

Occurrence of matrine-type alkaloids in honey from different botanical and geographical origins: a natural problem for beekeeping?

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Wang Z. Food Chem 2023, doi:10.1016/j.foodchem.2022.134254



Joint EURLs/NRLs
Workshop | 18-20
October 2023
(Stuttgart/Germany)

Quality Services International (QSI): Profile



FOUNDED IN 1954

SINCE 2013 MEMBER OF
THE TENTAMUS GROUP

170 STAFF
(FOOD) CHEMISTS,
BIOLOGISTS, PHARMACISTS
AND OTHER EMPLOYEES



LAB WITH FOCUS ON
AUTHENTICITY AND
ANALYTICAL TESTING
FOR HONEY,
PHARMA/GMP, TEA,
COFFEE & CANNABIS



ISO 17025 ACCREDITED
GMP CERTIFIED



HUB SERVICES via
QSI AMERICA, QSI MEXICO,
QSI INDIA, QSI VIETNAM



QSI - A LAB FOR LIFE

Main Lab Equipment

CHROMATOGRAPHY

- LC-MS/MS 7500, 5500+, 5500 (Sciex), 6495C (Agilent)
- LC-HRMS 6600, X500R (Sciex), Orbitrap Exploris 240 (Thermo)
- GC-MS/MS 7010B (Agilent)
- HPLC-DAD/FLD/ELSD/RID, HPAEC-PAD,...(Thermo, Shimadzu, Agilent)

ISOTOPE RATIO ANALYSIS

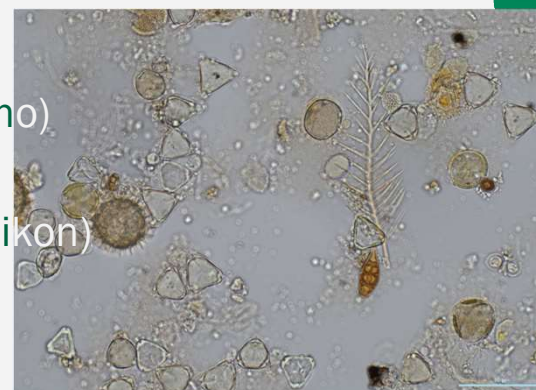
- LC-IRMS LC IsoLink™ II (Thermo)
- EA-IRMS CM-CRDS (Picarro Inc)

NMR

- AVANCE NEO FOODSCREENER SYSTEMS (400 MHz/Bruker)

FURTHER IMPORTANT EQUIPMENT:

- Colorimetric/enzymatic robotics Gallery™ Discrete Analyzers (Thermo)
- Microscope Ni-U/DIC (for melissopalynology/Nikon)



Honey...

...an animal-derived commodity...

Honey – Definition

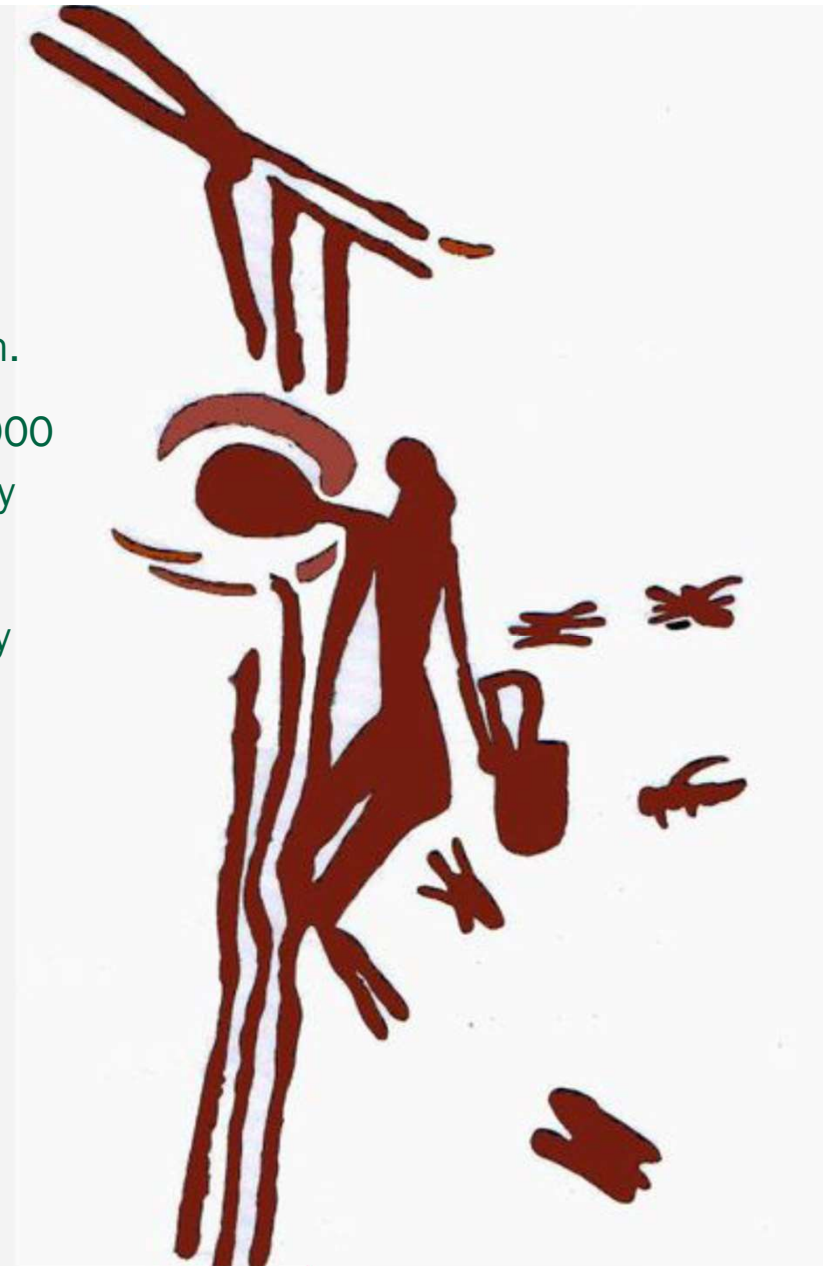
- “Honey is the natural-sweet substance produced by honeybees by the bees taking in nectar from plants or secretions of living plant parts or secretions from insects sucking on plants, transforming it by combination with their own specific substances, storing it, dehydrating it and storing it in the honeycombs of the beehive and allowing it to mature.
- Honey consists essentially of various sugars, in particular fructose and glucose, as well as organic acids, enzymes and solid particles absorbed during nectar collection.
- The colour of the honey ranges from almost colourless to dark brown. It can be of liquid, viscous or partly to fully crystalline consistency. The differences in taste and aroma are determined by the respective botanical origin”

AOAC - Association of Official Agricultural Chemists

The Honey: Beekeeper | Analysis | Law | Health (Horn, H; Lüllmann, C. 2019)

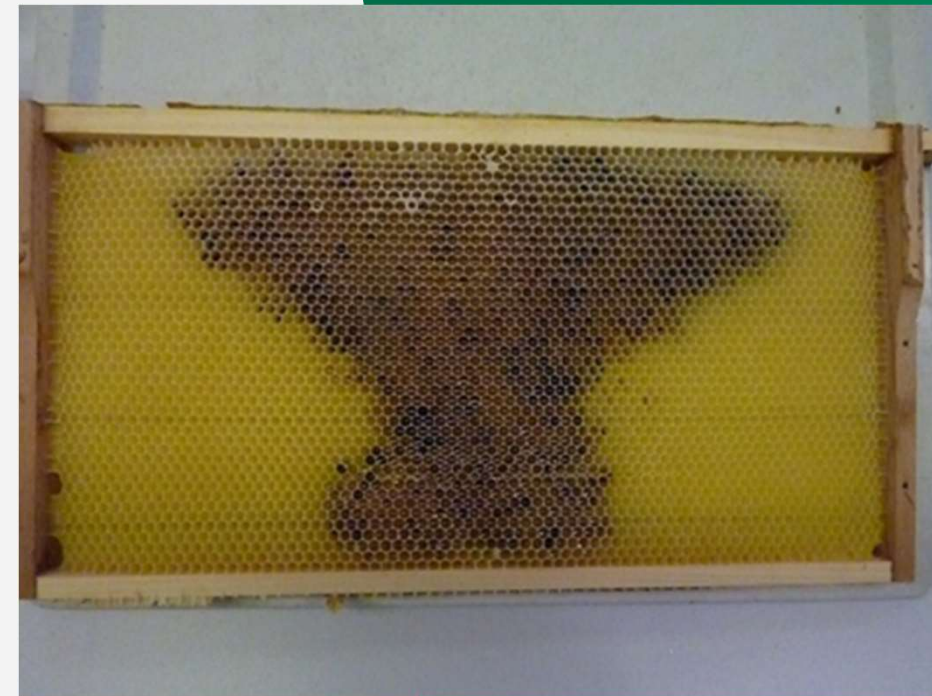
Honey – in ancient times

- The use of honey dates back to prehistoric times. An impressive testimony to this is the stone-age cave painting of la Araña in Spain.
- The petroglyph showing a honey hunter at work is dated around 7000 B.C. and is still considered to be the oldest document on the history of honey
- This type of honey production is still successfully practiced today by the honey hunters in Nepal. *Apis dorsata*, the local bee, which is found in large parts of Asia, produces abundant honey and nests preferably under rocky outcrops that are difficult to access.
- In India alone, honey yields from *Apis dorsata* populations are reported at 14,000 tons/a



Raw materials of honey

- The definition shows that honey is a very complex product produced exclusively by bees. All the raw materials of a honey are of botanical origin with different origins and compositions. **These are directly or indirectly the sifting tube sap of higher plants, which in the case of blossom honey is secreted by certain mechanisms of the plant or is collected and introduced by the bees in the form of honeydew as a sugar containing excretion product of plant-sucking insects.**
- In exceptional cases, bees occasionally use **other sources such as plant bleeding juices (sugar cane) or sweet juices of berries or fruits** as raw materials for honey.
- **These products...contain[ing] vegetable additives, must not be called honey.**



Honeycomb with parts of cherry juice...

Honey – today's analytical routine scope for analysis of residues & contaminants

Substance group	Substance
Toxins	Pyrrolizidine alkaloids, tropane alkaloids, matrine/oxymatrine
Plant protection products	Chlorine/phosphorous pesticides, neonicotinoids
Heavy metals	Lead, cadmium, mercury
Radioactivity	$^{137}\text{Caesium}$, ($^{135}\text{Caesium}$)
Bee medicine	legally authorized/not authorized
Antibiotics	Tetracyclines, sulfonamides etc.
Smoker Substances	phenolic compounds
Bee repellents	Phenols
Wax moth repellent	1,4-Dichlorobenzene

- Furthermore, isoquinoline alkaloids, quinolone alkaloids, gelsedine-type alkaloids, aconitine and other alkaloids have been detected in honey...
...but no routine monitoring of honey, yet.

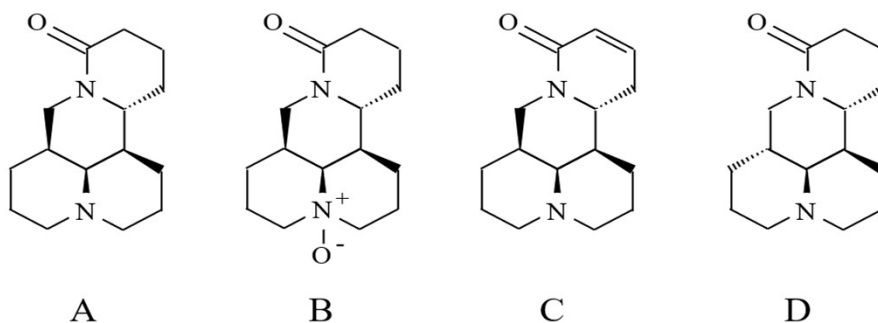
The Honey: Beekeeper | Analysis | Law | Health (Horn, H; Lüllmann, C. 2019;
Wang Z. Food Chem 2023, doi:10.1016/j.foodchem.2022.134254)

Matrine-type alkaloids

chemistry, occurrence

Matrine-type alkaloids: Chemistry, Occurrence

Matrine-type alkaloids belong to quinolizidine alkaloids, including matrine (A), oxymatrine (B), sophocarpine (C) and sophoridine (D)



Compound	pka	log P	Source
Matrine	9.75	1.08	ChemAxon
Oxymatrine	3.82	-0.048	ChemAxon

Occurrence

- rich in plants of Sophora genus, such as *Sophora flavescens*, *Sophora alopecuroides*, and *Sophora viciifolia* Hance

Wang Z. Food Chem 2023, doi:10.1016/j.foodchem.2022.134254



Matrine

pharmacological effects

Matrine: multiple studies depicting individual pharmacological effects

- Cardiovascular and cerebrovascular protection activity
 - Anti-atherosclerosis, anti-hypertension, anti-ischemia reperfusion injury, anti-arrhythmia, anti-diabetic cardiovascular complications
- Anti-cancer activity
 - Anti-lung cancer, anti-hepatoma, anti-breast cancer, anti-leukemia
- Anti-inflammatory, anti-bacterial and anti-viral effects
- Analgesic activity
- Immunosuppressive activity
- ☹ Although matrine exhibited multiple bioactivities, its low bioavailability and high dosage may limit its clinical application...

Sun X.Y. Front. Chem. 2022, doi: 10.3389/fchem.2022.867318 (review paper with many references)



Matrine

human health and protection

Human health and protection

General			
Property ⓘ	Value	Source; quality score; and other information ⓘ	Interpretation ⓘ
Threshold of Toxicological Concern (Cramer Class) ⚠	-	-	-
Mammals - Acute oral LD ₅₀ (mg kg ⁻¹)	> 10000	Q3 Rat	Low
Mammals - Dermal LD ₅₀ (mg kg ⁻¹ body weight)	2000	Q3 Rat	-
Mammals - Inhalation LC ₅₀ (mg l ⁻¹)	-	-	-
Other Mammal toxicity endpoints	-	-	-
ADI - Acceptable Daily Intake (mg kg ⁻¹ bw day ⁻¹)	-	-	-
ARfD - Acute Reference Dose (mg kg ⁻¹ bw day ⁻¹)	-	-	-

Health issues			
Specific human health issues	Carcinogen	Genotoxic ⓘ	Endocrine disruptor
	X	A0; B0; C0; D0; E3	No data found
	Reproduction / development effects	Acetyl cholinesterase inhibitor	Neurotoxicant
	No data found	X	No data found
	Respiratory tract irritant	Skin irritant	Skin sensitiser
	✓	✓	No data found
	Eye irritant	Phototoxicant	
	✓	No data found	
General human health issues	Considered safe May exhibit anti-tumour properties		

BPDB: Bio-Pesticides DataBase - <http://sitem.herts.ac.uk/aeru/bpdb/Reports/3135.htm> (last update:14/09/2023);
Background and Support: http://sitem.herts.ac.uk/aeru/bpdb/docs/3_4.pdf



LEGEND

Q Miscellaneous Data from On-line Sources
3 Unverified data of known source

Genotoxicity codes
A = Chromosome aberration (EFSA database)
B = DNA damage/repair (EFSA database)
C = Gene mutation (EFSA database)
D = Genome mutation (EFSA database)
E = Unspecified genotoxicity type (miscellaneous data source)
0 = No data
1 = Positive
2 = Mixed/ambiguous results
3 = Negative



Matrine

Pesticide use

Matrine: pesticide use

Mode of action	Acts on insect pest central nervous system causing breath inhibition and motion imbalance. Curative and preventative.
Example pests controlled	Aphids; Carmine spider mite; Apple & various leaf mites; Leek maggot; Grubs; Wireworms; Cutowrms; Diamondback moth; Caterpillars
Example applications	Glass house crops; Crucifers
Efficacy & activity	Matrine-based biopesticides have demonstrated acaricidal and insecticidal activity in both field and laboratory studies.
EC Regulation 1107/2009 status	Not approved (Never notified and authorised in the EU)
MRL according to Art 18(1)(b) Reg 396/2005	Default MRL of 0.01 mg/kg
Known to be used in the following countries	China

BPDB: Bio-Pesticides DataBase - <http://sitem.herts.ac.uk/aeru/bpdb/Reports/3135.htm> (last update:14/09/2023); <https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/active-substances/details/1244> (access to database on 12OCT2023)

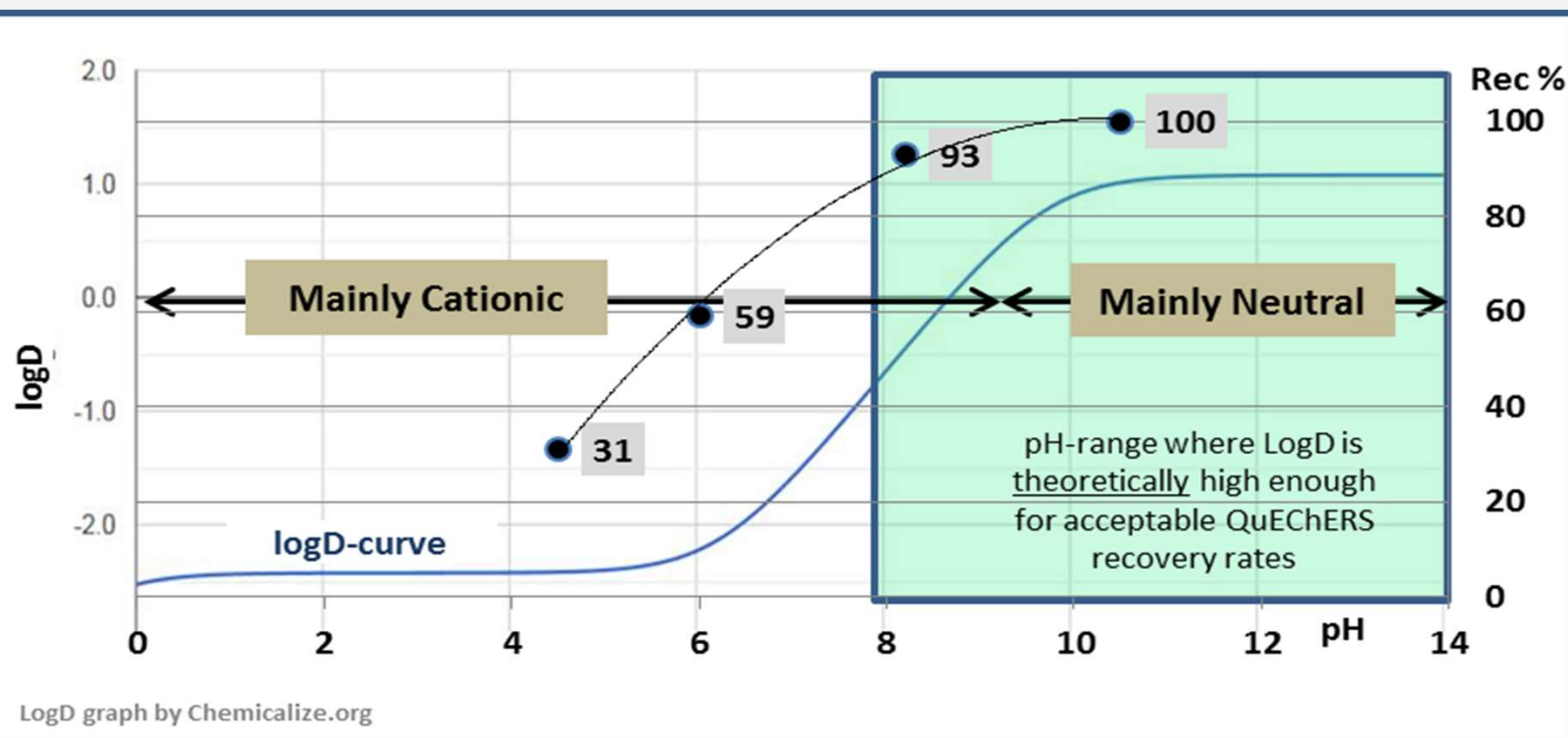
Common name	Plant species	Active ingredient(s)	Country of manufacture	Commercial product(s)
Matrine	Sophora flavescens	Matrine and related quinolizidine alkaloids	PR China	Matrine 0.3% EC



Matrin-type alkaloids

analytical methods

The main problem in sample prep by CEN QuEChERS :
bad recoveries of matrine due to high pka value (9.75)!



Anastassiades M: EURL-SRM - Analytical Observations Report on matrine/oxymatrine (unpublished)

Possible Solutions...

- QuPPe module M 4.2: “Quats & Co BEH Amide” using ILIS
- Alk-QuEChERS (matrix-matched calibration and/or ILIS)
- **CEN-QuEChERS using ILIS**
- At QSI: CEN-QuEChERS using ILIS oxymatrine D₃ in quantitative MRM pesticide screening scope. Validation for different honey types.

Matrix	Matrine D ₃ (as IS)	QuEChERS		Alk. QuEChERS		QuPPe	
		Mean Rec.	RSD %	Mean Rec.	RSD %	Mean Rec.	RSD %
Cucumber	No	35 %	2.2	90 %	1.8	92 %	4.5
	Yes*	94 %	2.4	100 %	2.1	95 %	3.4
Grape	No	24 %	0.7	41 %	3.2	93 %	3.5
	Yes	94 %	2.6	103 %	4.0	97 %	1.2
Lemon	No	39 %	1.3	63 %	9.3	89 %	5.1
	Yes	102 %	1.9	104 %	0.8	98 %	2.8

Table I: Matrine validation data at 0.01 mg/kg using different approaches

* Calc. using matrix matched calibration and nicotine D₄ as IS as matrine D₃ was not available at that time

Anastassiades M.:QuPPe-PO-Method V12.1 (17.03.2023); Zechmann, S: EPRW2020-PO-Suppl-Matrine; Marks H.: EPRW 2020-PO40

Matrin-type alkaloids in honeys

...a dilemma in beekeeping?

Detection of matrine-type alkaloids in acacia honeys

- Matrine and oxymatrine are classified as risky substances with maximum residue limits (MRLs) set as 0.01 mg/kg
- Since 2020, matrine and oxymatrine have been detected in Chinese honey by Spain, Belgium and other countries, and five notifications have been reported by the European Commission through the Rapid Alert System for Food and Feed (RASFF) which has aroused widespread concern.



Detection rates of matrine/oxymatrine in honeys

QSI data (2021)



Origin	No. of samples	< LOQ [%]	Positive [%]	≥ 0,02 mg/kg [% of positives]
Total	2716	94,6	5,4	80,5
China	167	65,9	34,1	42,1

- The highest levels observed were 0.13 mg/kg for matrine and 0.22 mg/kg for oxymatrine

Annual Honey Report

2021



Negotiations on matrine/oxymatrine in honey between PR China and EU authorities: actual situation

Standing Committee on Plants, Animals, Food and Feed (ScoPAFF) committee meeting (Section Phytopharmaceuticals – Pesticide Residues) on 23 – 24 September 2021

4. Matrine/Oxymatrine

- The Commission recalled that during previous meetings of this Committee it had been concluded that the default level of 0.01 mg/kg was the applicable MRL for matrine and oxymatrine.
- However, the Chinese authorities had sent a letter concerning findings of matrine and oxymatrine in honey, claiming that these levels would lack a scientific basis and would be disruptive for trade.
- A relevant research report mentioned that both substances would naturally occur in plants of the Sophora species and would not be artificially added in the honey-making process.
- Those plants blossom at the same time as the flowers of the black locust (*Robinia pseudoacacia*, Fabaceae) and acacia (*Acacia* spp., Fabaceae), thus when producing honey, bees collecting nectar from the Sophora flowers, contaminate the robinia and the acacia honeys with those substances.

Standing Committee on Plants, Animals, Food and Feed (ScoPAFF) committee meeting (Section Phytopharmaceuticals – Pesticide Residues) on 23 – 24 September 2021

4. Matrine/Oxymatrine (cont'd)

- A Member State proposed that the Chinese Authorities can submit an import tolerance request in accordance with Article 6 of Regulation (EC) 396/2005 and if, following an assessment from EFSA the proposed MRLs are safe for consumers, a MRL higher than the default MRL could be established.
- EFSA suggested to also check the EU monitoring data on honey to see whether the substances would also be found in honey in the EU, which could be assumed in case of their natural occurrence.
- The Commission invited the Member States to provide analytical data for potential findings of matrine and oxymatrine in honeys by 15 October 2021.



World Trade Organization (WTO) - Committee meeting on Sanitary and Phytosanitary Measures on 26 September 2022

4.1.4 EU notifications of matrine and oxymatrine in honey (ID 546) – Concerns of China

- 4.9. China raised questions regarding the tests conducted by the European Union on honey imported from China with a residue limit of 0.01 mg/kg according to the Regulation (EC) No 396/2005.
- China explained that Chinese honey was mainly acacia honey and that the matrine and oxymatrine were derived from the nectar of *Sophora vicifoliai*. Hence, a plant flowering at the same time as acacia, and not by artificial addition or contamination. China further indicated that there was no evidence that matrine and oxymatrine in honey might cause food safety risks. China questioned the scope and limit of application, the notification procedure and the scientific rationality of the measure, and hoped that the European Union took into account the clarifications provided.



WTO - Committee meeting on Sanitary and Phytosanitary Measures on 26 September 2022

4.1.4 EU notifications of matrine and oxymatrine in honey (ID 546) – Concerns of China (cont'd)

- 4.10. Thanking China for sharing the relevant data, the European Union highlighted the following points from the bilateral discussions held in November: matrine and oxymatrine, used as pesticides China, were not approved for use in the European Union; a recent evaluation by the German Federal Institute for Risk Assessment (BfR) indicated that the genotoxic potential of the substances could not be excluded and, therefore, no health-based guidance value could be derived for them; the default maximum residue limit (MRL) of 0.01 mg/kg was applied in accordance to of Regulation (EC) No 396/2005.
- The European Union invited China to submit a request for an import tolerance for matrine and oxymatrine in honey, which would be granted if it received a favourable assessment by the European Food Safety Authority (EFSA). The European Union expressed its willingness to continue bilateral discussions.



The source of matrine-type alkaloids in honeys – evidence from a scientific publication

Wang Z. Food Chem 2023, Doi:10.1016/j.foodchem.2022.134254

- Wang et al. established a novel LC-MS/MS method for simultaneous determination of matrine, oxymatrine, sophocarpine and sophoridine in honey.
- Application for the determination of these four matrine-type alkaloids in 567 honey samples collected from various botanical and geographical origins in China.
- The quantitative results, combined with melissopalynological analysis and nectariferous plants analysis, revealed:
 - The origin of matrine-type alkaloids in Chinese honey was assumed to be Sophora plant *S. viciifolia* Hance.

Wang Z. Food Chem 2023, doi:10.1016/j.foodchem.2022.134254



Contents of matrine-type alkaloids in honey samples from different botanical origins

The detection rates and average contents of four alkaloids in honey samples of different botanical origins.

Honey types	Number of samples	Σ4 MAs ^a Detection rate (%)	Average content ^c (μg/kg)	Matrine Detection rate (%)	Average content (μg/kg)	Oxymatrine Detection rate (%)	Average content (μg/kg)	Sophocarpine Detection rate (%)	Average content (μg/kg)	Sophoridine Detection rate (%)	Average content (μg/kg)
Multifloral honey	15	33.3	187.2	26.7	5.8	26.7	123.7	26.7	42.0	13.3	124.8
Linden honey	16	ND ^b	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Lycium barbarum</i> honey	12	66.6	133.0	41.7	23.6	58.3	92.1	50.0	50.2	ND	ND
Fennel honey	15	93.3	16.5	73.3	4.0	66.7	5.6	86.7	8.6	13.3	9.2
Vitex honey	17	29.4	14.0	11.8	1.4	29.4	9.0	17.6	2.6	5.9	14.8
Sunflower honey	14	92.9	27.8	71.4	7.9	85.7	8.3	92.9	12.7	21.4	5.8
<i>S. viciifolia</i> Hance honey	8	100.0	1183.3	100.0	92.8	100.0	967.2	100.0	123.3	ND	ND
Litchi honey	12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Vicia villosa</i> Roth honey	11	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
<i>Hevea brasiliensis</i> honey	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Acacia honey	383	72.8	74.1	40.7	7.6	72.3	59.6	66.1	15.9	0.5	10.5
Rape honey	25	8.0	8.9	8.0	2.5	4.0	4.5	8.0	4.1	ND	ND
Jujube honey	29	41.4	78.5	34.5	47.5	20.7	9.6	37.9	34.7	3.4	26.8

^a the sum of four matrine-type alkaloids;

^b not detected;

^c average content for the positive samples.

Interlaboratory test of QSI and 3 Chinese labs on matrine/ oxymatrine determination honeys in 2022 (northwest China - Gansu and Shaanxi provinces)



geogr. Origin	Unit	Tentamus Lab		QinHuangDao CIQ		Lab Tianjia (CN)		Nanjing CIQ	
		Matrine	Oxymatrine	Matrine	Oxymatrine	Matrine	Oxymatrine	Matrine	Oxymatrine
Yaodian, Huanglong, Yan'an, Shaanxi	[mg/kg]	0,015	0,278	0,008	0,231	0,01	0,165	0,01	0,218
Yaodian, Huanglong, Yan'an, Shaanxi	[mg/kg]	0,006	0,085	0,003	0,066	0,004	0,068	0,004	0,056
Yan'an, Shaanxi	[mg/kg]	0,006	0,108	0,004	0,132	0,006	0,15	0,004	0,097
Yan'an, Shaanx	[mg/kg]	0,004	0,078	0,003	0,066	0,004	0,083	0,003	0,053
Qingyang, Gansu	[mg/kg]	0,003	0,041	0,002	0,033	0,002	0,044	0,002	0,033
Qingyang, Gansu	[mg/kg]	0,003	0,036	0,002	0,025	0,003	0,037	0,002	0,026
Qingyang, Gansu	[mg/kg]	0,006	0,152	0,003	0,077	0,005	0,121	0,005	0,091
Qingyang, Gansu	[mg/kg]	0,009	0,042	0,006	0,031	0,008	0,038	0,008	0,027
Jingchuan, Pingliang, Gansu	[mg/kg]	ND	0,007	ND	0,004	ND	0,006	ND	0,003
Jingchuan, Pingliang, Gansu	[mg/kg]	0,01	0,019	0,009	0,009	0,009	0,011	0,008	0,011
Pingliang, Gansu	[mg/kg]	0,002	0,02	ND	0,013	ND	0,017	ND	0,013
Pingliang, Gansu	[mg/kg]	ND	0,013	ND	0,009	ND	0,013	ND	0,009

➤ Good comparability between results from individual labs!

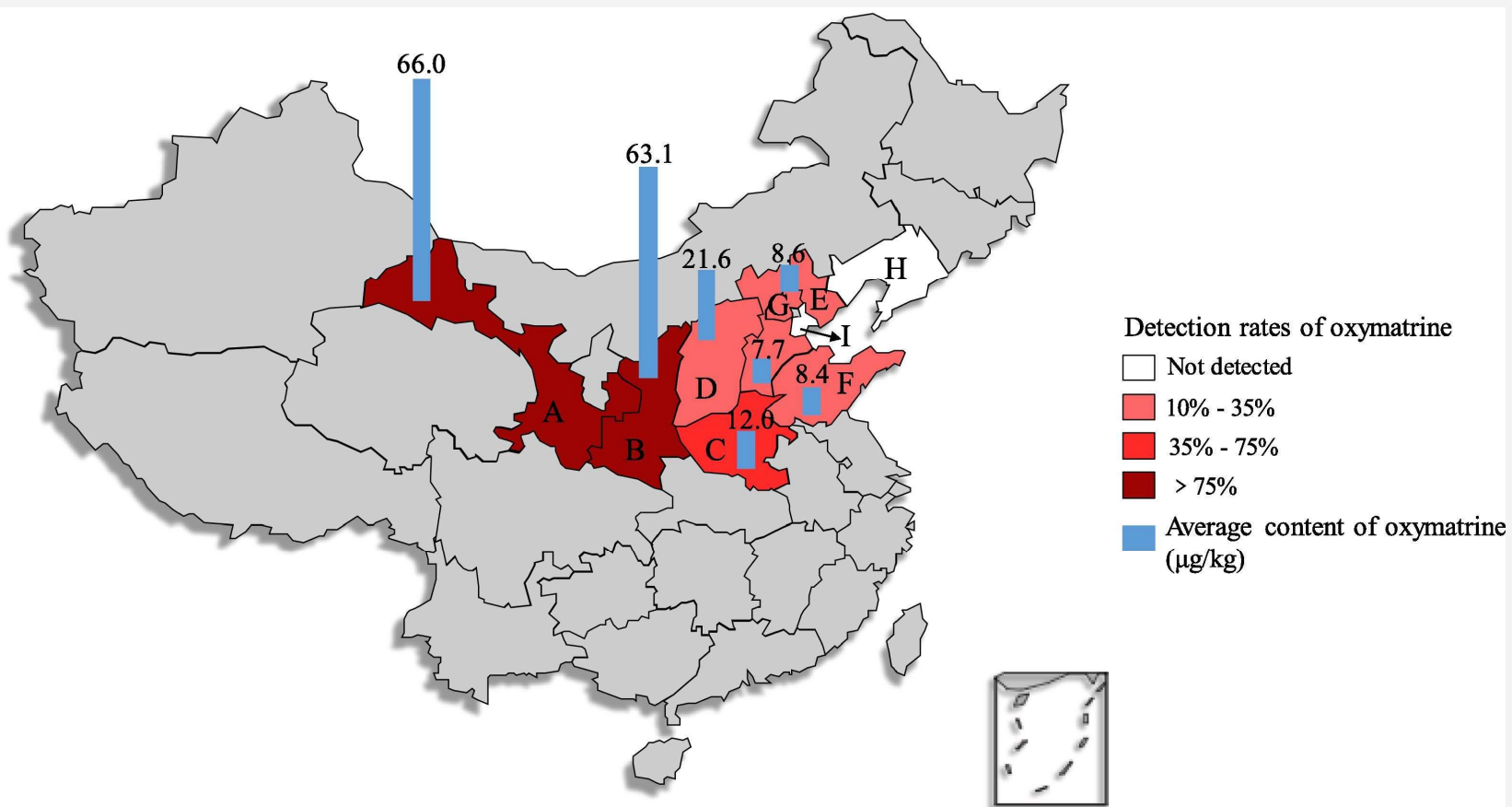
Differences in matrine-type alkaloids contents in different parts of Acacia and *S. viciifolia* Hance

Matrine-type alkaloids contents in different parts of Acacia and *S. viciifolia* Hance (mg/kg, $n = 3$).

Plant	Part	Matrine	Oxymatrine	Sophocarpine	Sophoridine
Acacia	Stems	ND ^a	0.48 ± 0.11	ND	ND
	Leaves	ND	0.20 ± 0.04	ND	ND
	Fruits	ND	0.31 ± 0.13	ND	ND
	Flowers	ND	ND	ND	ND
<i>S. viciifolia</i> Hance	Stems	73.6 ± 16.9	1319 ± 118.8	36.7 ± 8.2	ND
	Leaves	7.1 ± 4.4	224.7 ± 80.7	56.4 ± 14.1	ND
	Fruits	389.2 ± 23.7	6138 ± 622.3	535.8 ± 56.5	ND
	Flowers	251.3 ± 9.0	1564 ± 95.8	836.1 ± 157.8	ND

^a not detected.

High detection rates/contents of matrine-type alkaloids in acacia honeys in Gansu and Shaanxi provinces; a matter of localization with *Sophora viciifolia* Hance

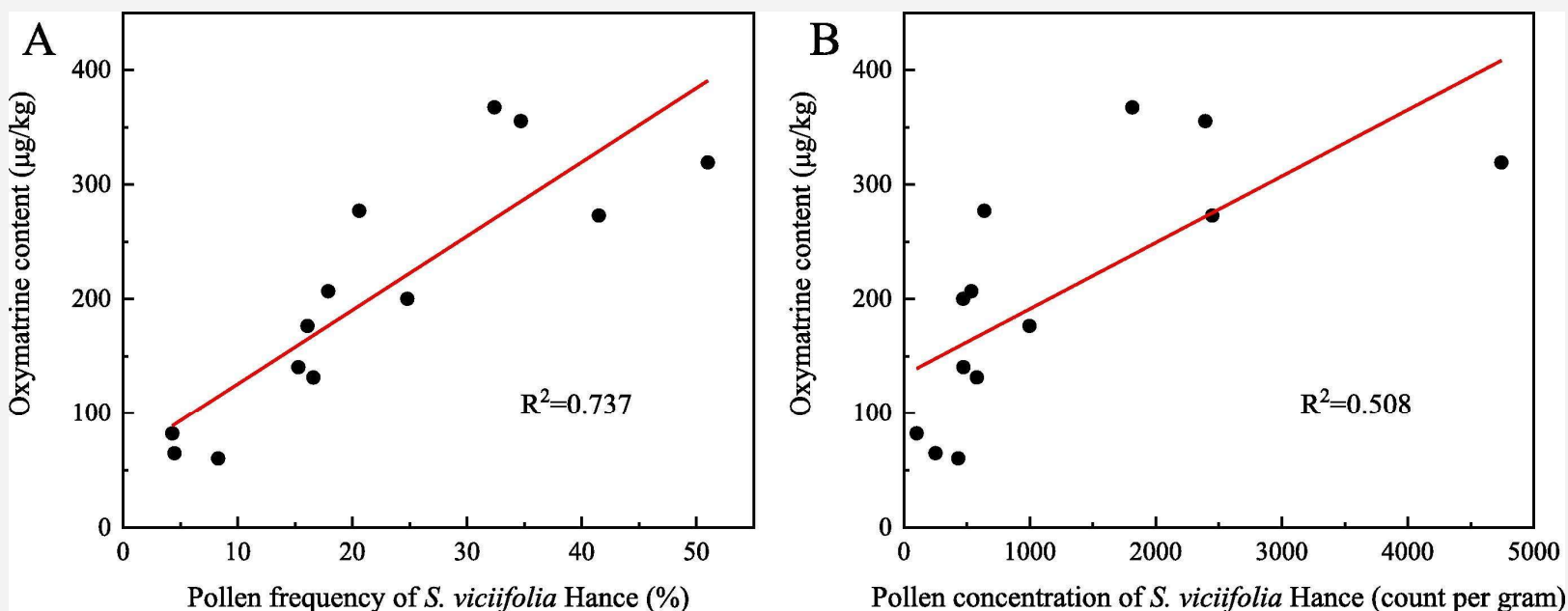


Melissopalynology of *S. viciifolia* Hance and Acacia pollen



S. viciifolia Hance pollen in red circle, Acacia pollen in yellow circle

Correlation between the oxymatrine content and pollen frequency (A) and pollen concentration (B) of *S. viciifolia* Hance in Acacia honey



- The origin of matrine-type alkaloids in Chinese honey was assumed to be Sophora plant *S. viciifolia* Hance.

Possible solutions of the matrine/oxymatrine problem?

Eradication of the problem?

- wiping out Sophora plants?
- use of herbicides?
- planting *acacia* or *robinia* plants kilometers from the sophora trees?
- ...hardly feasible!

Change of legislation?

- Adaption of MRLs to natural occurrence in the commodity honey according to ML system
- also applied to the pyrrolizine alkaloids (contaminants), chlorate (pesticide/contaminant issue)
- ...need for an assessment by EFSA

Take home messages

- Matrine-type alkaloids belong to quinolizidine alkaloids, including matrine, oxymatrine, sophocarpine and sophoridine
- Matrine-type alkaloids are rich in plants of Sophora genus
- Matrine and oxymatrine are regulated by EU pesticide legislation and classified as pesticide at the default MRL of 0.01 mg/kg
- Since 2020 high levels (> 0.01 mg/kg) of matrine and oxymatrine were found especially in acacia honey from specific Chinese provinces leading to limited marketability in the EU
- The studies of Wang and coauthors indicated that the origin of matrine-type alkaloids in Chinese honey may come from nectariferous plants *Sophora viciifolia* Hance co-flowering with acacia
- For facilitated marketability of matrine/oxymatrine containing honeys:
 - Need for an import tolerance for matrine and oxymatrine in honey after a favorable assessment by EFSA

Thank you for your attention!



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