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An alternative approach for analysing fat digestion products in small volume biological fluids

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Sensitive and affordable analytical techniques are critical for the development of novel foods and dietary strategies to improve human health. Key to this is the assessment of macronutrient bioaccessibility *in vitro*, using simulated digestion models[1] and bioavailability, by assessing postprandial responses *in vivo*. Current analytical procedures are expensive, need large sample volumes, which require storage, and extensive sample preparation. This can be cost-prohibitive, particularly in countries of lower socioeconomic status, where diet related non-communicable diseases are on the rise.

The gold standard method for free fatty acid analysis and quantification is gas chromatography-mass spectrometry (GC-MS)[2]. However, this requires extraction and derivatisation to enhance volatility and reduce polarity, enabling separation and detection. Whilst being a sensitive and powerful analytical technique, the use and maintenance of GC-MS instruments can be expensive. There is need for a simple, rugged and robust, 'fool-proof' method that can be carried out by non-specialists or potentially in remote research settings.

Our goal is to develop a comparable method using sample processing techniques to suit a range of substrates and matrices tested by nutritionists, such as *in vitro* digests and dried blood spots, and analyse using high performance liquid chromatography with diode array detection (HPLC-DAD). This widely available analytical technique requires less infrastructure and can be miniaturised and field deployable[3]. Free fatty acids have no chromophore - complicating detection by absorbance - so an additional derivatisation step is required (like in GC) to selectively tag the analytes. Ideally this uses commercially available reagents[4]. As with most analytical methods, analyte extraction from the matrix is also required and here we demonstrate the efficacy of a method using methyl-*tert*-butyl ether, methanol and water[5] instead of (toxic) chloroform/methanol mixtures widely reported in literature[6,7]. The chromatographic method restricts itself to standard conditions to fit capabilities of many HPLC models (reversed phase C^{18} column; modest run times, flow rates and back pressures; standard mobile phase solvents - acetonitrile and water).

Initial studies utilising *in vitro* digested peanut samples (INFOGEST)[1] demonstrated that HPLC-DAD is a reproducible analytical technique for detecting linoleic, palmitic, oleic, and stearic acid, with the maximum relative standard deviation (RSD) being low at 1.74% for the peak area of the palmitic acid derivative in digests. Currently we observe greater variance between different digest extracts (RSD up to 25.55%). We attribute this issue to preanalytical sample preparation of very inhomogeneous digests, and current work focusses on improving sample preparation robustness (to be assessed by testing a range of biological matrices and extraction protocols, comparing RSD analysis to existing methodologies).

In conclusion this method has potential to increase the accessibility of robust analysis so that high sensitivity, standard methods can be used in a range of research facilities regardless of location or economic status.

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