This is an interesting paper that looks at deamidation as a marker of parchment quality, and allows the comparison of deamidation levels with other variables such as species and thickness of parchment to gain insight into the parchment making process. It is interesting that this baseline correction appears to improve the s/n of some peaks, but not others. I think the paper could be improved by acknowledging the limitations of the background correction technique and if possible, to draw some conclusions as to why this works for some, but not other peaks, is it to do with the s/n for the peak in the raw data? I am unable to comment on the mathematical/statistical aspects as this is outside my area of expertise.

Some minor corrections are recommended.

Lines 116-119: I don't think this is true? You have a charge in MS2 as well, you can only see charged product ions and the product ion spectrum would be used for confirming the peptide sequence. The reason you have overlapping distributions in MS1 is due to the resolution of the instrument not the fact is the parent ion. In the future, since you have identified clear biomarkers it would be interesting to make a targeted MS method to carry out absolute quantitation using SRM on your 8 peptides.

Line 183: A s/n ratio of 1.5 is very low. Usually this is set at 3 for identification and higher than this for quantitative analysis (around 10) and what you are trying to do here is semi-quantitative.

Line 318 and 322: You could de-novo this to confirm?

Line 365: I'm not sure if authors mean that the base line correction did not work for this peptide in the sense that even after baseline correction, the s/n was too low?

Line 308: Why would a high level of deamidation in a peptide result in higher background if the background is caused by chemical interference from the MALDI matrix?

Line 384: MADI-TOF and ZooMS aren't interchangeable. One is describing the ionisation technique/mass analyser and one is an application of MS to a sample type. Maybe better to say something like in the field of bioarcheology the application of MALDI-TOF to ancient samples is commonly referred to as ZooMS.

Line 391: I wonder if this variation due to the poor quality spectra or biological variation?

Line 400: It would be very difficult, if not impossible to validate this to EMA or FDA regulations.

Figure 2: It's interesting that that for some peptides you can see an improvement in signal to noise when you compare them with the spectra in A vs B, but some still look poor quality after the background correction. Do you see any patterns in peptides where the correction works well for and which is doesn't? I suspect that rather than the ionisation type, it's the amount of collagen you are extracting in terms of s/n. If you remove deamidation levels from poor s/n spectra do your error bars change?

Figure 6: I'm not familiar with what level of spread of deamidation values you would expect for these samples but the error bars here seem quite high.

General comments: *m/z* should be italicised throughout.