

SigMa Milk: an efficient method for the quantification of milk metabolites from ¹H NMR spectra using the Signature Mapping (SigMa) approach

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The milk metabolome carries valuable information about the cow's health status, diet, lactation stage, parity, genetic background and geographical origin. The molecular profile of milk is routinely analyzed to assess its nutritional and technological quality. Proton (¹H) nuclear magnetic resonance (NMR) spectroscopy, is among the most robust, unbiased and quantitative analytical platforms used for molecular screening of milk. However, this technique is typically applied after removing fat and proteins from milk, with spectral data handling and metabolite annotation remaining the main bottleneck.

This study presents optimized and highly standardized protocols for measuring ¹H NMR spectra of whole milk, skim milk and ultrafiltered milk in a high-throughput manner. Additionally, an extensive milk metabolite library was developed, featuring the unique chemical shift ranges of signature signals corresponding to 63 milk metabolites. These ranges were identified through spiking experiments conducted on whole milk, skim milk, and milk serum using authentic metabolite standards. This library has been implemented into the SigMa (Signature Mapping) software [1, 2], a platform developed for comprehensive, unbiased and reproducible processing of ¹H NMR spectra from whole milk, skim milk and ultrafiltered milk. More specifically, the SigMa Milk platform converts raw NMR spectra into an informative metabolite table in a semi-automated and user-friendly manner, thus enabling unambiguous identification and unbiased quantification of milk metabolites. The quantification performance of SigMa was evaluated using gallic acid as an internal standard, and the applicability of the platform is demonstrated in a metabolomic analysis of cow's milk and colostrum. The fewest metabolites were identified and quantified in whole milk (33 and 3, respectively) followed by skim (53 and 46) and ultrafiltered (63 and 62) milk. The quantification performance of the methodology showed quantification uncertainty below 3.8% for ultrafiltered and skim milk, and below 1.7% for whole milk. Absolute quantification with gallic acid as internal standard had an uncertainty of 9%, showing the method to be robust, and providing reliable and precise concentrations of milk metabolites. Additionally, SigMa allowed the absolute quantification of 44 metabolites in 88 ultrafiltered colostrum samples, simultaneously, demonstrating how SigMa can be used to better understand the impact of pre-calving diet on colostrum composition in dairy cows. Finally, the proposed SigMa method can facilitate augmenting knowledge through generating comparable quantitative data across studies.