Electronic Supplementary Material (ESI) for Analyst.

Detection of the Adulteration of Coconut Water via NMR Spectroscopy and Chemometrics

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Supplementary information

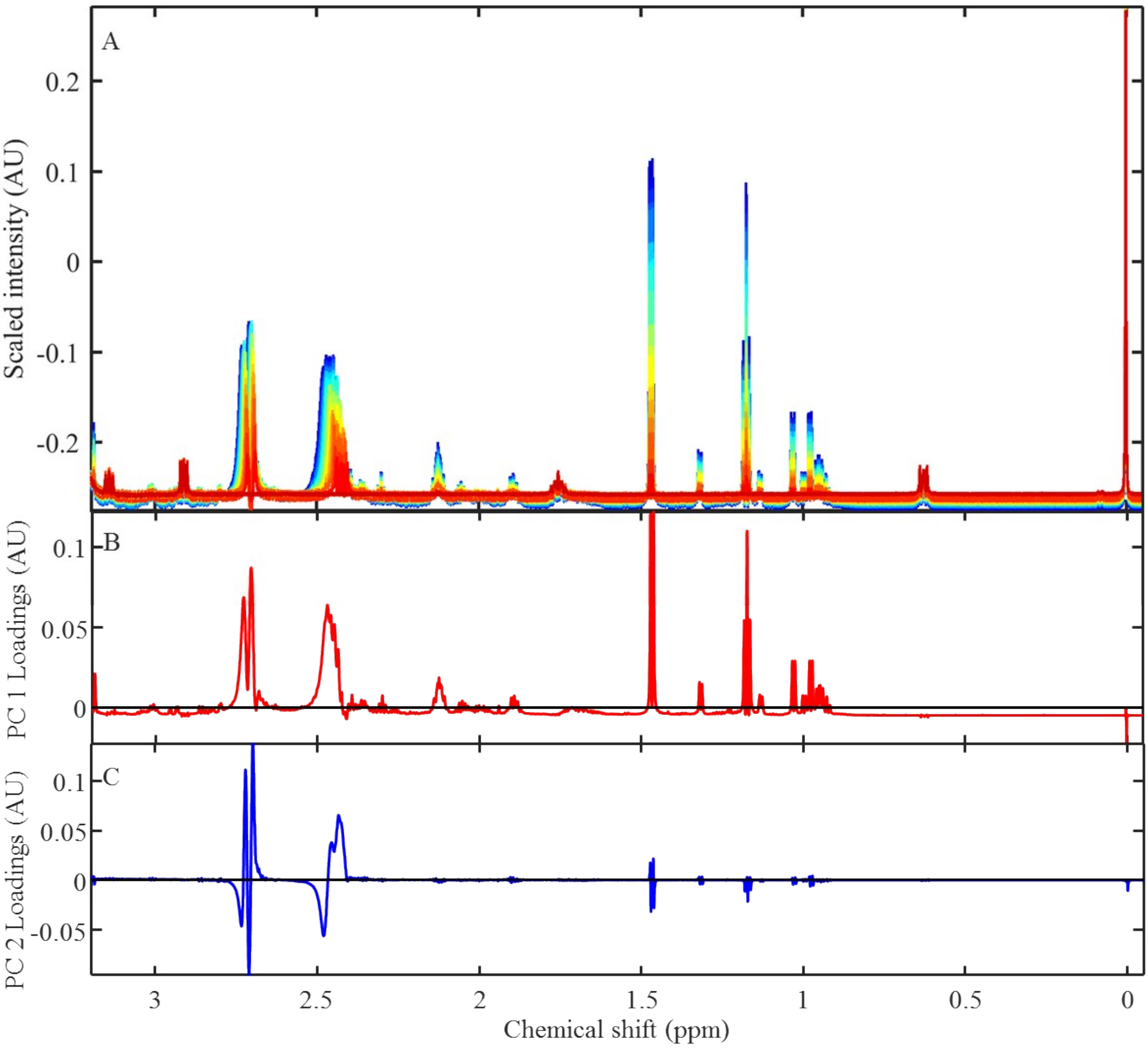

Figure S1: Overlaid ^{1}H NMR spectra for the adulteration of coconut water with our mixed sugar solution ranging from 0% (blue) to 100% (red), truncated to show the coconut-specific signal region (3.2 to -0.05ppm). Spectra have beenbaseline corrected. B: Loadings plot for PC 1, demonstrating the variance associated with each chemical shift value measured. C: Loadings plot for PC 2.

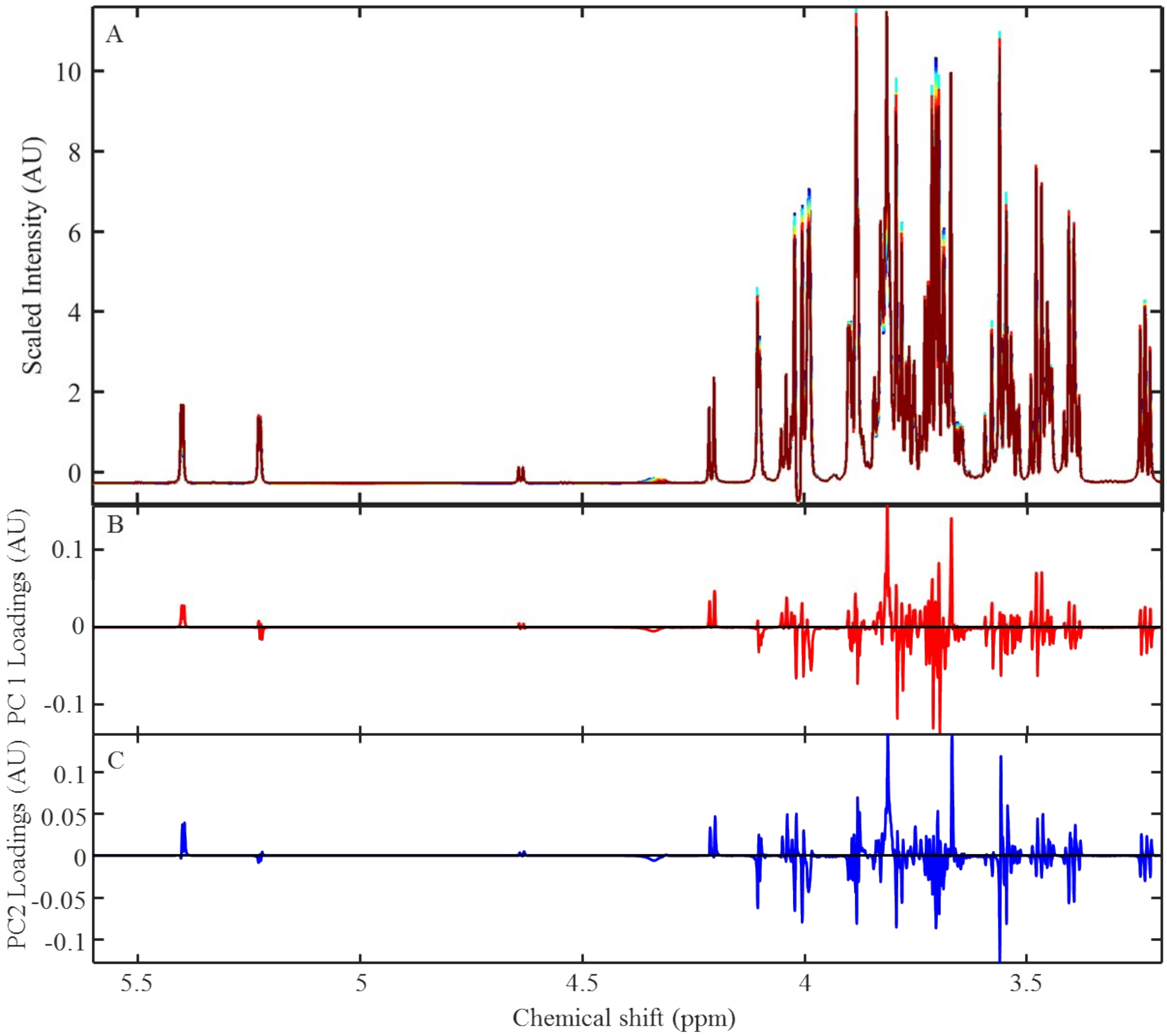

Figure S2: A: Overlaid ^1H NMR spectra for the adulteration of coconut water with our mixed sugar solution ranging from 0% (blue) to 100% (red), truncated to show the sugar-specific signal region (5.6 to3.2 ppm). Spectra have been baseline corrected. B: Loadings plot for PC 1, demonstrating the variance associated with each chemical shift value measured. C: Loadings plot for PC 2.

Evaluating the effect of pH on the chemical shift of malic acid signals

Although pH was controlled during initial experiments, the pH of coconut water (5.32) is very close to the calculated second pKa of malic acid (5.2). Furthermore, malic acid signals have been found to drift as a result of varying pH levels. To ensure that the source of drift was not a result of small pH variation, coconut water was spiked with HCl and NaOH to examine the effect of a small fluctuation inpH.

Methodology:

Three 1mL aliquots were prepared: one was spiked with 13 µL 1M NaOH (pH 5.43), one was spiked with 8 µL 1M HCl(Fischer Scientific) along with 5 µL water for normalisation (pH 5.21) and one was spiked with 13 µL water (pH: 5.32) and used as a control. Each sample was further spiked with 20 µL 4 mg.mL^{-1} DSS in D_2O for standardisation (earlier checks showed a negligible effect on pH). NMR data was obtained using identical conditions as prior, and data were realigned upon importation into Matlab.

Results:

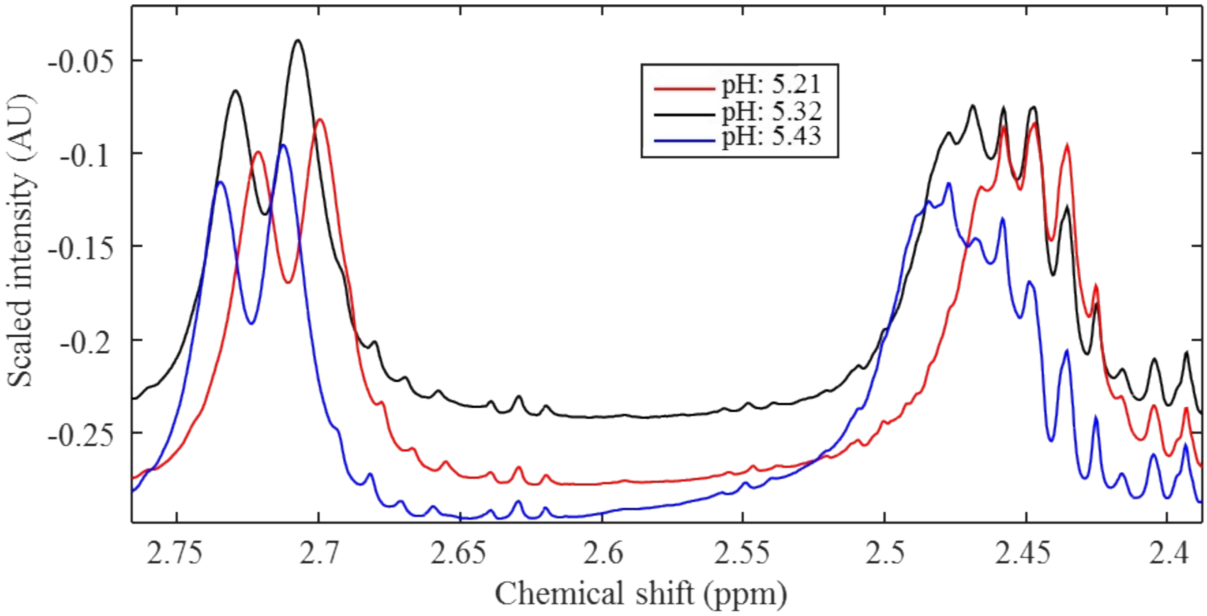

Figure S3: Overlaid ^{1}H NMR spectra of coconut water depicting the effect of differing pH values on malic acid signals. Spectra were not baseline-corrected.

Table S1: Table outlining the effect of pH on the two peaks in the ~2.7 malic acid signal. Values in parentheses are the shift relative to the coconut water(CW) sample at natural pH

pH	Shift peak 1	Shift peak 2
5.21 (-0.11)	2.721 (-0.008)	2.700 (-0.007)
5.32 (untouched CW)*	2.729	2.707
5.43 (+0.11)	2.735 (+0.006)	2.712 (+0.005)

*untouched CW is natural/freshly prepared coconut water

Figure S1 and Table S1 both confirm that a small change in pH (±0.11) results in a small drift in chemical shift, with an increase in pH leading to an increase in chemical shift. However, it should be noted that the magnitude of this drift does not match that of the drift observed in our original adulteration experiment. Where a variation of 0.11 pH units induced a change of <0.01 ppm, we originally found that a change of ~0.03 pH units led to a drift of ~0.02 ppm. We can therefore conclude that our original observations for malate signal shifts in the original adulteration experiments are not appropriately explained by pH alone.

Examining the effect of Malic acid concentration on the chemical shift of its signals

Along with pH, malate aggregation and cluster formation was considered as a feasible cause for the change in chemical shift. Before examining the possibility of other constituents in coconut water leading to this effect, it was important to check whether a decrease in the concentration of malate alone led to any alterations in chemical shift.

Methodology:

To determine whether the concentration of malate itself had an effect on its chemical shift, a strictly pH controlled dilution set was made. A "100% malate" solution meant to emulate the natural concentration of sodium malate in coconut water using the same proportions as with the addition of Mg^{2+} experiment(pH 5.32). Aliquots of this solution were then diluted to 75%, 50%, 25%, and 5% in phosphate buffer, and each aliquot was spiked with 20 μL of 4 $mg.mL^{-1}$ DSS in D_2O for standardisation.

Results:

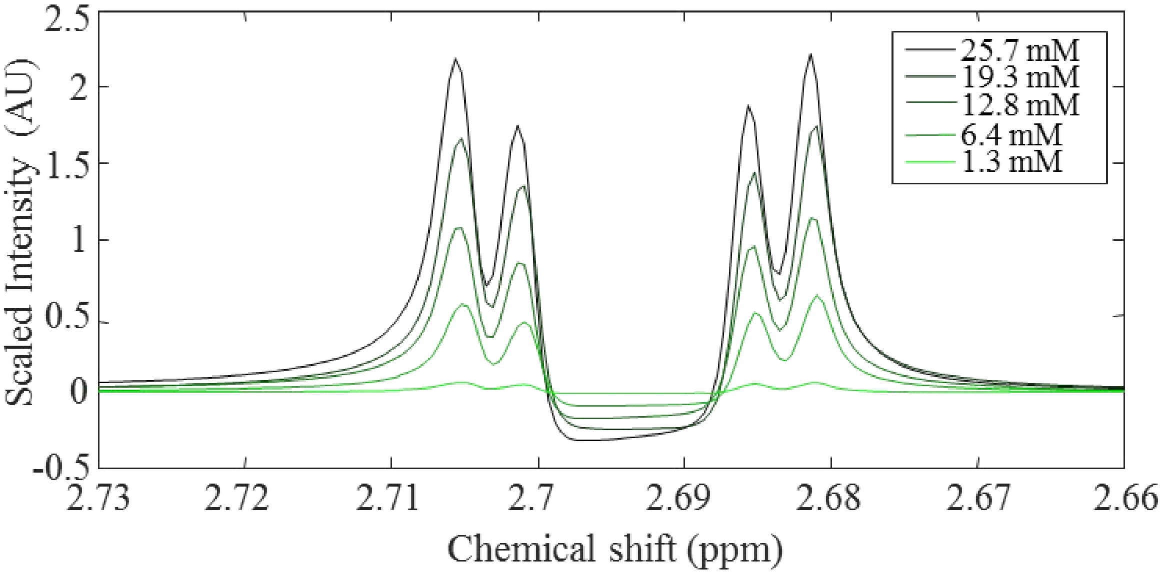

Figure S4: Overlaid ^{1}H NMR spectra of sodium malate at different concentrations emulating those of coconut water at different adulteration levels ranging from 0% adulteration (25.7 mM, black) to 95% adulteration (1.3 mM, green). Spectra have been baseline corrected and truncated to only show the malate signal.

It is clear from Figure S4 above that the concentration of malic acid alone has no effect on chemical shift. Once other constituents from coconut water are removed and the pH is tightly controlled, the peaks remain stable, only decreasing in intensity upon dilution. Furthermore, we see that the signals are not visibly sharpened or broadened, further confirming that other constituents in coconut water are responsible for our observations.

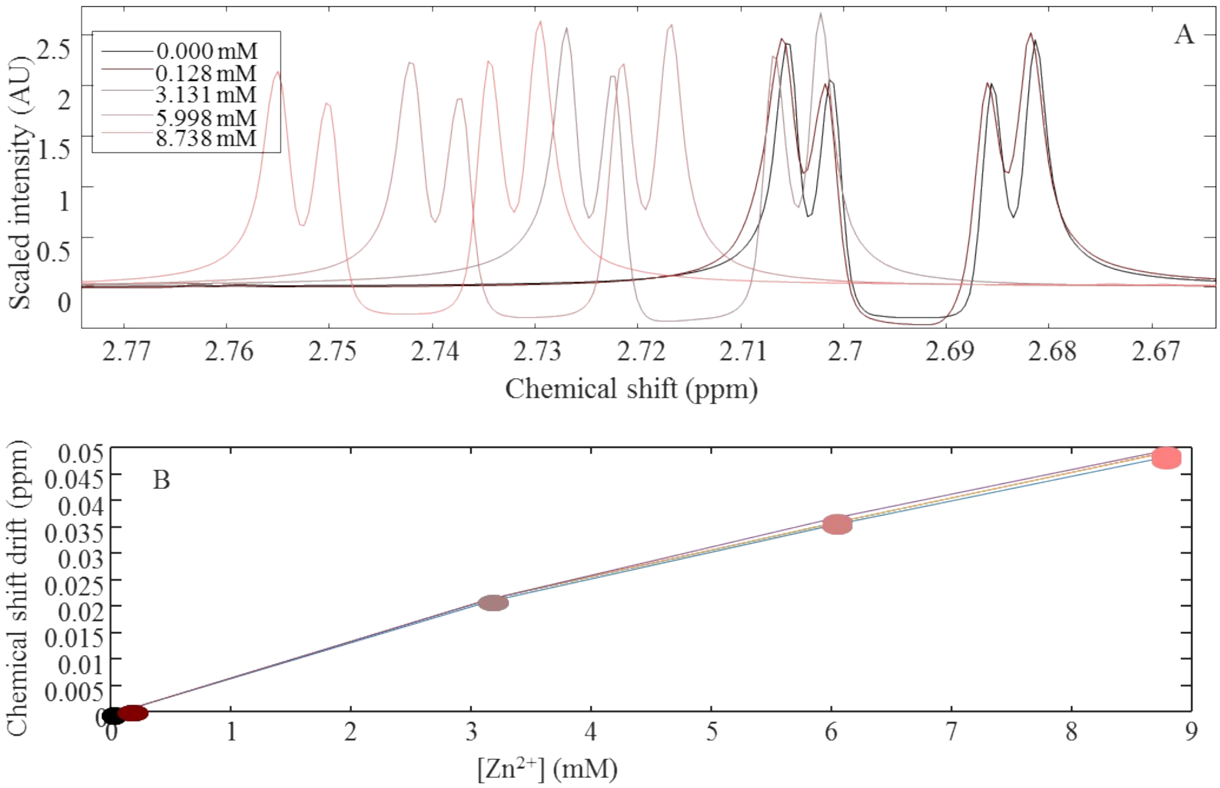

Figure S5: A: Overlaid ^{1}H NMR spectra depicting the effect on the malate signal of the incremental addition of aqueous Zn^{2+} ($ZnCl_2$) to a buffered 3.44 mg.mL^{-1} solution of sodium malate ranging from 0 mM Zn^{2+} (black) to 8.738 mM Zn^{2+} (red) in solution. B: Plot comparing the drift in chemical shift of each peak in the signal relative to that of the initial signal prior to addition of Zn^{2+} ions.