Determination of volatile organic compounds from *Terfezia arenaria* ascocarps via SPME-GC-MS

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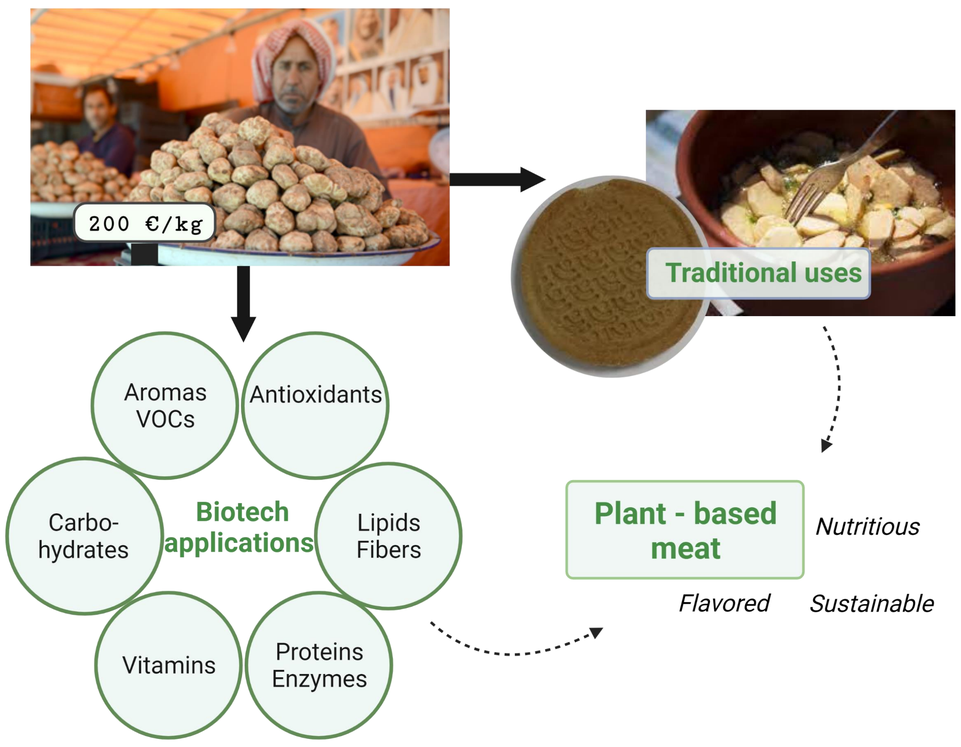
INTRODUCTION

Terfezia areanaria is a desert truffle, a seasonal edible ascomycete fungus with important ecological and socio-economic relevance. In addition to its economic value, the inclusion of *Terfezia* in our diet is also of high gastronomic and nutritional interest. If produced on a larger scale and at lower prices it may be an excellent product to use in the context of "plant based meat" concept, due to their nutritional and volatile composition. Few studies have demonstrated the chemical composition of volatile organic compounds (VOC) of desert truffles species, such as *T. claveryi, T. boudieri* and *Tirmania nivea*, and still there is no available data regarding *T. arenaria*, which is the one with highest productivity potential in Portugal. In this work we present the first analysis regarding the VOC composition of *T. arenaria* and compare it with the VOCs present in other truffles (*Tuber* species) and desert truffles.



METHODOLOGY

During the spring of 2019, fresh fruiting bodies of *Terfezia arenaria* were collected in the south of Portugal. Volatile analysis using HS-SPME was adapted from the protocol reported by Splivallo & Ebeler (2015)[1]. The fresh specimens were grounded and accurately weighed in 1.5 ml tightly sealed glass vials. A pre-extraction was performed in the vial at 60 ^{o}C for 10 minutes, then the SPME fiber (PDMS/DVB 65 um) was implanted manually and volatile compounds were extracted at 60 ◦C for 30 min. Afterwards, the SPME fiber was subsequently removed and placed manually in the injection port of the GC-MS. The analysis of volatile compounds was conducted on an GCMS-QP2010 (Shimadzu, Japan), with acquisition mode SCAN (35-600 m/z) and equipped with TRB-5MS column (Teknokroma, Spain). The injector and MS interface temperatures were both held at 250 ◦C. The analysis conditions were as the following: the constant flow of Helium in column was kept at 1.0 ml/min, the oven temperature was held at 40 ◦C for 10 min, then raised at a rate of 10 ◦C/min to 160 ◦C, and finally reached 260 ◦C with a rate of 50 ◦C/min and kept for 2 min. Blank GC-MS runs were performed during samples analyses.

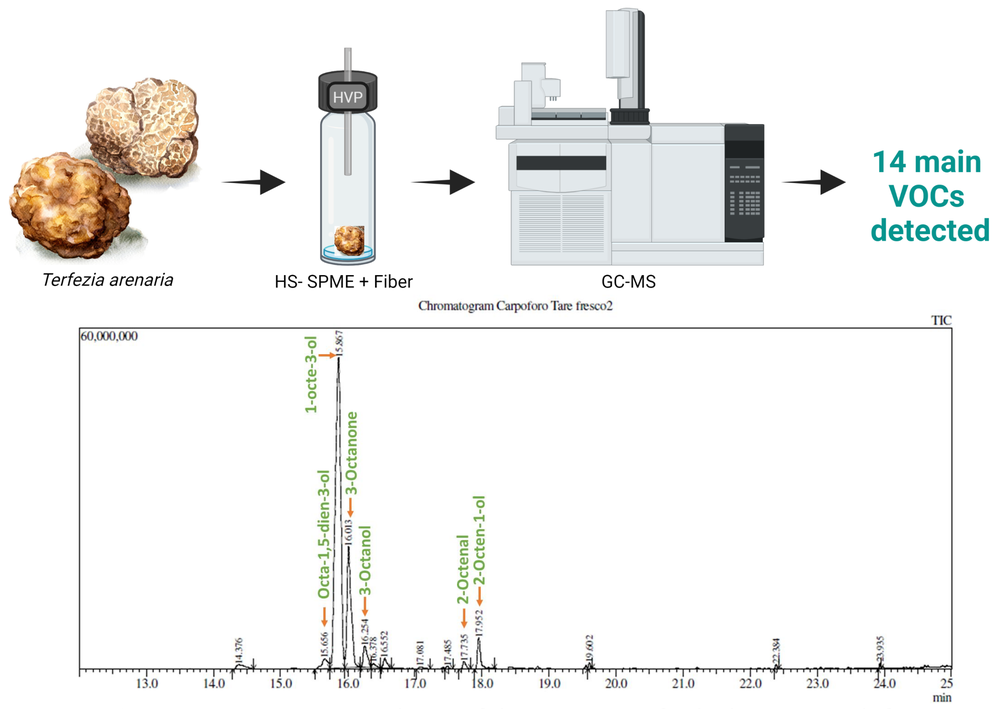
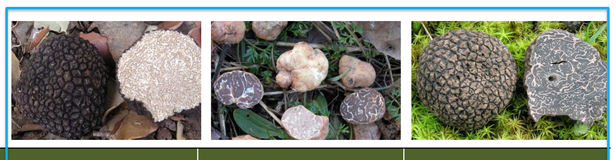


Figure 1 –Representative scheme of the extraction of volatile compounds from ascocarp samples by SPME-GC-MS, and the resulting chromatogram.

VOLATILE ORGANIC COMPOUNDS

Table 1 – Main volatile organic compounds of the fresh ascocarps collected in Alentejo. The results from the compounds with * could be influenced by external factors. Comparison of VOC's found in *Terfezia arenaria* with published data for truffles and desert truffles.



Terfezia arenaria VOC's	Retention Time	% Area	Metabolic pathway	Aroma Description	*Terfezia boudieri*	*Terfezia claveryi*	*Tirmania nivea*	*Tuber aestivum*	*Tuber borchii*	*Tuber indicum*
Hexanal	7.412	4.4%	Steroid hormone biosynthesis	grass, leafy, fruity, sweaty	X	X				
α-Pinene *	14.379	2.0%	Monoterpenoid biosynthesis	woody, spicy, oily	X	X				
L-Limonene *	17.086	0.4%		citrus, orange, fresh, sweet						
Octa-1,5-dien-3-ol	15.658	1.5%	Lipoxygenase pathway from linoleic acid	mushroom				X		
1-Octen 3-ol	15.847	66.4%		earthy, mushroom	X	X	X	X	X	X
3-Octanone	16.007	16.6%		herbal, lavender, mushroom	X	X		X	X	X
3-Octanol	16.256	2.0%		earthy, mushroom, herbal	X	X		X	X	X
2-Octenal	17.735	0.9%		green, citrus, peel, fatty	X	X	X	X		X
2-Octen-1-ol	17.952	3.4%		floral			X		X	X
Propanoic acid *	16.549	1.2%	n.a.	n.a.						
Benzeneacetaldehyde	17.417	0.2%	Phenylalanine metabolism	honey, sweet, floral	X					
Cyclopentane *	17.479	0.3%	n.a.	sweet, pungent, fruity						
Pyridine *	18.411	0.3%	Tropane, Piperidine, and Pyridine Alkaloid Biosynthesis	fishy						X
Benzaldehyde	18.831	0.2%	Aromatic compounds - Toluene degradation pathway	sweet, bitter, almond,	X	X	X	X		X
	TOTAL	**100%**	[2] [3]		[4] [5]	[4]	[5]	[6] [7] [8]	[6] [9] [10]	[9] [11]

CONCLUSION

This is the first Portuguese study reporting an overview of the VOCs present in fresh *T. arenaria* ascocarps. Collected ascocarps presented an aromatic profile similar to *T. boudieri* and *T. claveryi* and several compounds found in the *Tuber* species. The most abundant compound was 1-octen-3-ol. This compound can be found in greater quantity in the samples of *Terfezia arenaria*, unlike some species of *Tuber*, which appears in small amounts and is never the most representative element.
Terfezia arenaria samples do not have sulfur volatiles characteristic of *Tuber* species and are critical contributors to traditional truffle aroma. However, *Terfezia arenaria* has a milder aroma, making it more appealing to consumers who do not appreciate the strong flavour of truffles. Further analysis must be performed in future studies to obtain a complete aromatic profile of this endogenous desert truffle.

REFERENCES

[1] Splivallo R, Ebeler SE. (2015) Appl Microbiol and Biotechnology 99: 2583–2592.
[2] KEGG(2021) www.genome.jp/kegg/
[3] PathWhiz (2021) https://smpdb.ca/pathwhiz
[4] Farag MA, et al. (2021) *LWT* 142: 111046.
[5] Kamle M, et al. (2017) J Agric Food Chem 65:2977–2983.
[6] Mauriello G, et al. (2004) J Chromatogr Sci 42:299–305.
[7] Culleré L, et al (2010) Food Chem 122:300–306.
[8] Molinier V, et al (2015) Environ Microbiol 17: 3039–3050.
[9] Splivallo R, Bossi S, Maffei M, Bonfante P (2007) Phytochemistry 68:2584–2598.
[10] Zeppa S, et al. (2004) Rapid Commun Mass Spectrom 18:199–205.
[11] Culleré L, et al. (2013) Food Chem 141:105–110.

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