Investigating the metabolome of *Steatoda nobilis* by whole body mass spectrometry imaging

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Introduction

In this work, we focus our efforts on the study of the metabolome of Steatoda nobilis using whole-body MALDI-FT-ICR imaging. To our knowledge, arachnid metabolites as still poorly explored, even if it could yield to fascinating insights such as (i) a better understanding of the way used to metabolize different preys, shedding light on its ecological role, (ii) the alteration of her metabolism to adapt to various environmental conditions, or again (iii) the identification of the pheromones used for matting and territory marking.

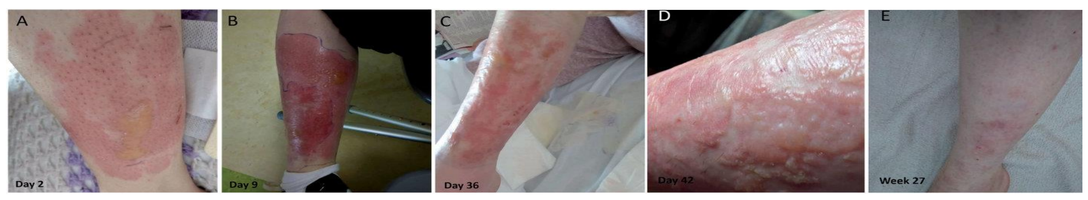
Mass Spectrometry Imaging (MSI) is a cutting-edge technology that has revolutionized the field of metabolomics. MSI combines the capabilities of mass spectrometry with spatial information, allowing researchers to visualize the distribution of metabolites within tissues and organisms. This technique has found applications in a wide range of fields, including biology, medicine, and environmental science. The application of MSI to investigate the metabolomics of spiders holds great promise as it enables the visualization of metabolite distribution within spider tissues. This can provide insights into the localization of specific metabolites within organs like silk glands, venom glands, and digestive systems. It can additionally help in the identification of novel and uncharacterized metabolites in spider tissues, potentially uncovering unique adaptations or bioactive molecules with ecological or biomedical significance.

Context

The Noble false widow spider, *Steatoda nobilis*, is a rapidly expanding species of the *Theridiidae* family, which includes the infamous black widows. This spider is increasing globally, in and around human dwellings.

Female *Steatoda nobilis*

It has recently been recognized as a medically significant species, due to growing numbers of reported severe envenomation. Moreover, recent studies analysing the venom characteristics of these spiders demonstrated the presence of α-latrotoxins, responsible for the latrodectism symptoms observed after black widow bites (shown below).



Materials & methods

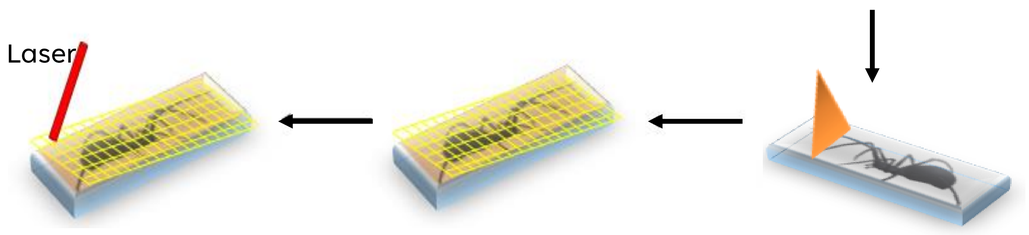
While MSI holds immense potential for advancing our understanding of spider metabolomics, the sample preparation of spider slices constitutes one of the main difficulties. The spider is made of very soft tissues and large proportion of biological fluids which makes the preparation of the sample a tough challenge, this problem has been solved by the mean of a gelatine-based sample fixation.

Sample preparation

Steatoda nobilis sacrifice (CO_2 chamber)

Incorporation in gelatin, then deep freezing

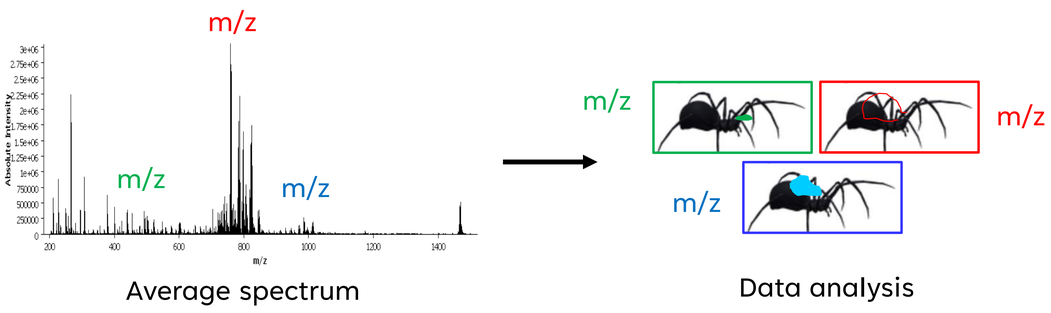
Series slicing (25 µm) Staining (Histology) / ITO Slide (MS)



Matrix deposition (HCCA ; 2-5 DHB) Suncollect

Pixel definition (Spatial resolution 50 µm)

Acquisition MALDI-FT-ICR 9.4T



Average spectrum

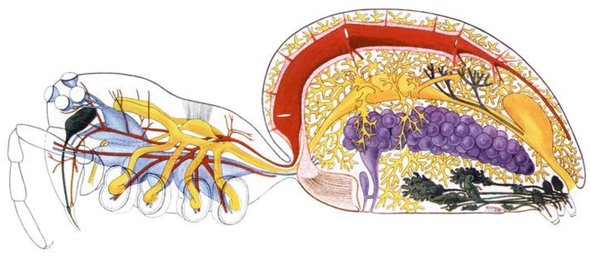
Data analysis

The slicing and matrix deposition are two critical steps of the sample preparation that can radically change the results obtained. These parts required a long optimisation process to yield interesting results.

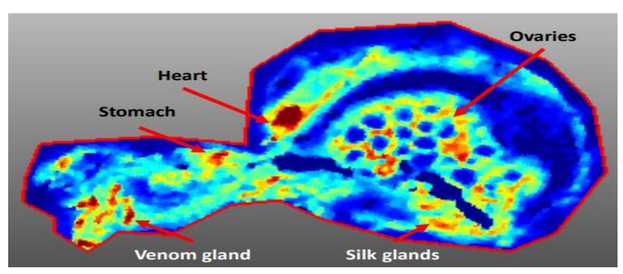
In addition, the parameters chosen for the acquisition have an even higher impact on the data. The optimisation parameters led to significant improvement of the data collection.

Results and discussion

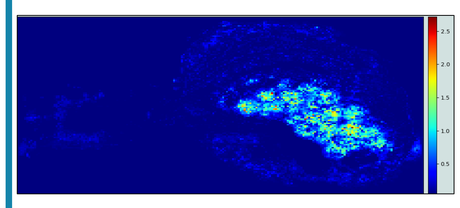
Organs specific metabolites allowed to rapidly identify various organs of the spiders, such as the silk glands, the ovaries, the venom glands or again the nervous systems.



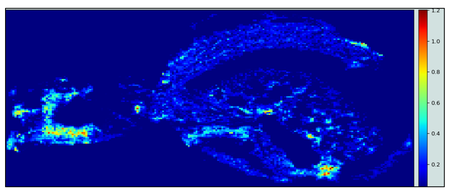
Spider anatomy



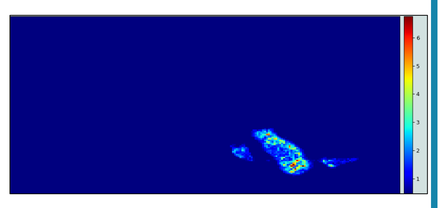
Compiled images



Ovaries



Vascular system



Silk glands

Kendrick plot

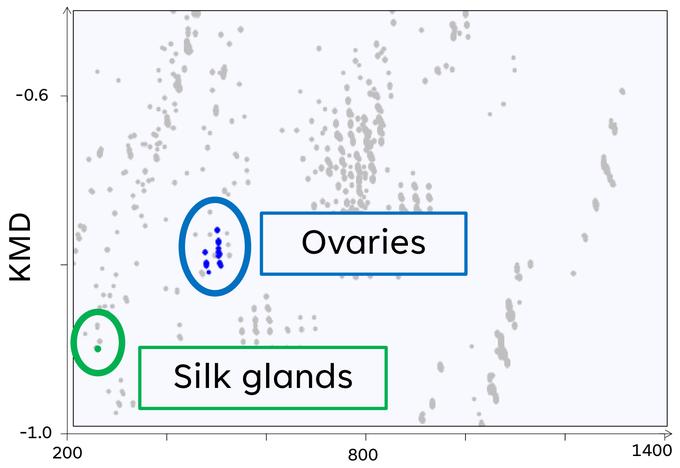
Use of a homemade program to solve data quantity issues allows to separate molecules based on their functional groups

Kendrick base = 1/14 of the $^{12}C^{1}H_2$ mass

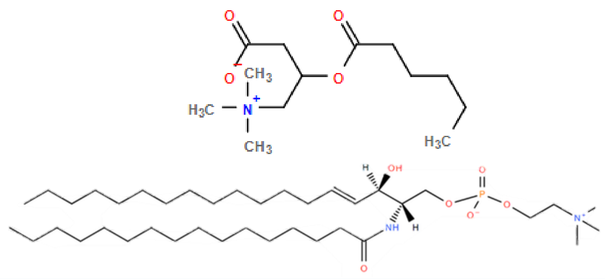
$$KM = \frac{m}{z} * \frac{\text{KRnominal mass}}{\text{KRexact mass}} = \frac{m}{z} * \frac{14}{14{,}01565}$$

KMD = round (KM) – KM

Advantage: Molecules are grouped by structural family, additionally to their location



Example of molecules found



Hexanoylcarnitine (260.1856 m/z):
Found in the silk gland, critical for the production of energy

Sphingomyelin SM(d34:1) (725.5568 m/z):
Found in the brain and nervous system

Conclusion

Globally, this project allowed the identification of a large number of molecules present in *Steatoda nobilis*, as well as their spatial distribution on the sections. This shows the potential of Mass spectrometry imaging for the metabolomic analysis of other arthropods and leads the path to other research that will be using the same method. The protocol for the sample preparation can be the same for any arthropods. Although the technique has been optimized for *Steatoda nobilis* but might require some adaptations for other species. If new studies are performed, this will resolve the problem of databases being incomplete and since computers keep getting better, since software are more and more advanced and since instruments are more and more powerful, the problem of the size of the files will disappear in the future.

References/Acknowledgements: Based on Q. Bastiaens' master thesis under the supervision of D. Redureau and V. Bertrand; promoter: L. Quinton