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Sources of Nicotine in dried mushrooms

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Introduction | Method Development | Results | Conclusion | References

Introduction

A discussion about the source of Nicotine (NIC) in boletus (*Boletus edulis*) was started in the EU in 2009. No clear conclusion about the nicotine source in wild dried mushrooms was reached [1,2]. In 2010 the specific MRL of 2.3 mg/kg for dried wild mushrooms was established.
Various theses have been developed and disproved. As ever, the highest nicotine levels can still be detected in Chinese mushrooms, especially from the province Yunnan [2].

Parallel to our study of nicotine findings in Indian tea [3], we postulated the ambient agro-economic situation in China and more precisely in Yunnan could be the cause (see additional information) [4] for high NIC levels.
To prove this, different products of boletus diverse origins were analysed, before and after the drying with different drying procedures. Other various dried mushrooms were also analysed for NIC and its main degradatoin product cotinine (COT).

Introduction | Method Development | Results | Conclusion | References

Method Development

The composition of the study:

- **Origin diff.**
- **Drying diff.**
- **Compartments sponge/base**
- **Sponge vs Lamella**
- **Further tobacco alkaloids/ degradation product COT**

method characteristica:

Equipment:
Infinity II-UHPLC – MS/MS 6500 QTRAP
ZORBAX Eclipse XDB- C18, 100 x 2,1 mm, 1,8 µm
Sample preparation:
Draft to § 64 LFGBL00.00-115/1, ISTD: NIC D4
Validation parameters

table 1. validation data

substance	LOQ, mg/kg	RSD at the LOQ, %	WFR at the LOQ %	linearity, R^2	linearity range, mg/kg
nicotine	0.01	15	89	0.998	0.01...2.0
anabasine	0.01	12	101	0.992	0.01...2.0
nornicotine	0.02	18	111	0.996	0.02...2.5
cotinine	0.005	11	98	0.999	0.005...1.0

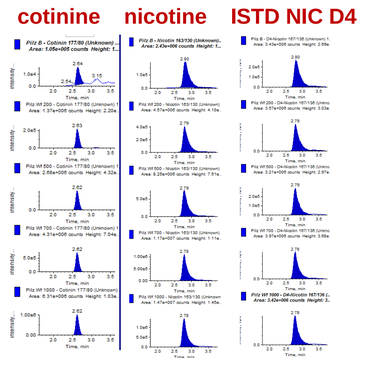

figure 1: chromatograms (examples)

additional information about Yunnan:
The province for special tobacco

figure 2: Yunnan at the map (southwestern China) and Yunnan boletus

Yunnan tobacco is a high quality tobacco quite popular among the cigarette smokers in China. In Yunnan, there are **16 tobacco brands**, altogether **21 kinds of tobacco products**. Yunnan tobacco has been highly applauded for its golden color, fragrant aroma, mild effect and pure taste. Yunnan tobacco has been exported and applied as raw materials in **84 tobacco factories** outside Yunnan in China [4].

Introduction | Method Development | Results | Conclusion | References

Results

1. ORIGIN
analysis for NIC in:

fresh boletus
from 2019 (july-oct)

UK
Russia
Germany

figure 3: self picked (bought) boletus

table 2. results table (10 products)

country	number of samples single mushrooms	amount min. mg/kg	amount max. mg/kg
Russia*	2	< 0.01	0.015
UK**	5	0.02	0.048
Germany*	3	< 0.01	0.025

* self picked
** self bought at the market (London)

resume 1: in fresh boletus amounts from negative, < 0.01 mg/kg, to max. 0.05 mg/kg (10 samples diff. European locations) are present

2. DRYING EXPERIMENTS

analysis for nicotine in self picked and dried russian boletus (NIC amount in fresh boletus < 0.01 mg/kg)

figure 4: domestic fruit/veg dryer
Photo: https://xn--grneliebe-r9a.de/getrocknete-steinpilze-ein-herrliches-aroma/

a) Dried in drying with dryer through steaming out
NIC/COT: < 0.01 mg/kg
NO positive findings

figure 5: air dried boletus

b) Dried outside after slicing at the air near the house
NIC: 0.053 mg/kg,
COT: 0.021 mg/kg

resume 2: smaller amounts in dried mushrooms (< 0.2 mg/kg) are possible due to the contamination from the air

3. COMPARTIMENT (imaging of forein particles)

Measured at the digital microscope Keyence VHX-6000

figure 6: 50 000 fold enlargement of boletus sponge and base

resume 3. forein particles (f.i. dust) are present, they are can be possible carrier of NIC

4. SPONGE (hat-) vs LAMELLA mushrooms

Lamella mushrooms: ***ouster and buttom mushrooms, chanterelle***
Hat mushrooms: ***boletus, Slippery Jack, orange birch bolete, cepe***

The european mushrooms (see fig. 7) were analysed with no clear tendency between COT and NIC levels

resume 4. positive and negative results present, COT/NIC if present, >10 %

5. SUMMARY of all results

Altogether we measured more than 20 different dried mushrooms for NIC, COT. The proportions COT/NIC were evaluated (fig 7).

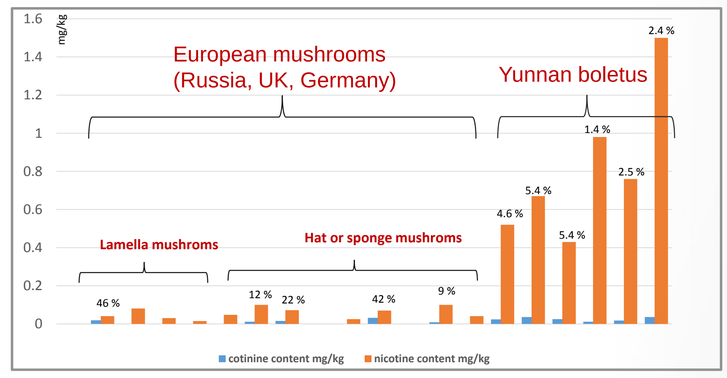

figure 7. COT/NIC findings in dried boletus products

resume 5. where the COT/NIC proportion was 1-5 % we would ascribe the contamination to tobacco producing/growing as a source, because this proportion is similar to tobacco. [4,5].

Introduction | Method Development | Results | Conclusion | References

Conclusion

After the summarizing of all five resumees and concedering of the tobacco industrial input in Yunnan area as well as findings of anabasine and cotinine, we could postulate two main different sources for the NIC presence in dried mushrooms:

1. **Lower NIC findings** (predominately european mushrooms)
   no differencies between lamella and sponge products
   negative results are present
   AIR drying of mushrooms slides is reponsible for pos. Findings
   -> NIC/COT Absorption from the AIR (dust particles visible)

   -> high COT/NIC proportion 10%-50% due to NIC oxidation n the air
   The numbers of both substances in caps are higher than in base.

AIR contamination with NIC is the source for small NIC results

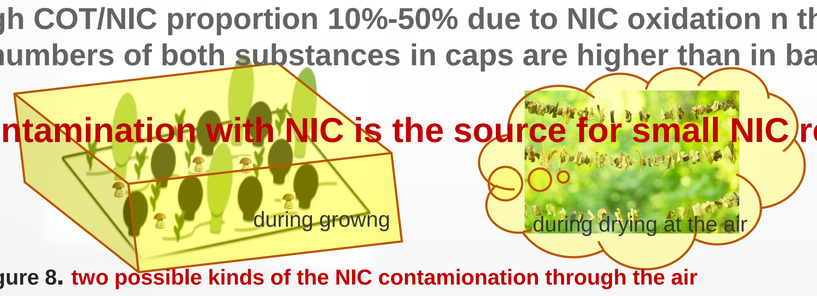

figure 8. two possible kinds of the NIC contamionation through the air

2. **Higher NIC findings** in boletus from Yunnan
   Yunnan is a very important Chinese area for tobacco and cigarette production. „Direct tobacco“ NIC is the source for the high NIC levels in mushrooms products from this region
   COT/NIC proportion is significant lower than in european products (between 1 to 5 %), it means, that
   -> Impact is **not only AIR** (fewer NIC oxydation), but the complete environment due to the huge concentration effects from huge exomycorrhiza
   -> **mycorrhizal (mushrooms hyphae) exchange and transport** with tobacco plants theoretically possible (figure 9, example with *nicotiana attenuata*: wild tobacco plant [6])

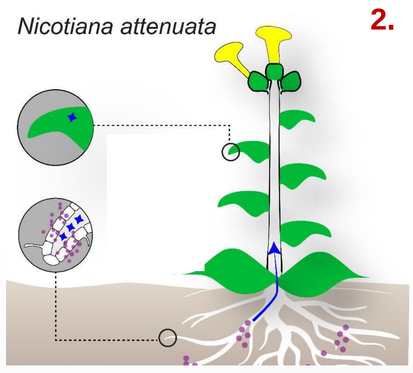

figure 9: substance exchange between root fungi and wild tobacco plant [6] Throuh mycorrhiza

Image source: https://www.labo.de/biotechnologie/bilder/marker-fuer-mykorrhiza-pilze-1.htm

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