Direct Analysis in Real-Time with High Resolution Mass Spectrometry: A Rapid Tool for Black Truffle Authentication

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Introduction

Food fraud is a major issue in the food industry leading to financial losses for food processors, inflicting lasting damage in the trust of consumers and in the most severe cases even threatening public health. With the aim for an excessive financial profit, particularly expensive products such as oils, spices and truffles are prone to food fraud.

Truffles are considered a luxury product with prices ranging up to 1000 – 2000 €/kg for the Périgord truffle (Tuber melanosporum Vittad.). Consequently, there is a concern about food fraud, which can encompass blending authentic truffles with lower-quality alternatives or employing artificial flavorings to replicate the truffle taste. Notably, the Asian truffle (Tuber indicum Cooke et Massee) is morphologically highly similar to the Périgord truffle but much cheaper in price. Also, while having little flavor on its own, the Asian truffle is able to take on the flavor of other truffles when stored together. Identifying adulterated truffles is a complex challenge for the food industry. To protect the customer, an efficient, simple and fast workflow for food authenticity and quality control analysis is of high interest.

In this study, we developed a comprehensive workflow for the differentiation of different black truffle species utilizing Direct Analysis in Real-Time (DART) ionization in combination with High Resolution Mass Spectrometry.

Methods

Sample preparation

The sample set consisted of 10 samples from each truffle species (*T.* melanosporum, *T.* indicum and *T.* aestivum). Samples were lyophilized for 24 h and grounded. 100 mg of the finely grounded truffles were extracted with 1 mL of ACN:H2O; 75:25 + 0.1% FA; v:v:v) for 15 min at 25 °C in an ultrasonic bath. The supernatant was collected after centrifugation at 17,000 rcf for 2 min and filtered using a H-PTFE-syringe filter. 3 µL of the extract were deposited onto a QuickStrip wire card for acquisition. Each sample was spotted and measured in duplicate.

DART source

The DART JumpShot source (Bruker Daltonics) was operated at 300 °C with He as ionization gas in pulsed gas flow mode with a pulse duration of 3 s.

QTOF-MS

For classification analysis the Impact II VIP QTOF mass spectrometer (Bruker Daltonics) was operated in negative ionization mode. MS/MS experiments were carried out using AutoMS/MS acquisition with a scheduled precursor list.

Software

Data processing and evaluation was carried out in MetaboScape (Bruker Daltonics). This included feature extraction, statistical analysis and tentative unknown annotation based on accurate mass, isotopic pattern and MS/MS spectra.

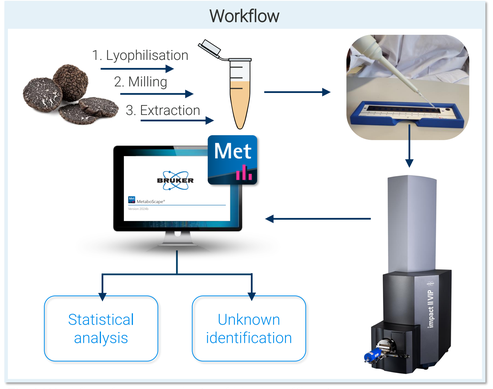

Fig.1 Workflow for the analysis of black truffles. It covers the data acquisition using the DART JumpShot source coupled to the impact II VIP QTOF-MS and the data evaluation in MetaboScape.

Results

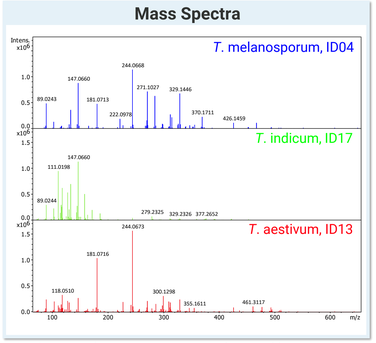

Fig. 2 Exemplary mass spectra obtained by DART-QTOF-MS in negative ionization mode.

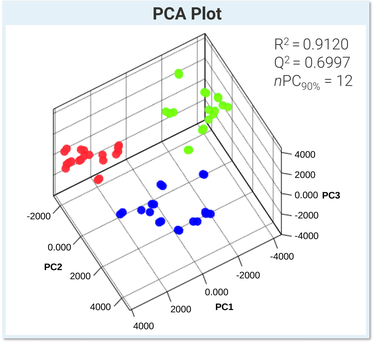

Fig. 3 3D PCA scores plot *T.* melanosporum (blue), *T.* indicum (green) and *T.* aestivum (red).

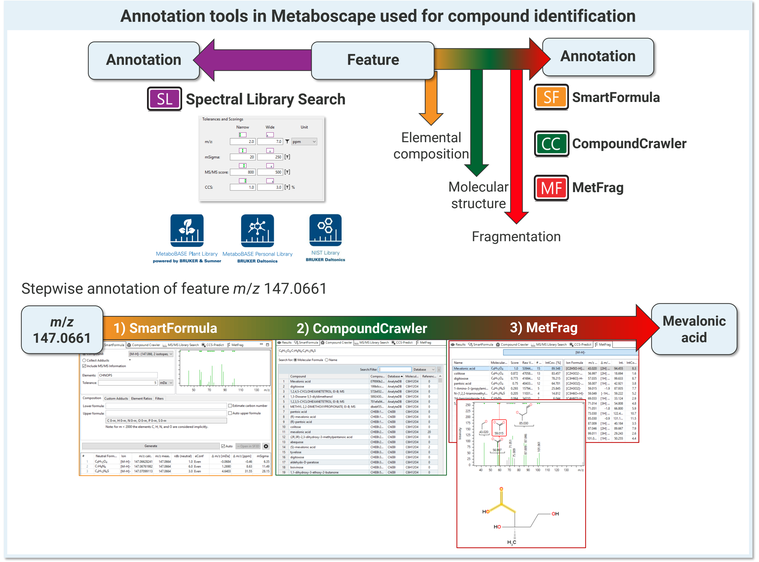

Fig. 4 Annotation tools in MetaboScape for the tentative annotation of the potential marker compounds. Annotation can be performed by either spectral library search or a workflow for complete unknowns based on HRMS information for the accurate mass, the isotope pattern as well as MS/MS fragments (Top). Illustration of the 3-Step workflow for the feature *m/z* 147.0661 (Bottom) .

Discussion

- Differences in the acquired mass spectra were observed between the different truffle species (Fig.2) as well as between samples from the same species
- Unsupervised principal component analysis (PCA) revealed distinct clustering of samples from one species and clear separation of different species (Fig. 3)
- Pairwise supervised partial least square analysis (PLS) was used to determine features with the highest variable importance in projection (VIP) responsible for group separation (Tab. 1)

- Tools for untargeted unknown identification included in MetaboScape were used for annotation of candidate marker compounds (Fig. 4):
  - Fully automated spectral library search
  - Semi-automated annotation workflow including elemental composition prediction, structure assignment and *in silico* fragmentation
- Six out of the nine top-scoring candidate markers could be assigned to a compound. The presence of those compounds in truffles was confirmed by literature search (Tab. 1)
- Other compounds identified in the truffle samples but less important for species discrimination were belonging to the groups of amino acids, sugars and organic acids

Tab. 1 Candidate marker compounds as determined by PCA and PLS-DA with putative annotation results.

PLS-DA	Feature	VIP / %	Ion	Molec. Formula	Name	|ppm|	mSigma
TM	*m/z* 329.1447	6.5	$[M-H]^-$	$C_{12}H_{26}O_{10}$	Unknown 2	1.88	6.5
	m/z 285.1185	6.2	$[M-H]^-$	$C_{10}H_{20}O_9$	Unknown 1	2.30	34
vs.	*m/z* 147.0661	6.2	$[M-H]^-$	$C_6H_{12}O_4$	Mevalonic acid[1]	0.46	6.4
TI+TA	*m/z* 133.0506	5.4	$[M-H_2O-H]^-$	$C_5H_{12}O_5$	Xylitol[a, 2, 4]	0.48	9.5
	m/z 111.0200	5.0	$[M-H]^-$	$C_4H_4N_2O_2$	Uracil	0.15	4.22
TA	*m/z* 181.0715	10	$[M-H]^-$	$C_6H_{14}O_6$	Mannitol[a, 2-4]	0.15	0.50
	m/z 147.0661	5.9	$[M-H]^-$	$C_6H_{12}O_4$	Mevalonic acid[1]	0.46	6.4
vs.	*m/z* 133.0506	5.3	$[M-H_2O-H]^-$	$C_5H_{12}O_5$	Xylitol[a, 2, 4]	0.48	9.5
TI+TM	*m/z* 227.0769	5.2	$[M-H]^-$	$C_7H_{16}O_8$	Unknown 3	1.50	0.73
	m/z 285.1185	3.9	$[M-H]^-$	$C_{10}H_{20}O_9$	Unknown 1	2.30	34
TI	*m/z* 181.0715	10	$[M-H]^-$	$C_6H_{14}O_6$	Mannitol[a, 2-4]	0.15	0.50
	m/z 111.0200	7.2	$[M-H]^-$	$C_4H_4N_2O_2$	Uracil	0.15	4.2
vs.	*m/z* 89.0243	6.3	$[M-H]^-$	$C_3H_6O_3$	Lactic acid[4]	1.01	0.36
TM+TA	*m/z* 103.0400	4.8	$[M-H]^-$	$C_4H_8O_3$	(±)-3-Hydroxy-butyric acid[4]	0.22	-
	m/z 227.0769	3.8	$[M-H]^-$	$C_7H_{16}O_8$	Unknown 3	1.50	0.73

*and/or epimers

[1]Splivallo *et al.*, New Phytologist, **2011**, 189, 688-699.
[2]Li *et al.*, Journal of Separation Science, **2023**, 46, 2200883
[3]Ceccaroli et al., New Phytologist, **2011**, 189, 751-764.
[4]Caboni *et al.*, *Food Chemistry*, **2020**, 319, 126573.

Conclusion

DART-QTOF-MS for truffle authentication offers:

- High throughput analysis enabled by short analysis times of 15 s per sample
- Chromatography-free workflow reducing solvent consumption
- High flexibility by support of various sample introduction options
- Increased information depth through high resolution mass spectrometry and MS/MS fragmentation capabilities

DART-QTOF-MS

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