

Diversity of active metabolites in stingless bee nest materials, a path for rational discoveries in apitherapy



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Main topics of Apitherapy, APIMONDIA 2023

- ❖ **Scientific based evidence supporting the nutritional, physiological and health claims of bee products.**
- ❖ **Preclinical research - safety, pharmacology and toxicology of bee products. Guidelines for medical applications.**
- ❖ **Clinical trials in apitherapy – doses, interactions, side effects (human and veterinary medicine). Update on the use of apitherapy in infectious diseases.**
- ❖ **Regulatory issues and clinical ethics related to the integration of apitherapy as TCM in healthcare systems.**

Acknowledgements

- ❖ To stingless bee keepers, entomologists (JMF Camargo[†], SRM Pedro, MS Engel) who identified our stingless bees along the years, and proactive colleagues.
- ❖ To M Linkogel, QSI Bremen, for the targeted ¹H-NMR of SBH.
- ❖ To G Meccia for the structures of chemical compounds.
- ❖ To my family for the unconditional support in my scientific research, D Vit collected *M. favosa* honey from Paraguana Peninsula, Venezuela.
- ❖ To CDCHTA-ULA, Welcome Trust, and Prometeo for financial support in diverse projects and scholarship.



APIMONDIA

48° CONGRESO INTERNACIONAL CHILE
DE APICULTURA 4 al 8 septiembre 2023

Desde el sur del mundo por una apicultura sostenible



UNIVERSIDAD
DE LOS ANDES
VENEZUELA

Gratitude as Invited Speaker

❖ To Dr. Cristina Mateescu



President

Scientific Commission on Apitherapy, APIMONDIA

Rumania

❖ To Dr. Walter Fierro

Local Apitherapy Commission, APIMONDIA

Uruguay

I wish to optimize my words and slides with this precious opportunity in the Central Symposium Value added products from Beekeeping

*I dedicate this talk to the memory of the Manuka honey scientist **Peter Molan**, from Waikato University, Hamilton, New Zealand, for his contributions in the UMF so important for microbial bee science*



Compared to *Apis mellifera* therapy
Apitherapy with stingless bees lack **APITOXIN**
but have a **BIODIVERSITY** of 605 species of bees
and air types of their nests...

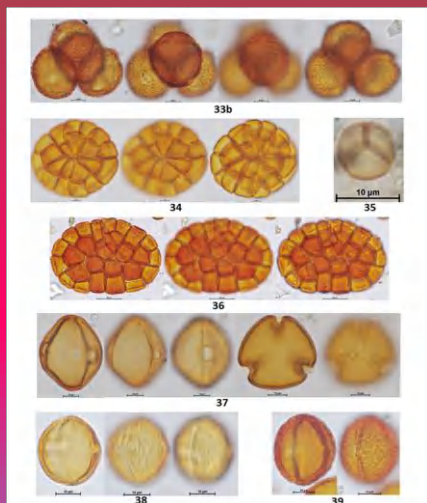
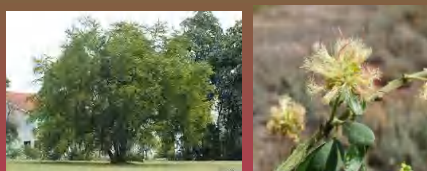


<https://www.ecocolmena.org/breathe-the-air-of-the-hive/>



IDs of stingless bee nest metabolites

Botanical



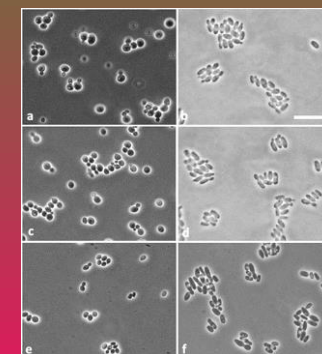
AIMS 2023; 8, 804-831

Entomological



JAR 2020; 60, 143-156

Microbial



Interciencia 2022; 47, 416-425
 Int J Syst Evol Microbiol 2018; 2675

“Man has known the curative properties of honey from the earliest times. We may have forgotten, in latter years, just what medicinal benefits the golden harvest of the hive can bring. Perhaps with bacteria ever more resistant to antibiotics and viruses that seemingly defeat the medical world it is time to look once again to one of nature’s own medicines that has a scientifically proven track record”.

International Bee Research Association
“Honey and healing: from the hive to the hospital”
Cardiff University
7 October 2000

Rationale for discoveries in apitherapy

- ❖ Significance to focus on a research, targetted or not
- ❖ What gaps the research intends to fill
- ❖ Synthesis of most bioactive metabolites for pharmaceutical applications

Slowly grow

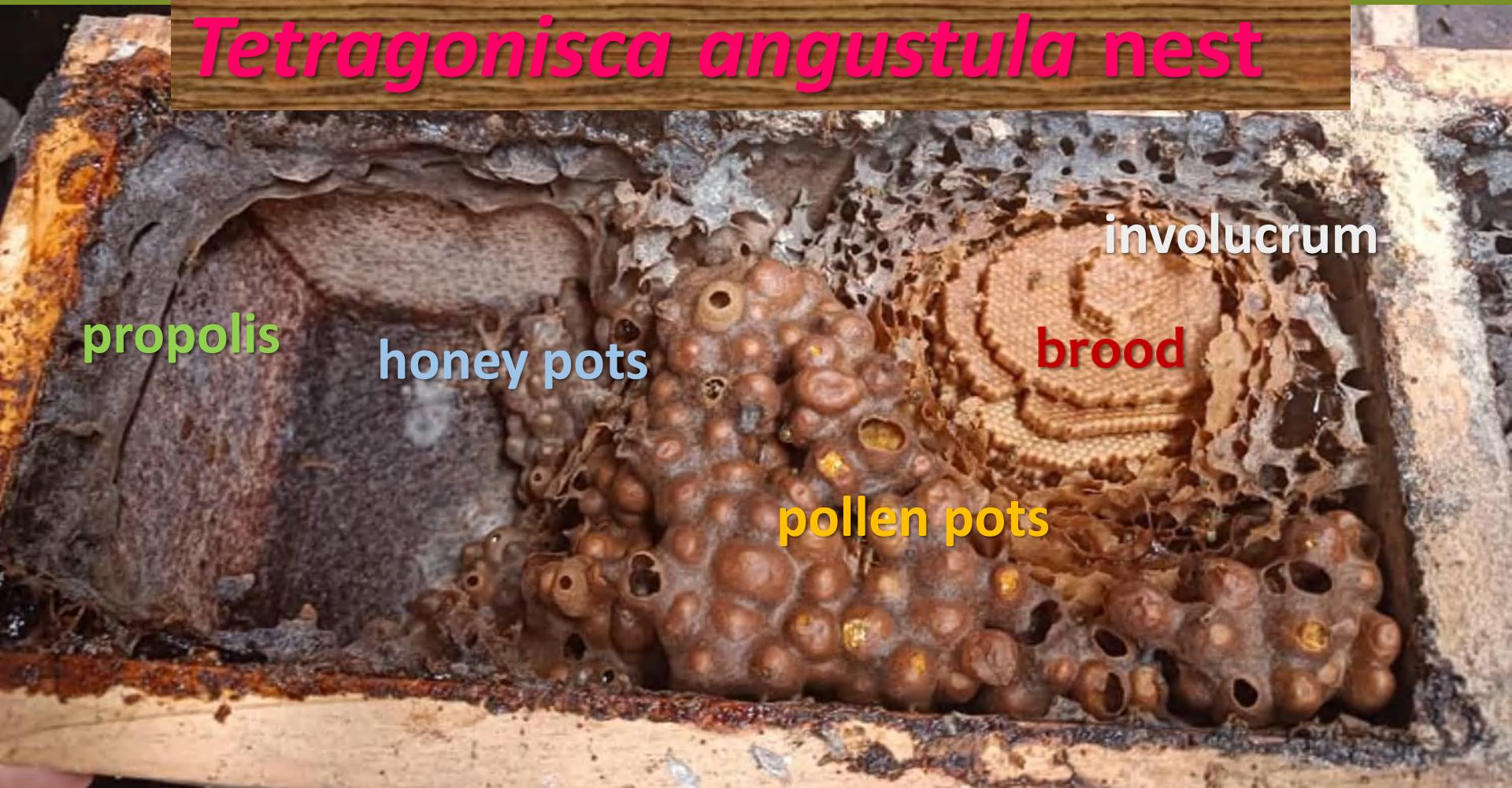
- ❖ Talent and dedication
- ❖ Multidisciplinary effort
- ❖ Ability of responsible team work



Have fun investing some of your family time in science



Tetragonisca angustula nest





1

Entrance
tube



2

Involucrum
of the
brood



3

Pillars



4

Empty
honey pots



5

Plant resin
deposit



6

Indoor
cover
propolis

Diverse nest materials have different chemical composition and added values on bioactive properties for pharmaceutical design



Diversity of **95 volatile organic compounds VOCs** detected and identified by HS-SPME/GC-MS. Nine chemical classes: 1. Acids (11), 2. Alcohols (16), 3. Aldehydes (7), 4. Esters (16), 5. Ketones (8), 6. Monoterpenes (17), 7. Oxides (5), 8. Sesquiterpenes (11), and 9. Others (4).

64	88	89	86	91	83
Entrance tube	Involucrum of the brood	Pillars	Empty honey pots	Plant resin deposit	Indoor cover propolis

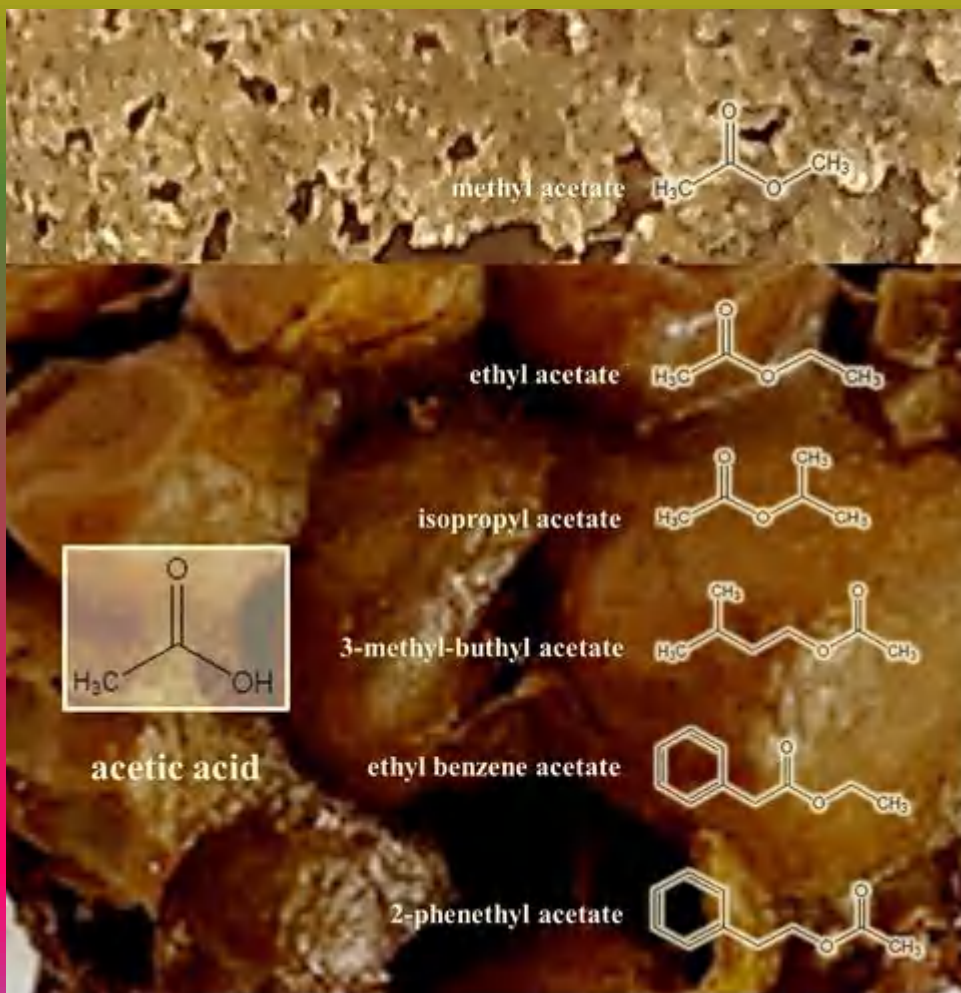
Diverse nest materials have different chemical composition and added values on bioactive properties for pharmaceutical design



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Entrance tube

Empty
honey pots

Acetic acid was accumulated in the cerumen of empty honey pots.

It was esterified into methyl acetate in the entrance tube, and five acetates in the honey pots

2015

Research Article



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Rapid Commun. Mass Spectrom. 2015, 29, 948–954
(wileyonlinelibrary.com) DOI: 10.1002/rcm.7184

Determination of interglycosidic linkages in *O*-glycosyl flavones by high-performance liquid chromatography/photodiode-array detection coupled to electrospray ionization ion trap mass spectrometry. Its application to *Tetragonula carbonaria* honey from Australia

Pilar Truchado¹, Patricia Vit^{2,3,4}, Tim A. Heard⁵, Francisco A. Tomás-Barberán¹ and Federico Ferreres^{1*}

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²Universidad Técnica de Machala, Provincia El Oro, Ecuador

³Faculty of Pharmacy, Universidad de Los Andes, Mérida, Venezuela

⁴University of Sydney, Lidcombe, NSW, Australia

⁵CSIRO Entomology, Long Pocket Lab, Indooroopilly, Queensland, Australia

2011

Journal of Chromatography A, 1218 (2011) 7601–7607

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Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma



Liquid chromatography–tandem mass spectrometry analysis allows the simultaneous characterization of C-glycosyl and O-glycosyl flavonoids in stingless bee honeys

Pilar Truchado^a, Patricia Vit^b, Federico Ferreres^a, Francisco Tomas-Barberan^{a,*}

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^b Bioactividad, Departamento Ciencia de Los Alimentos, Facultad de Farmacia y Bioanálisis, Universidad de los Andes, Merida, Venezuela

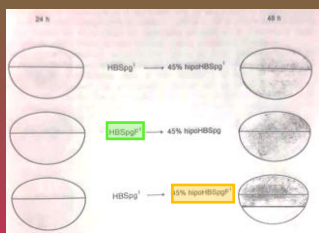
Flavonoid C-glycoside and O-glycosides in *Melipona favesa* Honeys from Venezuela.^a

Honey samples	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Flavonoids												
Flavone C-glycosides												
1	75.7	68.7	89.5	110.4	173.0	121.6	70.5	109.0	35.6	122.3	164.5	209.3
2	148.4	125.9	140.6	176.7	171.0	157.7	91.8	152.2	14.8	160.4	169.8	203.4
3+4	340.6	228.1	271.1	277.3	252.5	250.6	104.2	244.3	173.5	343.2	223.3	328.9
5	223.4	194.5	295.3	375.4	450.1	496.7	118.4	289.5	58.5	310.0	437.3	545.5
6	161.9	129.1	193.7	300.2	337.1	280.7	116.6	219.3	91.3	247.3	308.0	424.2
Total C-glyc	950.0	746.3	990.3	1240.1	1383.7	1307.4	501.6	1014.3	373.7	1183.3	1302.9	1711.3
Flavonol O-glycosides												
7	27.5	tr	26.6	21.4	16.6	tr	58.9	21.1	61.5	19.6	tr	20.8
8	52.1	nd	nd	nd	nd	1655.9	38.9	nd	227.9	252.5	50.3	87.6
9	314.7	50.3	43.7	tr	tr	9933.0	tr	tr	829.6	1208.3	229.0	443.3
10+11+12	97.9	202.4	56.0	74.9	61.3	1383.0	44.6	70.9	190.8	194.5	62.3	116.2
13	47.0	17.2	23.0	57.5	32.0	nd	22.5	29.4	nd	34.3	nd	24.2
14+15	48.8	135.4	35.2	nd	13.8	362.3	196.6	46.7	136.3	69.8	62.0	32.5
16	20.0	nd	nd	30.0	13.8	nd	21.4	16.0	31.5	12.3	nd	24.1
Total O-glyc	608.0	405.2	184.5	183.8	137.4	13334.2	382.9	184.1	1477.6	1791.2	403.7	748.7
Total glycosides	1558.0	1151.6	1174.8	1423.9	1521.09	14641.6	884.4	1198.4	1851.3	2974.6	1706.6	2459.9

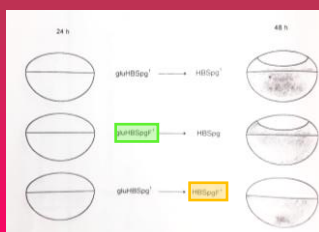


Ovine lens diagrams in equatorial view showed different opacification patterns according to the culture media causing osmotic stress.

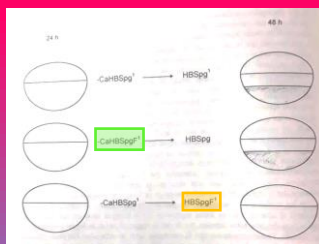
REGIONALIZED PROTECTION of tetramethyl ether luteolin applied **before** or **after** 24 h stress exposure, measured at 48 h.



45% Hypotonic



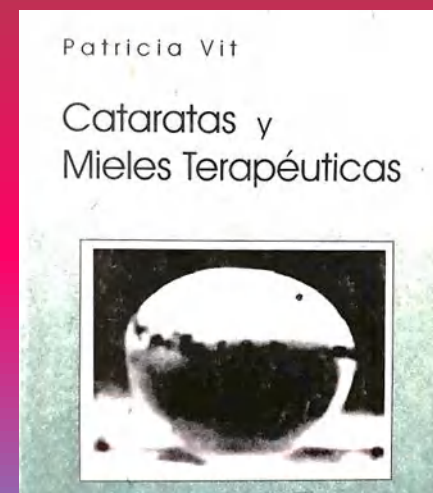
Hyperglycemic



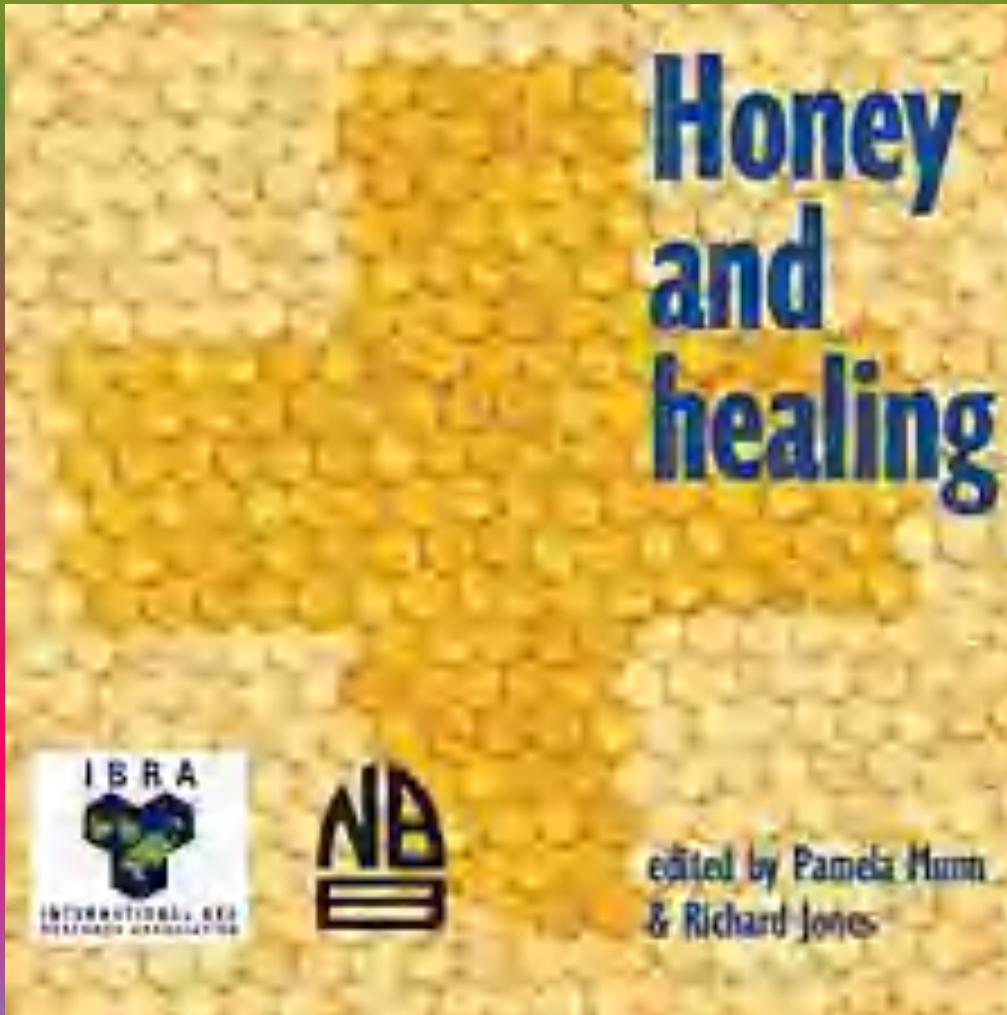
Hypocalcemic

Vit P
 1997

Cataratas y mieles terapéuticas.
 Consejo de Desarrollo Científico, Humanístico
 y Tecnológico, Universidad de Los Andes.
 Mérida, Venezuela. 79 pp.

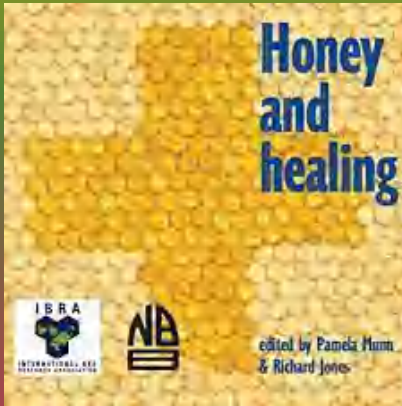


2001



This book was derived from papers given at the meeting organised by the International Bee Research Association entitled: **“Honey and healing: from the hive to the hospital”** which was held at the University of Cardiff on 7 October 2000. The programme for the meeting stated: **“Man has known the curative properties of honey from the earliest times.**

The book was originally published in 2001, but has been out of print for a number of years. Sadly, **Peter Molan**, who contributed two chapters, **died in 2015.**



Vit P 2004

Stingless bee honey and the treatment of cataracts.
International Bee Research Association. Cardiff, UK.
pp. 37-40



Apiservices Quality of the beehive for apitherapy 2000

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BOOST

<https://www.apiservices.biz/es/articulos/ordenar-por-popularidad/966-curso-calidad-colmena-para-apiterapia>

Curso Calidad de la colmena para la apiterapia (2000)

Curso "Calidad de la colmena para la apiterapia" - VII Congreso nacional de ciencias farmacéuticas

Patricia Vit
Coordinadora APIVA
Apiterapia y Vigilancia Ambiental
Facultad de Farmacia, Universidad de Los Andes, Mérida, Venezuela
vitpat@cantv.net
30-31 Marzo 2000

Este curso se organizó para enfatizar el concepto de autocalidad en el mundo apícola. Los cuidados de la colmena y de sus alrededores se reflejan en los productos de las abejas utilizados con fines terapéuticos. Visitamos el apiario "La Casita de la Miel" y la empresa de productos naturales "Vitaplant".

APITERAPIA EN EL DEPARTAMENTO CIENCIA DE LOS ALIMENTOS.

Isbelia González

. Patricia Vit.

Laboratorio Apiterapia y Vigilancia Ambiental, Departamento Ciencia de los Alimentos, Facultad de Farmacia, Universidad de Los Andes, Mérida.

El Departamento Ciencia de los Alimentos cumple funciones de docencia, investigación, extensión, producción y servicios. La apiterapia, entendida como tratamiento a base de productos de la colmena, sólo nos ocupa en labores de investigación. Además de haber organizado este curso sobre Calidad de la Colmena para la Apiterapia, en este VII Congreso Nacional de Ciencias Farmacéuticas, inauguraremos el primer laboratorio de investigación de nuestro departamento, APIVA (Apiterapia y Vigilancia Ambiental). En un espacio reducido, se han diseñado tres áreas para disponer de un bioterio experimental con temperatura controlada, un ambiente estéril para cultivo de órganos y el espacio para preparación y análisis de muestras. ¿Por qué apiterapia en un departamento de ciencia de los alimentos?. En 1985 se inició la investigación sobre calidad de mieles comerciales venezolanas, basada en las normas COVENIN del CT10 para alimentos. Posteriormente se orientó hacia el estudio de las mieles producidas por abejas sin aguijón. Las relaciones con instituciones especializadas en otras ciudades y en otros países, permitió avanzar con análisis que aun no figuran en las normas COVENIN para miel de abejas (Métodos de ensayo 2136-84 y Requisitos 2191-84), como la melitopalínología, el contenido de flavonoides, la evaluación sensorial y la actividad antibacteriana. Luego de haber evaluado el aspecto bromatológico de las mieles venezolanas, el siguiente paso era apuntar hacia la aplicación del conocimiento adquirido. La investigación en apiterapia es una de las avenidas que decidimos explorar. Nuestro primer objetivo ha sido explorar el vínculo tradicional entre miel de abejas y cataratas oculares, el cual ha recibido financiamiento regional, nacional e internacional y ha generado un título doctoral, diversas publicaciones y un libro especializado. Esperamos poder diversificarnos con el estudio de otras enfermedades y de los demás productos de la colmena. Así continuaremos la filosofía socrática de la medicina que nutre y el alimento que cura.

Palabras clave

alimentos, apiterapia, miel

2008

Journal of Health Science 54, 196-202

Putative Anticataract Properties of Honey Studied by the Action of Flavonoids on a Lens Culture Model

Patricia Vit^{a,*} and Tim John Jacob^b

^aApiterapy and Bioactivity (APIBA), Food Science Department, Faculty of Pharmacy and Bioanalysis, University of The Andes, Mérida 5101, Venezuela and ^bSchool of Biosciences, Cardiff University, Cardiff CF10 3US, U.K.

Table 2. Effect of Flavonoids 10⁻⁵ M in Cataract Model

Treatments	Grey scale ^{ab}	Treatments	Grey scale ^{ab}
Isonic HBS control	90.87 ± 0.39 ^c (90.10 – 91.40) [0.75]	Acacetin	142.95 ± 5.15 (137.80 – 148.10) [5.09]
Luteolin 4'-O-α-glucoside	116.00 ± 1.57 (113.70 – 119.00) [2.34]	Apigenin 7-O-α-glucoside	144.90 ± 5.10 (139.80 – 150.00) [4.98]
Luteolin 3'-7-O-α-diglucoside	117.20 ± 1.20 ^c (116.00 – 119.60) [1.77]	Quercetin 3,7,3',4'-tetramethyl ether	145.53 ± 2.13 (142.70 – 149.70) [2.54]
Luteolin tetramethyl ether	120.13 ± 1.87 ^c (116.5 – 122.70) [2.69]	Hyperside	145.67 ± 2.34 (141.00 – 148.40) [2.79]
Orientin	121.60 ± 7.92 ^c (107.20 – 134.50) [11.28]	45% hypotonic HBS osmotic cataract	145.83 ± 2.84 (142.80 – 151.50) [3.37]
Isoquercitrin	136.13 ± 3.20 (130.00 – 140.80) [4.08]	Avicularin	146.17 ± 2.59 (142.60 – 151.20) [3.06]
Kaempferol	139.45 ± 5.35 (134.10 – 144.80) [5.51]	Luteolin 7-O-α-glucoside	148.05 ± 1.85 (146.20 – 149.90) [1.77]
Luteolin	140.15 ± 9.95 (130.20 – 150.10) [10.04]	Acacetin 7-O-α-rutinoside	148.55 ± 3.35 (145.20 – 151.90) [3.19]
Quercetin 3-O-β-D-glucopyranosyl-6'-acetate	141.33 ± 4.96 (134.90 – 151.10) [6.09]	Peltatoside	151.33 ± 7.05 (138.90 – 163.30) [8.07]
Kaempferol 3-O-α-robinoside-7-O-α-rhamnoside	141.45 ± 2.65 (138.80 – 144.10) [2.65]	Apigenin	152.90 ± 1.90 (151.00 – 154.80) [1.76]
Homorientin	142.40 ± 6.35 (134.20 – 154.90) [7.73]	Quercetin	154.73 ± 9.42 (143.20 – 173.40) [10.54]

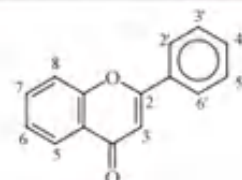
^a Values are grey scale mean ± SEM, (ranges) and [cv]. The grey scale varies between 0 (black) and 255 (white) ^b different from other treatments ($p < 0.050$) ^c different from isotonic HBS and 45% hypotonic HBS ($p < 0.050$) after *t*-student test.

Osmotic cataracts were induced by incubating ovine lenses in 45% hypotonic HBS for 24 h to test the anticataract action of 20 synthetic flavonoids 10⁻⁵ M concentration.

Luteolin tetramethyl ether, luteolin-4'-glucoside, luteolin-3'-7-diglucoside, and orientin significantly inhibited onset of induced cataracts. Preliminary evidence of SBH anticataract properties.



Table 1. Structures of Flavonoids Tested in Cataract Model



Flavonoids	Abbreviation	substituents						
		R ₃	R ₅	R ₆	R ₇	R ₈	R _{7'}	R _{4'}
Acacetin	aca	H	OH	H	OH	H	H	OCH ₃
Acacetin 7-O- α -rutinoside	aca7R	H	OH	H	Orut	H	H	OCH ₃
Apigenin	api	H	OH	H	OH	H	OH	H
Apigenin 7-O- α -glucoside	api7G	H	OH	H	Oglu	H	OH	H
Kaempferol	kae	OH	OH	H	OH	H	H	OH
Kaempferol 3-O- α -rabinoside-7-O- α -rhamnoside	kae3R7H	Orob	Orha	H	OH	H	H	OH
Luteolin	lut	H	OH	H	OH	H	OH	OH
Luteolin tetramethylether	lutMe4	H	OCH ₃	H	OCH ₃	H	OCH ₃	OCH ₃
Luteolin 6-C-glucoside (homorientin)	lut6G	H	OH	Cglu	OH	H	OH	OH
Luteolin 8-C-glucoside (orientin)	lut8G	H	OH	H	OH	Cglu	OH	OH
Luteolin 3',7-O- α -diglucoside	lut3'G7G	H	OH	H	Oglu	H	Oglu	OH
Luteolin 4'-O- α -glucoside	lut4'G	H	OH	H	OH	H	OH	O-glu
Luteolin 7-O- α -glucoside	lut7G	H	OH	H	Oglu	H	OH	OH
Quercetin	que	OH	OH	H	OH	H	OH	OH
Quercetin-3-O- α -arabinoside (avicularin)	que3A	Oara	OH	H	OH	H	OH	OH
Quercetin-3-O- α -arabinoglucoside (peltatoside)	que3AG	Oara/glu	OH	H	OH	H	OH	OH
Quercetin-3-O- α -galactoside (hyperoside)	que3L	Ogal	OH	H	OH	H	OH	OH
Quercetin 3-O- α -glucoside (isoquercitrin)	que3G	Oglu	OH	H	OH	H	OH	OH
Quercetin 3-O- β -D-glucopyranosyl-6"-acetate	que3GAc	Oglu/Ac	OH	H	OH	H	OH	OH
Quercetin 3,7,3',4'-tetramethylether	queMe4	OCH ₃	OH	H	OCH ₃	H	OCH ₃	OCH ₃



2013

Patricia Vit · Silvia R. M. Pedro
David Roubik *Editors*

Pot-Honey

A legacy of stingless bees

 Springer

Chapter 35 Use of Honey in Cancer Prevention and Therapy

Patricia Vit, Jun Qing Yu, and Fazlul Huq

*This chapter is dedicated to cancer sufferers and survivors, and
researchers engaged in its prevention and therapy*



Pot-honey cytotoxicity in human ovarian cancer cell model *in vitro*

Biodiversity of 13 species of stingless bee honeys from 4 countries

488

P. Vit et al.

Table 35.1 IC₅₀ values of pot-honeys in the human ovarian cancer cell lines

		Ovarian cancer cell lines				
		A2780	A2780 ^{CisR}	A2780 ^{CisR}	A2780 ^{ZDU473R}	A2780 ^{ZDU473R}
		IC ₅₀	IC ₅₀	RF	IC ₅₀	RF
Geographical origin, city, country		Cisplatin (control)				
		0.88	3.88	4.42	3.44	3.91
		Pot-honey bee species				
Australia	Brisbane, Australia	8.96	4.16	0.53	4.54	0.51
Brazil	Chetumal, Mexico	4.24	3.35	0.79	4.14	0.98
	El Reyeván, Mexico	6.17	4.72	0.77	4.28	0.69
	Mourá, Brazil	6.18	5.83	0.94	5.89	0.95
Mexico	Talocas, Brazil	8.00	3.97	0.50	5.15	0.64
	Prezinho, Brazil	13.56	6.69	0.49	7.69	0.57
Venezuela	Monuy, Venezuela	16.50	4.21	0.26	12.81	0.78
	Monuy, Venezuela	3.30	3.68	1.08	3.65	1.08
	Baldim, Brazil	< 10	4.68	0.92	3.80	0.74
	Jairá Pessoa, Brazil	24.37	25.72	1.06	27.64	1.31
	Chimpo, Mexico	2.74	1.66	0.61	0.79	0.29
	Natal, Brazil	17.54	27.60	1.57	54.39	3.98
	El Reyeván, Mexico	4.10	4.59	1.10	4.10	0.98
	Cuicatlan, Mexico	7.71	8.41	0.97	5.62	0.73
	Xingú, Brazil	3.60	1.54	0.43	1.36	0.38
	Brisbane, Australia	8.96	4.16	0.53	4.54	0.51
	Guernsey, Mexico	4.58	4.32	1.03	4.19	0.92

IC₅₀ honey (mg/mL), cisplatin (μM), RF resistance factor as the ratio IC₅₀ resistant cell line/IC₅₀ parent cell line

IC₅₀ mg honey/mL
μM cisplatin

RF Resistance Factor

IC₅₀ resistant cell lines/
IC₅₀ parent cell line

A2780

Cis R

ZDU473R

} resistant
cell
lines

Stingless bees process honey and pollen in cerumen pots

Patricia Vit & David W Roubik, editors



Fotografías: Dr. Cristiano Menezes
Abeja fotografiada: *Tetragonisca muelleri* (Friese, 1900)

2013 Open Acces

Chapter 8

Systematic reviews on interventions with honey in cancer.

Vit P, Huq F

Chapter 10

Antioxidant activity of nest products of *Tetragonisca angustula* from Mérida, Venezuela.
Pérez-Pérez EM, Suárez E, Peña-Vera MJ, González AC, Vit P

Chapter 20

Cosmetic properties of honey. 1. Antioxidant activity

Isla M, Cordero A, Díaz L, Pérez-Pérez EM, Vit P

“Fermented pot-honey is not a defect, but microbial associations with stingless bees preserving their wet honey with active metabolites”

Patricia Vit, EuroFoodChem-Food for Future, Copenhagen, Denmark (2009).

2011

Revista del Instituto Nacional de Higiene Rafael Rangel 12, 58-63

Presencia de ácido láctico y otros compuestos semivolátiles en mieles de Meliponini

Patricia Vit¹, Luis B Rojas², Alfredo Usubillaga², Rosa Aparicio², Gina Meccia², Miguel A Fernández Muiño³, María Teresa Sancho³

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3 Nutrición y Bromatología, Facultad de Ciencias, Universidad de Burgos, Burgos, España. Autor principal: Tlf. 0274-2403565 (of) Fax 0274-2711802 vit@ula.ve.

Lactic acid was found in the honey produced by four species of stingless bees.

Other compounds were identified: 2-ethyl-hydroxy-propanoic acid in *Melipona favosa*, *Tetragonisca angustula* honeys, 5-(hydroxymethyl)-2-furancarboxaldehyde and α -phenylmethyl benzene ethanol in *Scaptotrigona mexicana* honey, 2-butanol, heneicosane and heptacosane in *Tetragonula carbonaria* honey.

2004 Bee World 85, 2-5

Alcoholic Fermentation Yeasts



Etymology

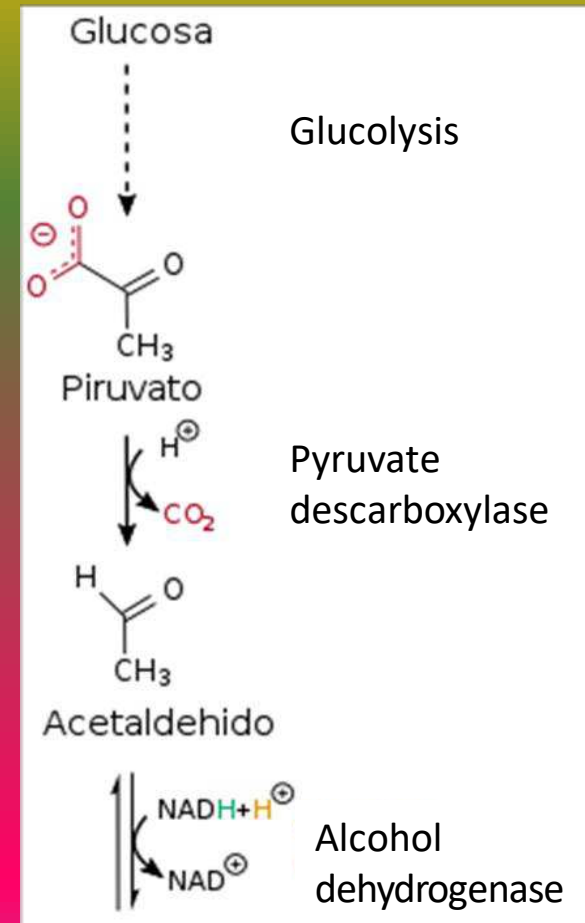
Fermentation Latin *fervere*, boiling forming bubbles



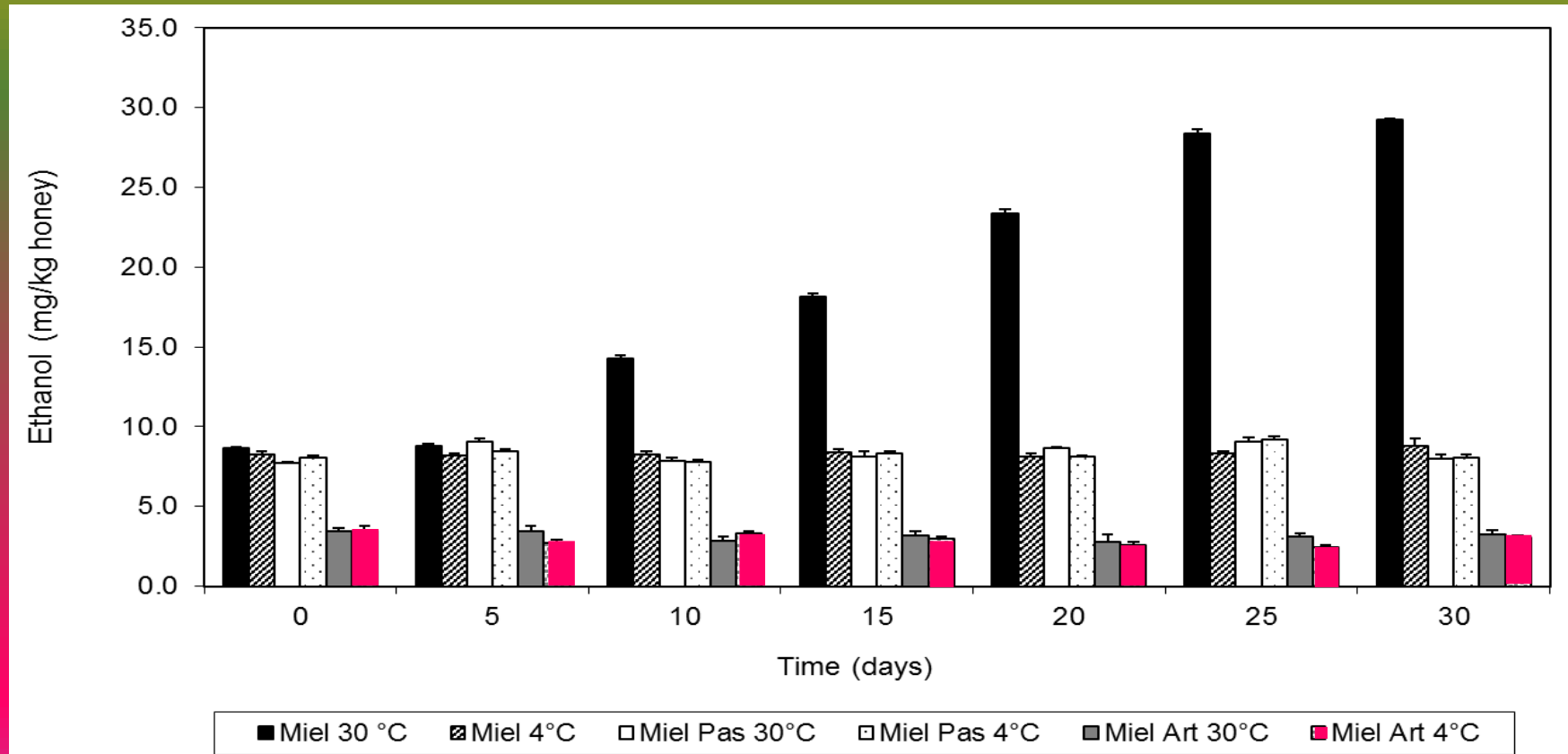
Post-harvest

Scaptotrigona mexicana
pot-honey

fermentation prior to bottling, Sierra Norte de Puebla, Mexico.



2007 BioTecnología 10, 14-22



Effect of storage temperature (30°C and 4°C) for one month on ethanol content of raw and pasteurized *Tetragonisca angustula* (Ta) honey, compared to artificial honey

2004

Bee World 85, 2-5

TABLE 2. Medicinal uses of stingless bee honey.

Species	Medicinal uses
<i>Melipona beechell</i>	Digestive disorders, eye diseases, respiratory infections, wound healing, post-birth recovery, fatigue, casts for fractures, skin ulcers
<i>Melipona favosa favosa</i>	Delivery enhancer
<i>Melipona paraensis</i>	Post-birth recovery
<i>Melipona trinitatis</i>	Gastritis
<i>Scaptotrigona mexicana</i>	Respiratory infections
<i>Nannotrigona perilampoides</i>	Cataract and pterygion treatment, stomach aches, bruises
<i>Plebeia jatifomis</i>	Cataract, pterygion, external injuries on the head, stomach aches
<i>Trigona (Tetragonisca) angustula</i>	Stomach disorders, cataract and pterygion, respiratory infections, wound healing

This was the first proposal of standards for SBH

2004 Bee World 85, 2-5

Do we need them for apitherapy?

TABLE 3. Suggested standards for stingless bee honeys, compared with official Codex Alimentarius Commission standards for *Apis mellifera* honey.

Honey composition	Standards			
	<i>Apis mellifera</i>	<i>Melipona</i>	<i>Scaptotrigona</i>	<i>Trigona</i>
Water content (g/100g)	max 20.0	max 30.0	max 30.0	max 30.0
Reducing sugars (g/100g)	min 65.0	min 50.0	min 50.0	min 50.0
Sucrose (g/100g)	max 5.0	max 6.0	max 2.0	max 6.0
Acidity (meq/100g)	max 40.0	max 70.0	max 85.0	max 75.0
Ash (g/100g)	max 0.5	max 0.5	max 0.5	max 0.5
HMF (mg/kg)	max 40.0	max 40.0	max 40.0	max 40.0
Diastase activity (DN)	min 8.0	min 3.0	min 3.0	min 7.0



This was the second proposal of standards for SBH, expanded
2023 Interciencia 48, 2-5 *Yes, we need them for apitherapy*

Chemical quality factors	<i>Apis mellifera</i> Honey standards ^(a)	Neotropical stingless bee genera				Paleotropical stingless bee genera		
		Suggested honey standards ^(d)			New suggested honey standards ^(a)	Indo-Malayan ^(b)	Australasian ^(c)	
		<i>Melipona</i>	<i>Scaptotrigona</i>	<i>Trigona</i>		New suggested honey standards		
				<i>Tetragonisca</i>	<i>Geniotrigona thoracica</i>	<i>Heterotrigona itama</i>	<i>Tetragonula carbonaria</i>	
Water (g/100g honey)	Maximum 20.0	Maximum 30.0	Maximum 30.0	Maximum 30.0	Maximum 30.0	Maximum 30.0	Maximum 30.0	
Reducing sugars (g/100g honey)	Minimum 65.0	Minimum 50.0	Minimum 50.0	Minimum 50.0	Minimum 50.0	Minimum 50.0	Minimum 50.0	
Sucrose (g/100g honey)	Maximum 5.0	Maximum 6.0	Maximum 2.0	Maximum 6.0	Maximum 5.0	ND	Maximum 2.0	
Free acidity (meq/100g honey)	Maximum 40.0	Maximum 70.0	Maximum 85.0	Maximum 75.0	Maximum 70.0	Maximum 300.0	Maximum 150.0	
Ash (g/100g honey)	Maximum 0.5	Maximum 0.5	Maximum 0.5	Maximum 0.5	Maximum 0.5	Maximum 0.2	Maximum 0.5	
Hydroxymethylfurfural (mg/kg honey)	Maximum 40.0	Maximum 40.0	Maximum 40.0	Maximum 40.0	Maximum 40.0	Maximum 20.0 ^(f)	Maximum 20.0 ^(g)	
Diastase activity (DN)	Minimum 8.0	Minimum 3.0	Minimum 3.0	Minimum 7.0	Minimum 8.0	-	Minimum 15.0 ^(h)	

ND: not detected. Source: ^(a) Vit (2023a,b); ^(b) Zawawi *et al.* (2022); ^(c) Persano-Oddo *et al.* (2008); ^(d) Vit *et al.* (2004); ^(e) Codex Stan (2019); ^(f) (Suhana Ahmad, personal communication).

Cytotoxicity of Ecuadorian Stingless Bee Honey



WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 7.632

Volume 10, Issue 9, 115-134

Research Article

ISSN 2278 – 4357

**CYTOTOXICITY OF *GEOTRIGONA*, *MELIPONA* AND
SCAPTOTRIGONA ECUADORIAN POT-HONEYS IN HUMAN
OVARIAN CANCER CELL MODEL**

Fazlul Huq¹, Jun Qing Yu¹, Silvia RM Pedro², Elizabeth Pérez-Pérez³, Favian Maza⁴,
Patricia Vit^{5,6*}

Cytotoxicity of 3 genera of Ecuadorian Stingless Bee Honeys

IC₅₀ values and resistance factors (RF) for honey and cisplatin as applied to A2780 and A2780^{cisR} human ovarian cancer cell lines

Cell lines	Stingless bee species IC ₅₀ (mg mL ⁻¹ honey)			Reference drug IC ₅₀ (μM)
	<i>Geotrigona leucogastra</i>	<i>Melipona grandis</i>	<i>Scaptotrigona</i> sp.	Cisplatin
A2780	19.28 ± 1.93	0.15 ± 0.02	4.25 ± 0.26	0.88 ± 0.05
A2780 ^{cisR}	22.67 ± 2.27	0.05 ± 0.002	4.29 ± 0.39	3.90 ± 0.50
RF	1.18	0.32	1.01	4.43

Cell survival fraction as IC₅₀ values were calculated in response to pot-honey dilutions. Resistance factor (RF) is the ratio of the IC₅₀ value in the resistant cell line to that in the parental cell line. Data are expressed as means ± SE (n = 3).



Pot-honey harvest from a *Melipona grandis* nest in Puyo, Pastaza province, Ecuador





Cytotoxicity of Ecuadorian Stingless Bee Honeys

Antioxidant activity and **biochemical components** of honey produced by three species of stingless bees did not explain the highest cytotoxicity of *M. grandis* in the ovarian cancer cell model

Honey parameters	Stingless bee species		
	<i>Geotrigona leucogastra</i>	<i>Melipona grandis</i>	<i>Scaptotrigona sp.</i>
Antioxidant activity			
AOA (mM UAE /100 g)	1.0 ± 0.1	1.1 ± 0.0	1.1 ± 0.0
HR (% inhibition HR)	85.8 ± 0.9	88.4 ± 0.9	87.5 ± 0.2
TAA (µmoles TE/100g)	148.8 ± 6.5	125.1 ± 1.5	170.1 ± 0.5
Biochemical components			
Flavonoids (mg QE/100 g)	80.0 ± 8.2	67.3 ± 2.2	122.0 ± 17.4
Polyphenols (mg GAE/100 g)	1444.2 ± 105.0	890.4 ± 163.7	1340.7 ± 29.7
Proteins (mg proteins/ 100 g)	593.4 ± 124.7	1097.1 ± 82.8	1374.9 ± 56.0

UAE uric acid equivalents, HR hydroxyl radical, TE Trolox equivalents, QE quercetin equivalents, GAE gallic acid equivalents. Data are expressed as means ± SE (n = 3).



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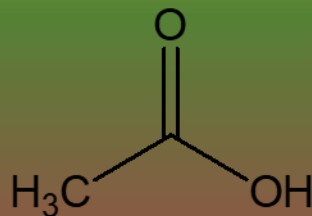
Research Paper

Impact of genus (*Geotrigona*, *Melipona*, *Scaptotrigona*) in the targeted $^1\text{H-NMR}$ organic profile and authenticity test by interphase emulsion of honey processed in cerumen pots by stingless bees in Ecuador

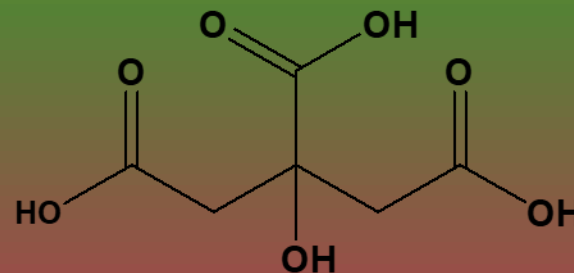
Patricia Vit^{a,*}, Jane van der Meulen^b, Maria Diaz^b, Silvia R.M. Pedro^c, Isabelle Esperança^d, Rahimah Zakaria^e, Gudrun Beckh^b, Favian Maza^f, Gina Meccia^g, Michael S. Engel^{h,i}

Supplement with 41 chemical structures of honey metabolites

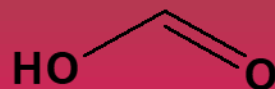
Aliphatic Organic Acids (AOA)



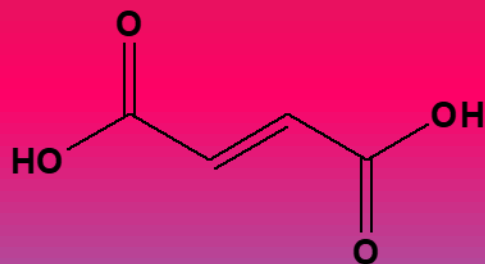
Acetic acid



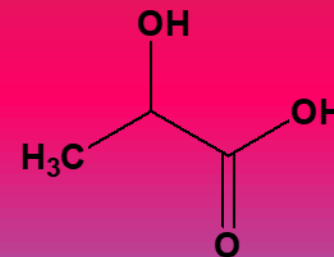
Citric acid



Formic acid

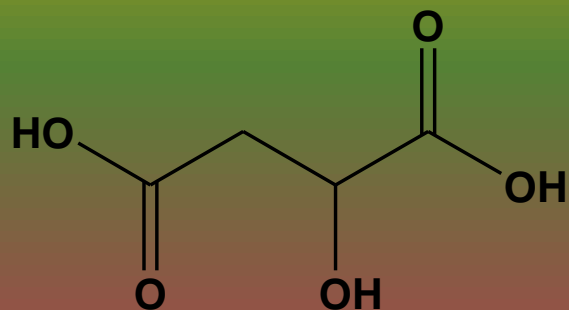


Fumaric acid

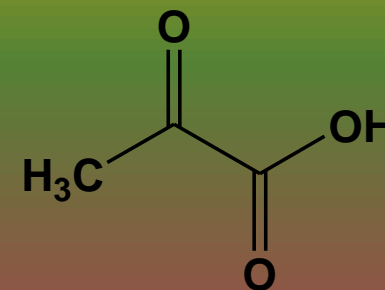


Lactic acid

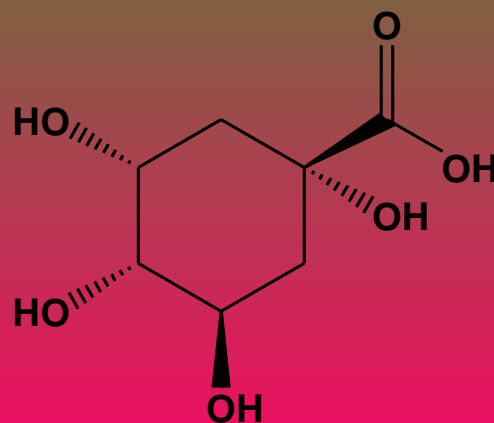
Aliphatic Organic Acids (AOA)



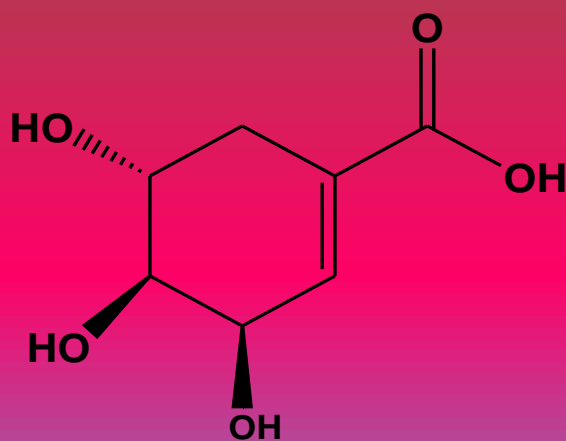
Malic acid



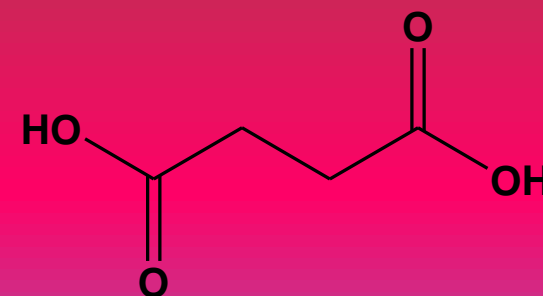
Pyruvic acid



Quinic acid



Shikimic acid



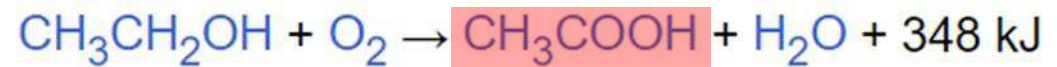
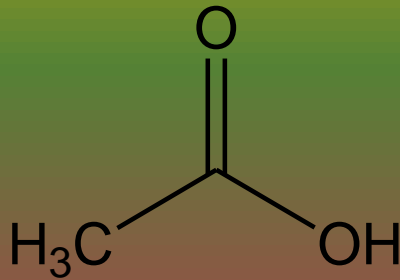
Succinic acid



Acetic acid

Acetic Fermentation

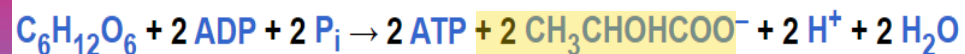
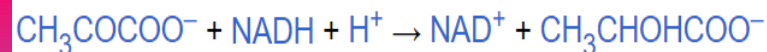
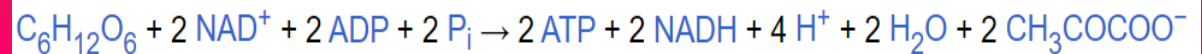
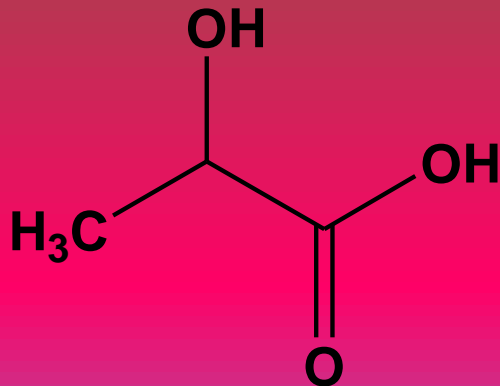
Acetobacter

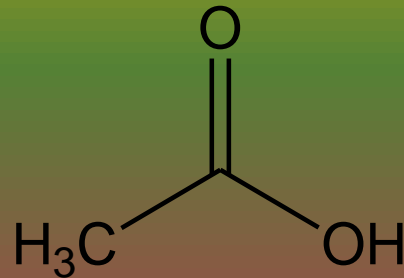


Lactic acid

Lactic Fermentation

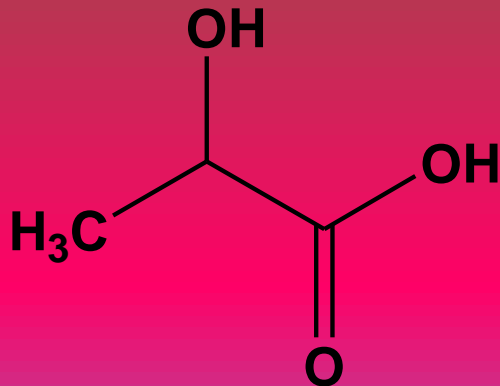
Lactic Acid Bacteria LAB





Acetic acid

Aliphatic organic acids (mg/kg honey)	Stingless bee genus		
	<i>Geotrigona</i> (n = 8)	<i>Melipona</i> (n = 4)	<i>Scaptotrigona</i> (n = 8)
Acetic acid (g/kg) Monocarboxylic acid	19.60 ± 1.45 ^b [15.23–25.56]	1.33 ± 0.33 ^a [0.46–2.06]	1.21 ± 0.34 ^a [0.21–2.85]



Lactic acid

Lactic acid (g/kg) Monocarboxylic acid	24.30 ± 1.65 ^b [19.28–30.45]	1.56 ± 0.45 ^a [0.41–2.63]	1.55 ± 0.39 ^a [0.43–3.39]
---	--	---	---

Gluconic acid is not measurable by NMR due to its 16 isomers But it was measured by HPIC-SCD high performance ion chromatography with suppressed conductivity detection in 111 SBH from Australia.

Journal of Food Composition and Analysis 122 (2023) 105466



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Journal of Food Composition and Analysis

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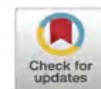
Organic acid profiles of Australian stingless bee honey samples determined by ion chromatography.

Natasha L. Hungerford ^{a,*}, Hans S.A. Yates ^b, Tobias J. Smith ^c, Mary T. Fletcher ^a

^a Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Health and Food Sciences Precinct, Coopers Plains, Qld 4108, Australia

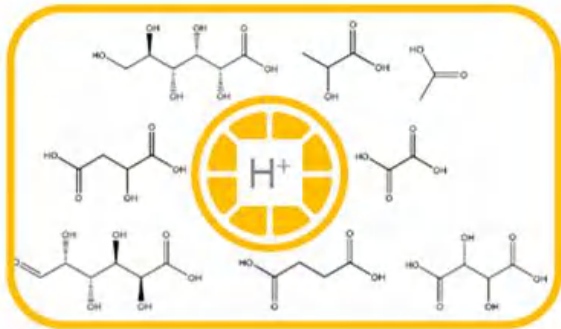
^b Forensic and Scientific Services, Queensland Health, Coopers Plains, Qld 4108, Australia

^c School of Biological Sciences, The University of Queensland, St Lucia, Qld 4072, Australia



Seminal review

The Review on Aliphatic Organic Acids (AOA) of Honey and Pot-honey for Bee Science



Patricia VIT • Svetlana SIMOVA

- ❖ **Harmonized AOA units** available in the literature, useful to compare a set of AOA in different honey types, based on the 100 g compositional standard of food science adopted as the best way to compare nutrients in similar products.
- ❖ **Updated reference for honey AOA contents**
 Visualization of **AOA < 2.5%** for *Apis mellifera* honey replacing the current < 0.5%, as well as **AOA < 5.8%** for **pot-honey**, represented by the *Axestotrigona* genus from Tanzania in this SBH dataset.
- ❖ Chronological data (1994–2023) selected refined bibliometric support of 919 samples, **710 *Apis mellifera* honey** and **209 Meliponini pot-honey**
 We eagerly anticipate a positive response from our esteemed readers networking between pot-honey experts and integrative multidisciplinary science.






antioxidants



Article

Chemical Components and Antioxidant Activity of *Geotrigona* sp. and *Tetragonisca fiebrigi* Stingless Bee Cerumen Reduce Juglone-Induced Oxidative Stress in *Caenorhabditis elegans*

Isamara Carvalho Ferreira ¹, Raíssa Cristina Darroz Côrrea ¹ , Sarah Lam Orué ¹, Daniel Ferreira Leite ¹, Paola dos Santos da Rocha ¹, Claudia Andrea Lima Cardoso ² , Rosilda Mara Mussury ³ , Patricia Vit ⁴, Kely de Picoli Souza ¹, Edson Lucas dos Santos ¹ and Jaqueline Ferreira Campos ^{1,*}

¹ Research Group on Biotechnology and Bioprospecting Applied to Metabolism (GEBBAM), Federal University of Grande Dourados, Rodovia Dourados-Itahum, Km 12, Dourados 79804-970, MS, Brazil; isamara.ferreira137@academico.ufgd.edu.br (I.C.F.); raissa.correa416@academico.ufgd.edu.br (R.C.D.C.); sarah.orue005@academico.ufgd.edu.br (S.L.O.); daniel.leite059@academico.ufgd.edu.br (D.F.L.); paolasantosrocha@ufgd.edu.br (P.d.S.d.R.); kelypicoli@ufgd.edu.br (K.d.P.S.); edsonsantos@ufgd.edu.br (E.L.d.S.)

² Course of Chemistry, State University of Mato Grosso do Sul, Rodovia Dourados-Itahum, Km 12, Dourados 79804-970, MS, Brazil; claudia@uems.br

³ Faculty of Biological and Environmental Sciences, Federal University of Grande Dourados, Rodovia Dourados-Itahum, Km 12, Dourados 79804-970, MS, Brazil; maramussury@ufgd.edu.br

⁴ Apitherapy and Bioactivity, Food Science Department, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Mérida 5101, Venezuela

* Correspondence: jaquelinefcampos@ufgd.edu.br

Phenolic compounds and fatty acids in ethanolic extracts of cerumen (mg/mL), and antioxidant activity IC₅₀ (mg/mL).

Compounds	<i>Geotrigona sp</i>	<i>Tetragonisca fiebrigi</i>
<i>Phenolic compounds</i>		
3,4-hydroxycinnamic acid	3.040 ± 0.032	-
Catechin	10.000 ± 0.044	-
Gallic acid	-	6.473 ± 0.020
Rutin	-	12.993 ± 0.022
Vanillin	4.020 ± 0.035	-
<i>Dicarboxylic acids</i>		
Maleic acid	1.970 ± 0.023	-
<i>Fatty acids</i>		
Caprylic acid	0.303 ± 0.003	0.347 ± 0.003
Capric acid	1.607 ± 0.007	1.503 ± 0.003
Lauric acid	2.590 ± 0.012	2.543 ± 0.009
DPPH	1.001 ± 0.062	1.251 ± 0.068
ABTS	0.496 ± 0.040	0.254 ± 0.023

Juglone is an organic compound generating large amounts of the radical superoxide anion, inducing oxidative stress and reducing nematode viability. EEC ameliorated juglone-induced oxidative stress during the redox imbalance. In other studies. Rutine, palmitic acid and oleic acid, increased the resistance to oxidative stress in *C. elegans* by activating DAF-16

Starmerella camargoi f.a., sp. nov., *Starmerella ilheusensis* f.a., sp. nov., *Starmerella litoralis* f.a., sp. nov., *Starmerella opuntiae* f.a., sp. nov., *Starmerella roubikii* f.a., sp. nov. and *Starmerella vitae* f.a., sp. nov., isolated from flowers and bees, and transfer of related *Candida* species to the genus *Starmerella* as new combinations

Ana Raquel O. Santos,¹ Marina P. Leon,¹ Katharina O. Barros,¹ Larissa F. D. Freitas,¹ Alice F. S. Hughes,¹ Paula B. Morais,² Marc-André Lachance³ and Carlos A. Rosa^{1,*}



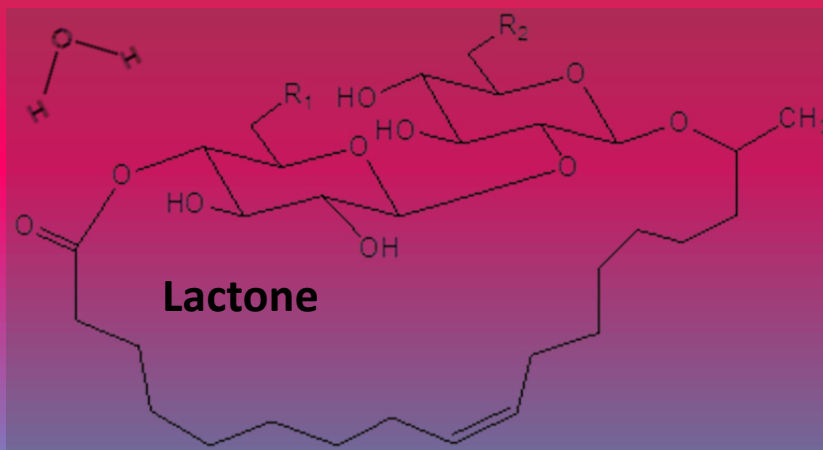
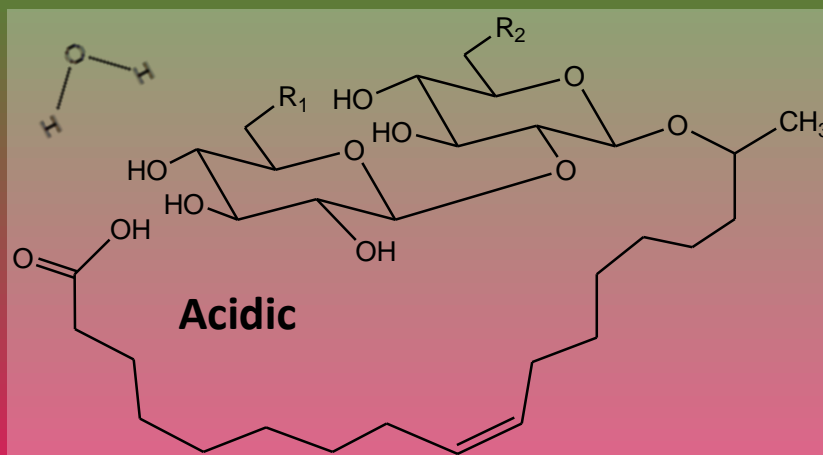
Sophorolipids

Industrial applications

- ❖ Food
- ❖ Bioremediation
- ❖ Cosmetics
- ❖ Pharmacy
- ❖ Ecology friendly home

Biotechnological optimization of culture media for microbial origin surfactants

*Scaptotrigona
vitorum*
honey





Metabolomics Categorizations

Categories of metabolites to **VISUALIZE** healing power

- ❖ Chemical type
- ❖ Quantity
- ❖ Entomological origin

MANUKA HONEY was the **seminal METABOLOMIC CATEGORY** for HONEY

With the **UMF**

The worldwide pharmaceutical attribute **UNIQUE MANUKA FACTOR**



APIMONDIA

48° CONGRESO INTERNACIONAL CHILE
DE APICULTURA 4 al 8 septiembre 2023

Desde el sur del mundo por una apicultura sostenible



Metabolomics Categorizations

2008 Acta Bioquímica Clínica Latinoamericana 42, 237-244

Bioquímica Clínica

Mieles checas categorizadas según su actividad antioxidante

Czech honey categorized according to their antioxidant activity

- ▶ Patricia Vit¹, María Gabriela Gutiérrez², Dalibor Titěra³, Michael Bednař⁴, Antonio Jesús Rodríguez-Malaver⁵

Categories of Antioxidant Activity

Tabla VI. Clasificación ampliada de mieles según su AAT (μ moles equivalentes Trolox/100 g miel).

Clases	Categoría AAT	AAT μ moles equivalentes Trolox	
		Desde	Hasta
1	Pro-oxidante	<1	
2	Muy baja	1	50
3	Baja	51	100
4	Moderadamente baja	101	150
5	Media	151	200
6	Moderadamente alta	201	250
7	Alta	251	300
8	Muy alta	>300	



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Trehalulose

2020

**SCIENTIFIC
REPORTS**

natureresearch



OPEN

Stingless bee honey, a novel source of trehalulose: a biologically active disaccharide with health benefits

Mary T. Fletcher¹, Natasha L. Hungerford¹, Dennis Webber²,
Matheus Carpinelli de Jesus³, Jiali Zhang¹, Isobella S. J. Stone^{2,3}, Joanne T. Blanchfield³ &
Norhasnida Zawawi^{1,4}



Trehalulose Sci. Rep. 2020

¹Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Brisbane, QLD 4072, Australia.

²Biosecurity Queensland, Department of Agriculture and Fisheries, Brisbane, QLD 4108, Australia.

³School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072, Australia.

⁴Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.



Trehalulose

2022

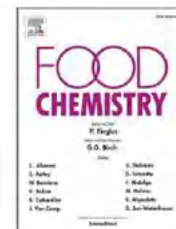


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Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Unique physicochemical properties and rare reducing sugar trehalulose mandate new international regulation for stingless bee honey

Norhasnida Zawawi^{a,b,*}, Jiali Zhang^b, Natasha L. Hungerford^b, Hans S.A. Yates^c,
Dennis C. Webber^e, Madeleine Farrell^c, Ujang Tinggi^c, Bhesh Bhandari^d, Mary T. Fletcher^b

^a Faculty of Food Science and Technology, University Putra Malaysia, 43400 UPM Serdang, Malaysia

^b Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Health and Food Sciences Precinct, Coopers Plains, Qld 4108, Australia

^c Forensic and Scientific Services, Queensland Health, Coopers Plains, Qld 4108, Australia

^d School of Agriculture and Food Sciences, The University of Queensland, St. Lucia, Qld 4067, Australia

^e Biosecurity Queensland, Department of Agriculture and Fisheries, Brisbane, QLD 4108, Australia



Trehalulose

Table 2
Average physicochemical properties of stingless bee honey across different species.

Physicochemical properties	Malaysian Stingless Bee Species <i>Heterotrigona itama</i> (n = 10)						Australian Stingless Bee Species <i>Tetragonula carbonaria</i> (n = 11)					
	<i>Geniotrigona thoracica</i> (n = 5)			<i>Tetragonula hockingsi</i> (n = 10)								
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Colour (mmPfund)	230.4 ^{2c}	111.6	107.85–450.3	148.5 ^a	58.8	95.34–315.32	436.4 ^b	131.5	297.83–718.11	365.7 ^{bc}	116.2	203.72–575.26
Moisture (%)	28.16 ^a	1.45	25.97–30.70	28.30 ^{2bc}	2.08	25.78–31.01	25.48 ^b	0.85	23.91–26.88	25.15 ^{bc}	1.16	23.75–27.66
pH	3.19 ^a	0.15	2.86–3.42	3.07 ^a	0.04	3.01–3.1	3.6 ^b	0.06	3.5–3.71	3.63 ^b	0.13	3.44–3.88
Free acidity (meq/kg)	211.5 ^a	93	17.0–336.2	235.6 ^a	95.8	95.3–315.3	167.8 ^a	32.8	98.5–212.3	125.4 ^a	40.9	74.1–202.0
Total Phenolic (mg GAE/kg)	358.9 ^a	88.2	251.1–541.8	325.0 ^a	69.5	223.4–410.4	1054.9 ^b	136.2	883.4–1246.3	1044.5 ^b	165.6	923.2–1321.3
Sugar (g/100 g)												
Fructose	16.23 ^a	6.21	4.89–25.01	4.11 ^b	2.61	0.69–7.07	17.43 ^a	2.87	12.98–22.40	16.58 ^a	3.39	10.48–20.60
Glucose	13.37 ^a	7.22	2.12–24.88	2.90 ^b	1.20	ND–4.26	12.10 ^a	2.51	7.81–15.26	12.71 ^a	2.91	6.30–16.91
Fructose + Glucose	29.61 ^a	13.32	7.01–49.59	6.29 ^b	4.3	0.69–11.32	29.54 ^a	5.21	20.79–37.66	29.29 ^a	6.10	17.34–36.47
Trehalulose	28.38 ^a	9.05	17.81–42.30	49.08 ^b	5.84	43.08–57.04	23.18 ^a	4.80	18.03–35.45	24.90 ^a	6.49	18.62–38.75

Categories of Trehalulose in SBH

Classes	Trehalulose Concentration ranges (g/100g SBH)	Stingless bee species
1	10.0–19.9	<i>Heterotrigona itama</i> , <i>Tetragonula carbonaria</i> , <i>Tetragonula hockingsi</i>
2	20.0–29.9	<i>Heterotrigona itama</i> , <i>Tetragonula carbonaria</i> , <i>Tetragonula hockingsi</i>
3	30.0–39.9	<i>Heterotrigona itama</i> , <i>Tetragonula carbonaria</i> , <i>Tetragonula hockingsi</i>
4	40.0–49.9	<i>Heterotrigona itama</i> , <i>Geniotrigona thoracica</i>
5	50.0–59.9	<i>Geniotrigona thoracica</i>

Valued stingless bee products sustain social projects

Review Article

Female stingless bee keepers promoting nutraceutical and health benefits of meliponine honey, pollen, cerumen, and propolis

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Single mothers' stingless bee keeper project in Terengganu, Malaysia by UMT

Conclusions

- ❖ Besides the chemical composition, the antioxidant activity and the antimicrobial activity are important overall added values for stingless bee products, to ground a medicinal approach for both nutritional and pharmaceutical applications.
- ❖ Focused targets continue developing investigations on respiratory, cardiovascular, digestive systems boosting the the immune system, protecting against cancer, COVID, and dilucidating mechanisms of action.



Muy apreciada la hospitalidad chilena en el exitoso Congreso APIMONDIA 2023



*desde la línea aérea LATAM
la asistencia y recepción en el aeropuerto
la distinguida atención en el Hotel Double Tree en Vitacura
los uber taxi y los choferes de los autobuses
las recepciones con deliciosos alimentos y bebidas
la producción y gastronomía chilena
organizadores, coordinadores y asistentes
la generosidad de su gente
la miel de ulmo, de avellano y de todas sus latitudes*

Te llevamos en nuestros corazones Chile



Thank you for your kind attention