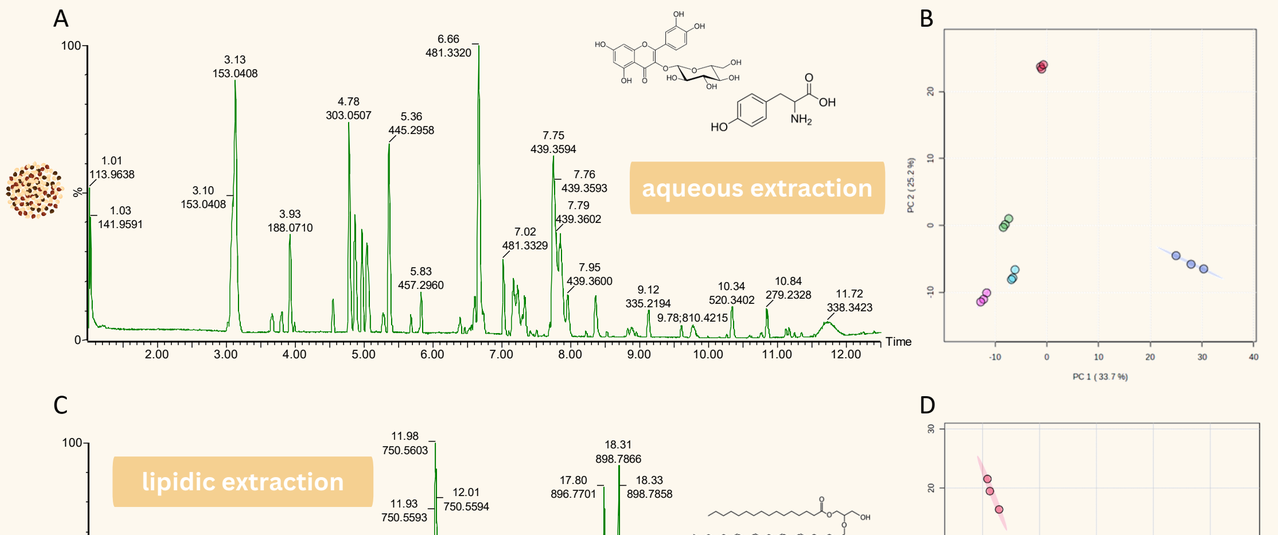


Fig. 4. Full scan chromatogram of quinoa fluor (A,C) and PCA score plot of five food flours (B,D) extracted by aqueous (A,B) and Blight-Dyer extraction (C,D) methods and analysed by LC-HRMS. ● Rice ● Lentils ● Quinoa ● Faba beans ● Chickpeas

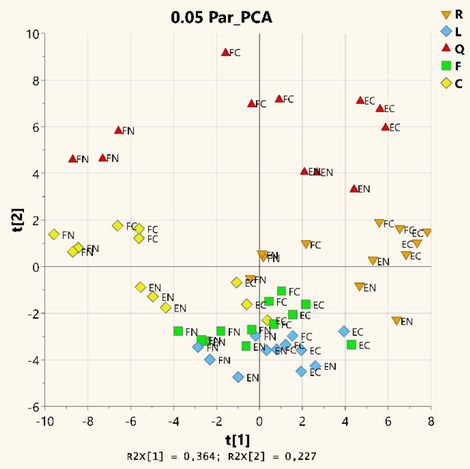


Fig. 2. Principal Component Analysis (PCA) applied to all 60 observations: t1/t2 score plots, relative to the PC1/PC2, for 0.05 ppm-size binned data, by applying Pareto scaling

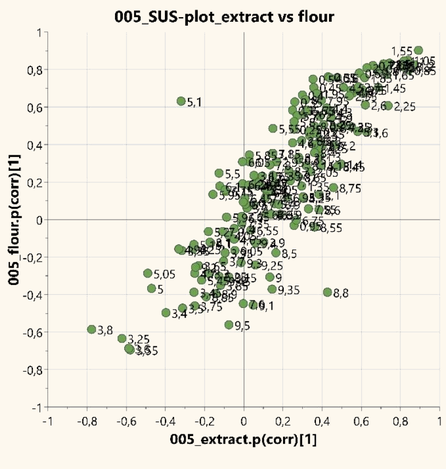


Fig. 3. SUS-plots obtained by comparing the two OPLS-DA models built on extract and flour 0.05 ppm-size binned data by setting the NMR experiment as class.

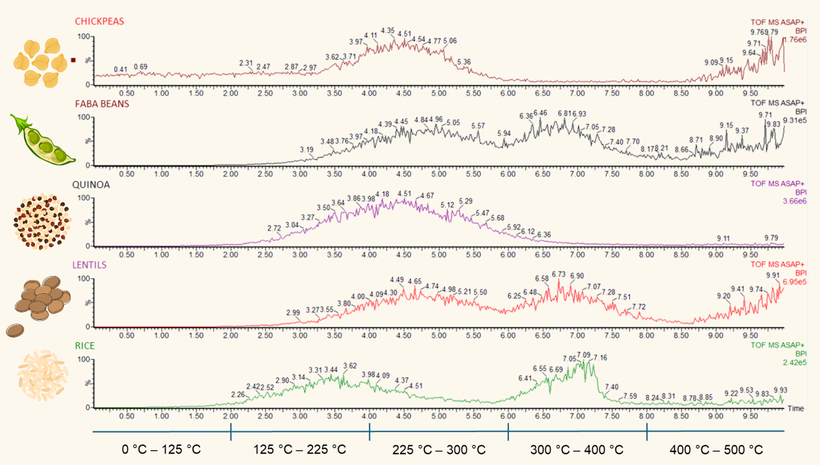


Fig. 5. Representative base peak intensities (BPI) «thermograms» of five different food flour samples at increasing temperature obtained by ASAP-HRMS.

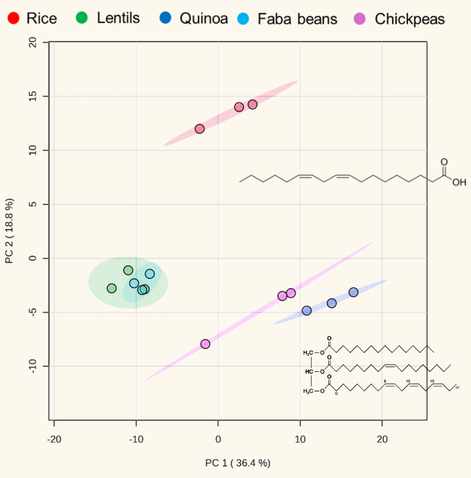


Fig. 6. PCA score plot of the five food flours analysed by ASAP HR-MS (temperature range 300-400 °C).

4

Both **NMR and MS** analyses have achieved a **specific metabolic profile** for the analyzed foodstuffs, providing **complementary information** regarding their chemical composition and demonstrating **HR-MAS NMR and ASAP HR-MS** application in the **rapid and versatile analysis of solid complex matrices**.

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2. Theodoridis, G.A., Gika, H.G., Want, E.J., Wilson, I.D., 2012. Analytica Chimica Acta 711, 7–16.
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