Novel food-omics: exploring a fingerprinting approach to insect species identification

Belluco, S[1], Miano B[2], Piro R[2].

1. Laboratorio di tecnologie alimentari - Istituto Zooprofilattico Sperimentale delle Venezie, Vicenza (VI), ITALY sbelluco@izsvenezie.it;

2. Laboratorio di chimica sperimentale - Istituto Zooprofilattico Sperimentale delle Venezie, Vicenza (VI), ITALY

Objective Edible insects are a promising food source but attention should be paid to their safety and authenticity [1,2]. In this work we have explored the performances of DART-HRMS (Direct Analysis Real Time – High Resolution Mass Spectrometry) to differentiate insects' powder of known origin and composition. In particular, analyses were performed to evaluate the characteristic fingerprinting profile of the insects samples and to identify chemical markers able to differentiate insects. All the collected data were used to build a statistical model able to discriminate samples according to composition (species) and to classify insects samples (both single species and multispecies) of unknown origins to identify adulterated powders. Results suggest the possibility to use DART-HRMS as rapid techniques for authenticity evaluation at least as a first screening technique.

Results

Data from MeOH extraction (positive ionization) were used to run statistical analyses according to the results of preliminary tests. Statistical analyses were run on all samples, on samples of crickets and mealworms and on crickets only samples. Relevant compounds were annotated through the online tool Metlin (XCMS - https://metlin.scripps.edu/) and are presented in the box plots. Results, despite the exploratory nature of this study, show a correct clusterization of samples according to PLS-DA, with the identification of relevant discriminating compounds among samples, as shown in the box plots (fig. 3, 4, 8). Statistical analyses were repeated also including only crickets and mealworms and only crickets to further explore the discriminating ability.

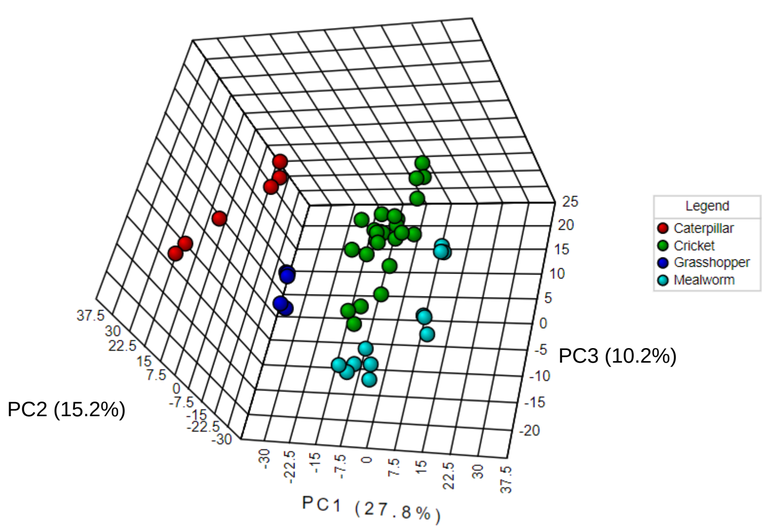


Figure 1: PCA - 3D score plot of the selected PCs. Explained variance in brackets.

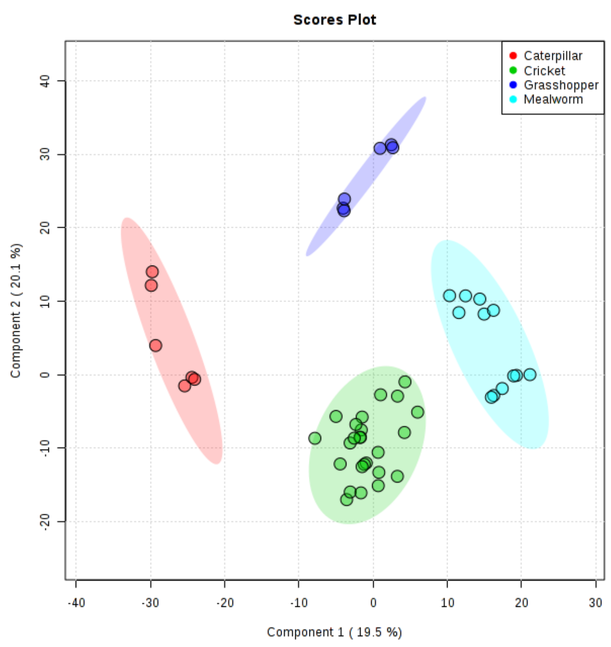


Figure 2: PLS-DA 2D score plot of the selected PCs. Explained variance in brackets.

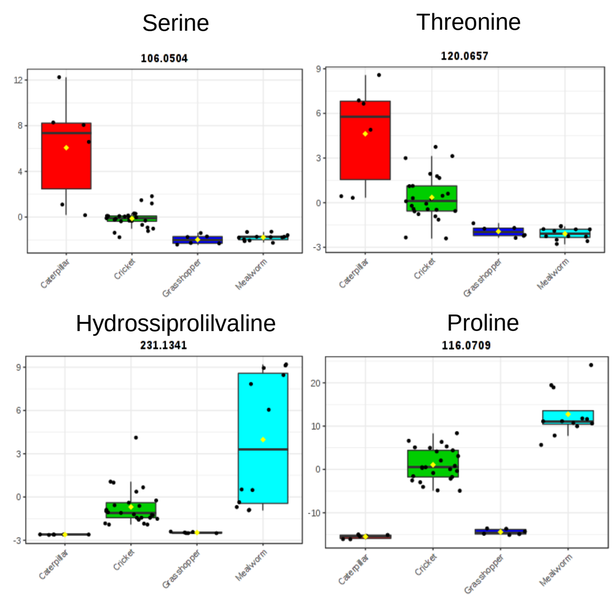


Figure 3: PLS-DA. Box plot of relevant compounds. Tentative annotations through Metlin online tool.

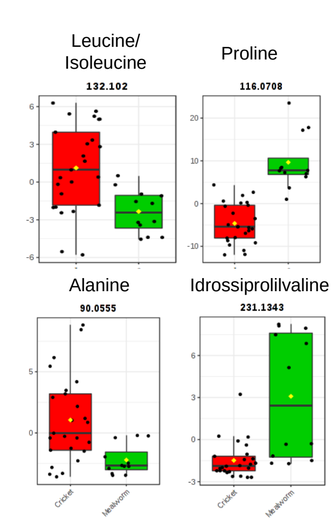


Figure 4: PLS-DA. Box plot of relevant compounds. Tentative annotations through Metlin online tool.

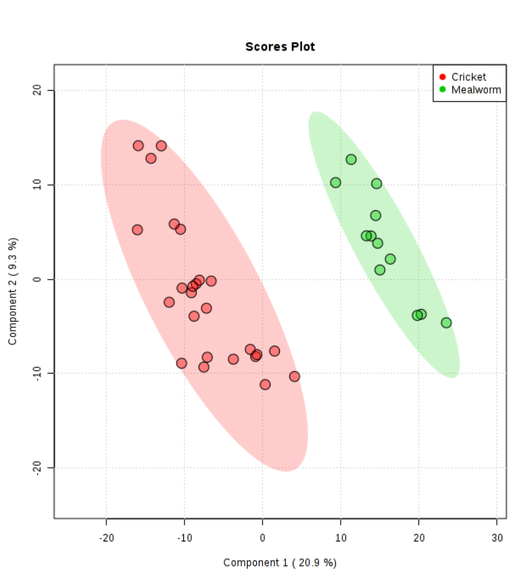


Figure 5: Mealworms vs crickets. PLS-DA 2D score plot of the selected PCs. Explained variance in brackets.

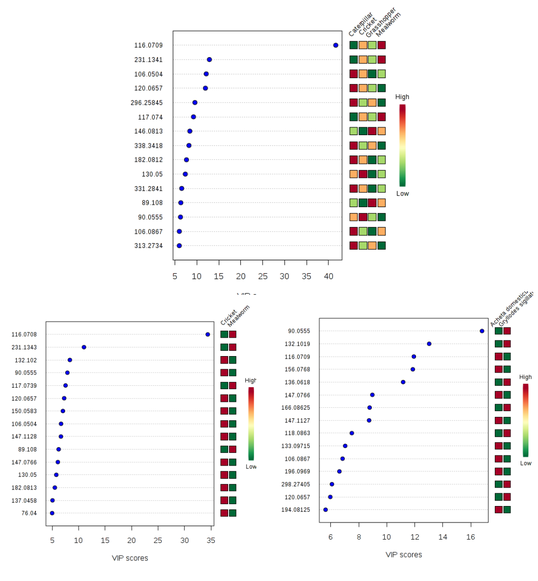


Figure 6: VIP scores of different PLS-DA with relevant compound (expressed in m/z) identified.

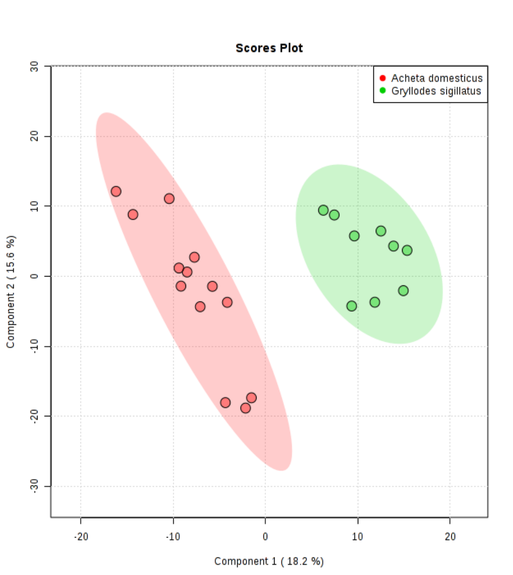


Figure 7: *Acheta domesticus* Vs *Gryllodes sigillatus*. PLS-DA 2D score plot of the selected PCs. Explained variance in brackets.

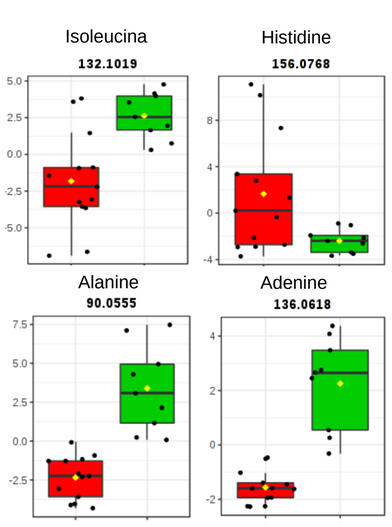


Figure 8: PLS-DA. Box plot of relevant compounds. Tentative annotations through Metlin online tool.

Conclusions The study show promising results but, due to the pilot nature of the investigation, no final conclusions can be reached. All analyses carried out show the presence of discriminants compounds in the tested species and, due to the different origins, such compounds can be considered as species dependent. Further studies are needed to evaluate to which extent discriminating ability is useful to predict the species content of insects powder or to evaluate any other difference in marketed products influencing composition.

Materials

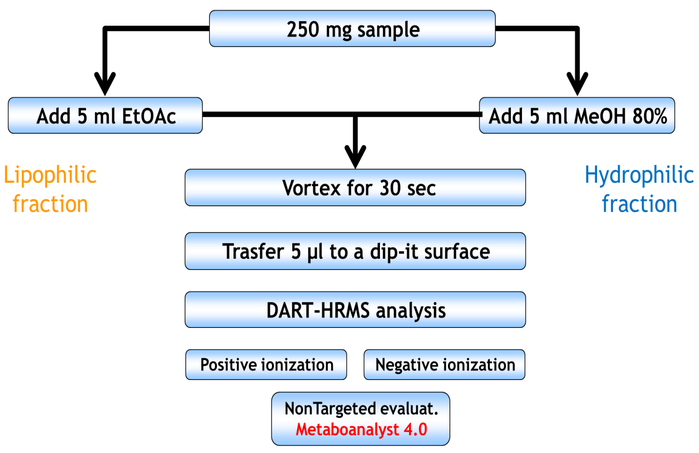
Insects samples originated both from commercial and research farms. Commercial samples in particular originated from the collaboration with the Entotrust global certification program for food safety and environmental sustainability. Details in table:

	Common name	Species	Origin	Details
1	Banded cricket	*Gryllodes sigillatus*	The Netherlands	Dried, powdered.
2	Banded cricket	*Gryllodes sigillatus*	Canada	Dried, powdered.
3	Banded cricket	*Gryllodes sigillatus*	Canada	Dried, powdered.
4	House cricket	*Acheta domesticus*	Italy	Dried, powdered.
5	-	*Gryllus madagascariensis*	Madagascar	Dried, powdered.
6	House cricket	*Acheta domesticus*	Italy	Dried, powdered.
7	House cricket	*Acheta domesticus*	Italy	Dried, powdered.
8	House cricket	*Acheta domesticus*	Italy	Dried, powdered.
9	House cricket	*Acheta domesticus*	Italy	Dried, powdered.
10	Chapulines	*Sphenarium purpurascens*	Mexico	Dried, spiced.
11	Chapulines	*Sphenarium purpurascens*	Mexico	Dried, spiced.
12	Chenille de karite	*Cirina butyrospermii*	Burkina Faso	Boiled, fried and dried.
13	Chenille de karite	*Cirina butyrospermii*	Burkina Faso	Boiled, fried and dried.
14	Mealworm	*Tenebrio molitor*	Unknown	Dried, powdered.
15	Mealworm	*Tenebrio molitor*	France	Dried.
16	Mealworm	*Tenebrio molitor*	Italy	Dried, powdered.
17	Mealworm	*Tenebrio molitor*	Italy	Dried, powdered.

Methods

Analyses were carried out through DART-HRMS with Orbitrap technology according to the flow in picture. riefly, powdered samples (250 mg) were extracted in Ethilacetate (lipophilic extract) and methanol-water (hydrophilic extract). All the mixtures underwent ultrasound bath at room temperature. Finally, 5 µl of the solutions was introduced to instrument using Dip-it tips (IonSense, Saugus, MA, USA). Mass between 75 and 1125 m/z were recorded at a resolution of 70000 FHWD. A total of 12 spectra samples were acquired (3 for each combination extraction/ioniziation).

Statistical analyses (PLS-DA) were carried out with the online tool metaboanalyst [4] according to the following specifications: mass tolerance 0,008, IQR data filtering, normalization by sum with Pareto scaling (More details about methods in [6]).



References

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