May 20, 2013

*Journal of Visualized Experiments*

Editorial Office

Dear Michelle Kinahan:

Enclosed is a revised version of the manuscript “Profiling the triacylglyceride contents in bat integumentary lipids by preparative thin layer chromatography and MALDI-TOF mass spectrometry”, which I co-authored with Thomas Risch and Brett Savary. The reviewers provided helpful comments that we used to strengthen the manuscript. All reviewers felt that this was a well written manuscript that thoroughly explained the techniques of TLC and MALDI-TOF MS in a fashion that novice readers could understand. There were only minor edits and formatting changes, which we were able to make. Listed below are responses to the reviewers comments and changes have been highlighted in the manuscript with the Track Changes under Review option in Microsoft Word.

**Response to the reviewers:**

Many of the revisions were minor and most were corrected accordingly. Referee 1 supplied a thorough review on MALDI procedures and data interpretation. Both Referees felt that it would be beneficial to include diseases in the introduction associated with altered sebaceous profiles. A list of diseases was included with a 2012 review paper cited. Some specific responses to Referee comments are included below.

EDITORIAL COMMENTS

The formatting issues have been addressed including the deletion of brand or company names.

REFEREE 1

4. Intro, p. 2: "free fatty acyls" should be changed to "free fatty acids"

In keeping with the terminology proposed by Fahy et al. 2005 and 2009, the usage of acyl was retained.

10. Protocol, 1 - It is unclear if the procedure is being performed on live animals or on dissected animal tissue. A clarification is needed, along with a few additional comments about handling live bats, if necessary (ie, how to hold them, are they anesthetized, etc.)

This was clarified in the procedures along with the IACUC approval and the citation for proper use of wild mammals in research, which was followed.

13. Protocol, 1, 1.3 - Is there a specific grade of filter paper required?

There is no particular grade that is required; however, we used qualitative P8 filter paper.

16. Protocol 3 - It might be helpful to make a new subheading called "Instrument calibration and optimization" or something like that, and have steps 3.1 to 3.5 under that subheading, since they are separate from preparing the samples for analysis.

While we feel that the Referee is correct in this suggestion, the protocol is at the length limit established by JoVE without incurring additional editorial processing.

18. Protocol 3, 3.11 and 3.12 - This could use a little more details...are peak lists exported from the instrument after smoothing and background subtraction? What are the typical settings for S/N or minimum intensity? Are there any restrictions on how the peaks are entered into the glycerolipids\_batch tool? A screen shot of the tool would be helpful in the manuscript.

Peak lists are exported after smoothing and background subtraction, which is stated in 3.11. Restrictions on how peaks are processed in LIPID MAPS is dependent on the equipment operator, therefore operator proficiency is required for data acquisition and interpretation. The authors feel the level of detail given is sufficient for the manuscript.

17. Protocol 3, p. 6 - It is surprising that you are analyzing TG's in positive mode but you are adding NaOH...have you looked at any other sources of Na for complexation? Does the NaOH work best? If so, a comment to that effect should be added.

This topic has been addressed in detail in Gidden et al. 2007, which is cited in the manuscript. “Various concentrations (from 0.025 to 14 M) of NaOH, NH3, NaC2H3O2, and NH4HCO3 were prepared in water”, added to samples of vegetable oil, and analyzed with a Bruker Ultraflex II. They concluded that the presence of base promoted Na+ ion formation while suppressing H+ ion formation. The H+ ion is more unstable and prone to fragmentation, so the addition of the base promotes singly charged molecular ions that are more stable. A 1.0 M addition of NaOH was determined to give optimal results. A sentence was added to the results to explain the addition of NaOH.

20. Results, p. 6 - The discussion of the differences between prep and HP TLC plates is appreciated, but it needs clarification. It reads as though with prep plates there are 4 bands: sterols, FFA's, TAGs, and sterol esters/wax esters/squalene. But with HP plates there are only 3 bands, but it is stated that "the sterol esters, waxy esters and squalene will appear as only 3 separate bands" - are those 3 one band with prep plates and 3 bands with HP plates? Or are sterols, FFAs, TAGs, and sterol esters/wax esters/squalene 3 bands with HP plates, with sterols and waxy esters not separated? Please clarify.

We feel that the separation of broad lipid class was not clarified thoroughly and was confusing to read as the Referee commented. We added more specific terminology and explanation of broad lipid class separation should be less confusing.

23. Results - One problem with lipid analysis in positive mode is the spreading out of signal between protonated, sodiated, and potassiated species. Do you know how much protonated or potassiated signal you might be getting? Does the addition of NaOH allow only Na+ ions? A sentence or two addressing this topic is needed.

Please refer to the comment to number 17.

24. Discussion, p. 9 - add a source/vendor for Sebotape; also change "...Sebutape call also provide..." to "...Sebutape can also provide..."

Please refer to the editor’s comment below. Sebutape was changed to “specialized tape products” and was included in the table of materials and reagents.

*Editorial Comment: To comply with JoVE guidelines, please do not add any vendor information or company or brand names to the manuscript text but include this information in the table of materials and reagents.*

REFEREE 2

Referee 2 had similar comments as Referee 1. All of the minor concerns have been addressed.

I would like to thank the referees for their time to improve the quality of the manuscript. Thank you for considering this manuscript for publication in *Journal of Visualized Experiments*.

Sincerely,

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