

42° INTERNATIONAL APICULTURAL CONGRESS APIMONDIA 2011 BUENOS AIRES – ARGENTINA Congress: From 21st to 25th, September 2011



POSSIBLE INTERNATIONAL DIRECTIVE FOR QUALITY CONTROL OF BEE POLLEN

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Maria Graça Campos

Head of the Bee Pollen Working Group at International Honey Commission (IHC)



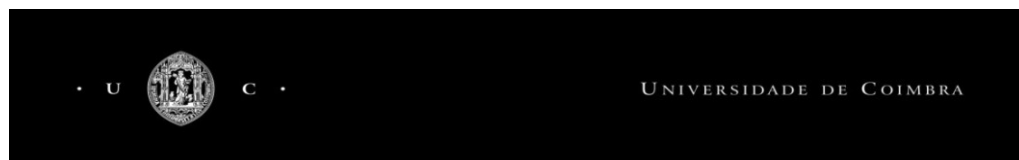
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DRUG DISCOVERY Quality Control Risk assessment

Specialist at European Scientific
Cooperation Working Group on Isoflavones, at the
European Community Agency, European Food
Safety Authority – EFSA, (2009- ...)



Specialist at Technical Commission of Autoridade
de Segurança Alimentar e Económica
(ASAE/PORTUGAL) - Dietetic Products,
Nutrition and allergies (2008- ...)



SUMMARY

- Urgent need to have a International Directive for quality Control of Bee pollen to **avoid International Trade Conflicts**
- Bee pollen as Medicinal Plant (**add value**)
Bioactivity correlated to the floral source
- Data from various countries around the World
Denomination by region or by floral source
- Future Challenges ~ ISO norm draft for **BEE POLLEN**



REVIEW ARTICLE

Pollen composition and standardisation of analytical methods.



High Quality

Maria G. R. Campos¹, Stefan Bogdanov², Ligia Bicudo de Almeida-Muradian³, Teresa Szczesna⁴, Yanina Mancebo⁵, Christian Frigerio¹, Francisco Ferreira¹.

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Summary

Honey bee pollen is considered to be a food, and national pollen standards exist in different countries such as Brazil, Bulgaria, Poland and Switzerland. It is the aim of the present work to review pollen composition and the analytical methods used for the evaluation of high quality bee pollen. Based on the experience of different countries and on the results of published research, we propose quality criteria for bee pollen, hoping that in the future they will be used as world wide bee pollen standards.

Keywords: Standardisation of bee products; bee pollen; quality control



Proposed technical regulation for the identity and quality of bee pollen

Technical regulation for identity

Objective: To establish the identity and the minimum quality requirements for bee pollen.

Target: The regulation will be applied to bee pollen sold in national and international markets.

1. Description

1.1. Definition: Bee pollen is the result of the agglutination of flower pollens, made by worker honey bees, with nectar (and/or honey) and salivary substances, and collected at the hive entrance.

1.2. Classification:

1.2.1. According to water content:

1.2.1.1. Bee pollen: The product collected in the original form, with water content between 20-30 %. Storage of such pollen should be in a freezer to avoid bacterial and mould contamination.

1.2.1.2. Desiccated bee pollen: The product submitted to a drying out process in temperatures not higher than 42°C, with water content not higher than 6%.

1.2.2. According to the floral source content:

1.2.2.1 Monofloral bee pollen: the major *taxon* need to be not less than 80% (different *taxa* can be used for specific nutritional and therapeutic purposes).

1.2.2.2 Multifloral bee pollen: include different *taxa*.

1.3. Denomination for sales purposes will include classification according the water and floral source content.



- **FUNCTIONAL FOOD**
- **DIETARY SUPPLEMENT**

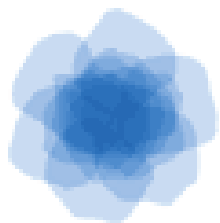


- **DRUG**

Medicinal Plant

Depending of the floral origin





PORTUGAL 2007
Presidência do Conselho da União Europeia

Reunião Informal do Comité dos Medicamentos à Base de Plantas (HMPC) da Agência Europeia de Medicamentos (EMA) - 27/28 Setembro 2007

<http://www.emea.europa.eu/>



Human Medicines - Herbal Medicinal Products

Directive 2004/24/EC1 introduced a new pathway for marketing traditional herbal medicinal products, the "simplified registration".

The Committee on Herbal Medicinal Products (HMPC) has been established in the framework of Directive 2004/24/EC and Regulation (EC) No 726/20042.

The main task of the Committee is to establish Community herbal monographs and entries to the 'list of herbal substances, preparations and combinations thereof for use in traditional medicinal products'. Community monographs and entries to the Community List established by the HMPC

<http://www.emea.europa.eu/Patients/herbals.htm>

Bee-Pollen Therapeutical Value

MARIA GRAÇA R. CAMPOS*, CHRISTIAN FRIGERIO
AND FRANCISCO FERREIRA

REVIEW ART

What is

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Received 29 July

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ABSTRACT

Around the world many things have been related to the health benefits of bee-pollen and different therapeutic bioactivities. Few researches have been done in a way to prove the importance to explore the potential of bee-pollen as a source of new drugs. In the 90ths the interest for this product increased and relevant research has been undertaken. The major lack on the most of the results published is the relationship between the pharmacological activity and the compounds involved. And in some cases it can only be verified through the pharmacodynamic activity because the assay with a well correlated dose effect and the quantification of the molecules and the pharmacokinetic of them is very hard to attribute. Different therapeutic bioactivities attributed to bee-pollen have been reviewed in this paper presenting the more relevant research published so far. From the data published here it's obvious that pollen itself and bee-pollen have a good potential of bioactivity that can be explored with different approaches. However, research is still needed to confirm many of these preliminary findings. Nevertheless it still remains a promising remedy to cure various human ailments..

Key words : Antiatherosclerotic, anti inflammatory, antioxidant, bee-pollen, osteoporosis, prostatitis



orte -

t, Porto



UNIVERSIDADE DE COIMBRA

Many research as been done in a way to prove the importance to explore the potential of bee-pollen for **therapeutical purposes**



Available online at www.sciencedirect.com



Food Chemistry 100 (2007) 237–240

Food
Chemistry

www.elsevier.com/locate/foodchem

Antioxidative properties of bee pollen in selected plant species

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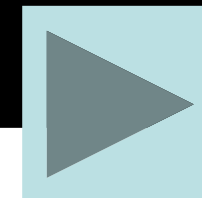
^b *Becarios COFAA, México*

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Received 22 September 2005; received in revised form 26 July 2006; accepted 4 August 2006



Floral origin – bioactivity compounds



Anaemia and the hemopoietic system – bee pollen - Iron



Antioxidant activity – *Eucalytus globulus* – flavonoids + ??



Anti inflammatory activity - *Pinus densiflora* – flavonoids ??



Antitumoral activity – *Brassica napus* – polysaccharide LBPP



Antiatherosclerotic activity - fat-soluble pollen extract



Activity on osteoporosis – *Cistus ladaniferus* – PM \leq 1000



Activity in Disorders of the Prostate – Hidroxamic acid



Antidiarrhoeal activity - *Eucalytus globulus*; *Salix atrocinera*



Antimicrobial activity – *Ranunculus* sp – herbacetin (flavonoid)



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RISK ASSESSMENT

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

J. Agric. Food Chem. XXXX, .

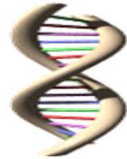
Hepatotoxic Pyrrolizidine Alkaloids in Pollen and Drying-Related Implications for Commercial Processing of Bee Pollen

MICHAEL BOPPRÉ,[‡] STEVEN M. COLEGATE,^{*,†} JOHN A. EDGAR,[†] AND
OTTMAR W. FISCHER[‡]

CSIRO Livestock Industries, Plant Toxins Research Group, Private Bag 24, Geelong, Victoria 3220,
Australia, and Forstzoologisches Institut, Albert-Ludwigs-Universität,
D-79085 Freiburg i.Br., Germany



Bee Pollen Standardization & Quality



Analytical Methods



Data Base

Identification of the worlds
main floral sources

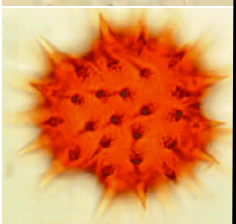


Microscopic analysis

(Barth et al, 2010 Anales de Academia Brasileira de Ciências, 82(4))

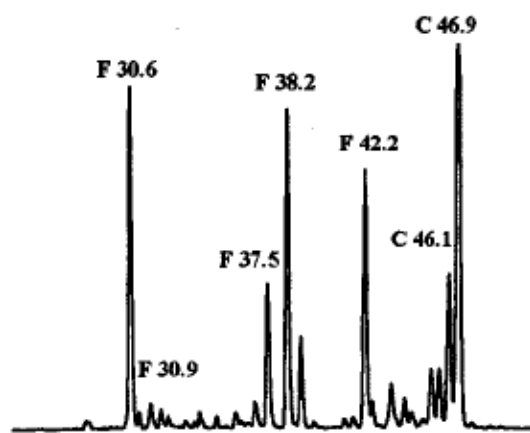
HPLC/DAD phenolic profiles

(Campos et al, 1996 Phytochemical Analysis)

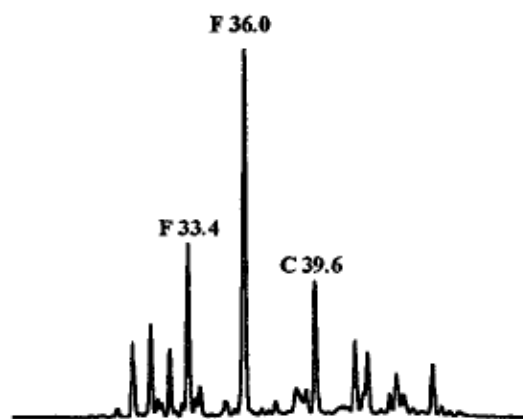


PHYTOCHEMICAL ANALYSIS, VOL. 8, 181-185 (1997)

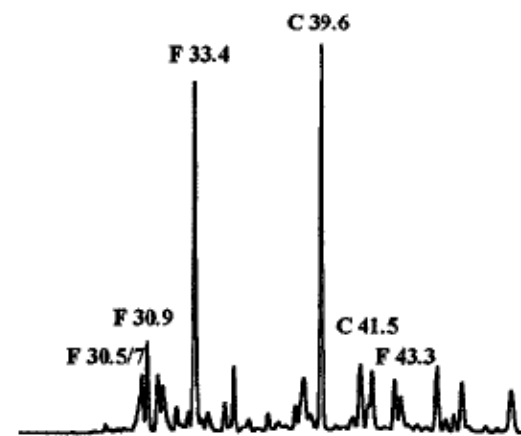
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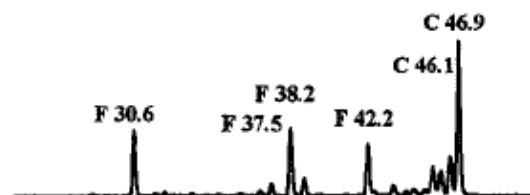
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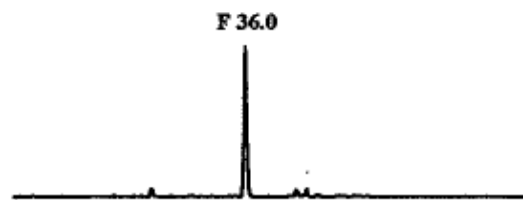
10.95 NZ



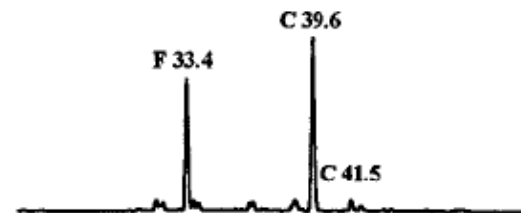
Eucalyptus globulus 92%

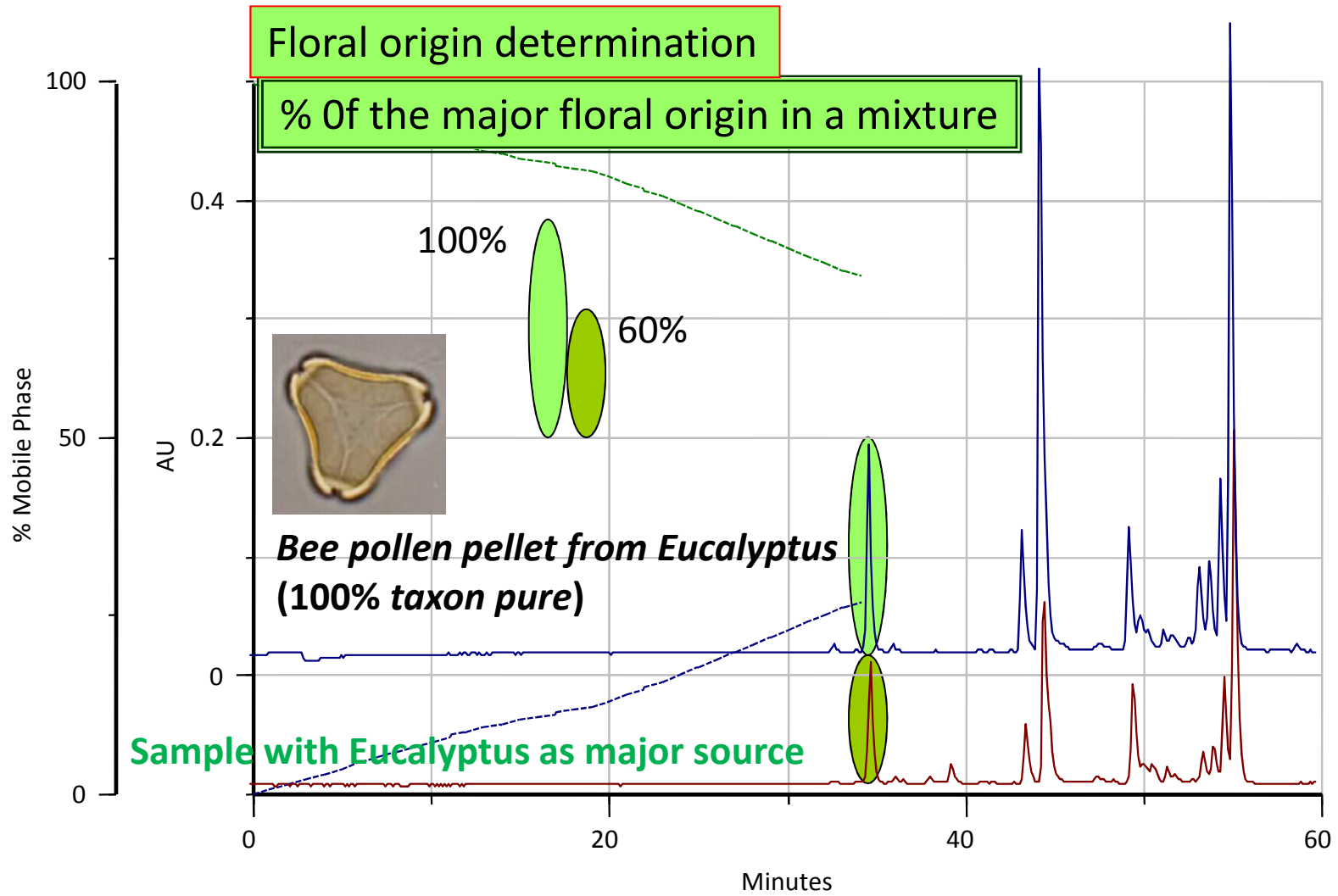


Erica australis 35%



Salix atrocineria (NZ) 60%





— c:\amine\hplc\magui\estroge3.001\estroge3.gdt : 340nm, 0.01 sensitivity : amostra inteira: Inj. Number: 2

— c:\amine\hplc\magui\estroge3.001\estroge3.gdt : 340nm, 0.01 sensitivity : carga polinica: Inj. Number: 3

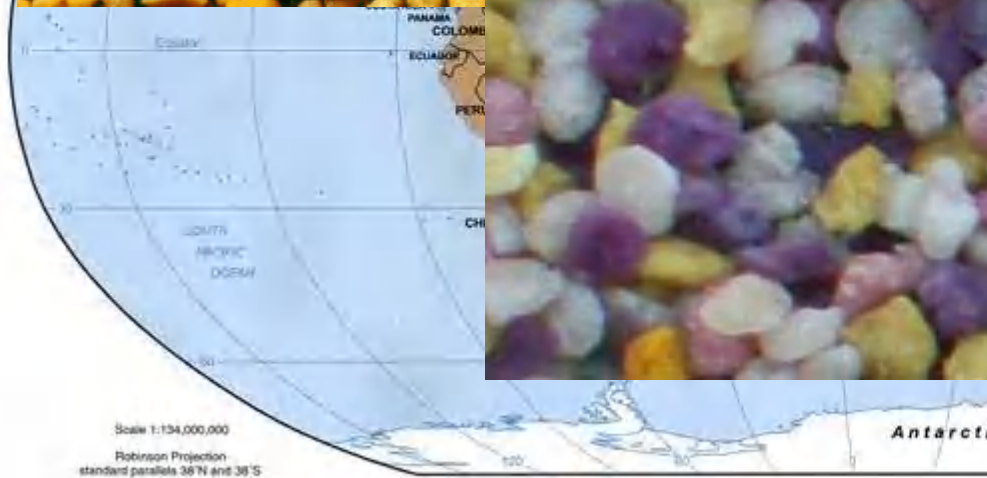
PORTUGAL



BULGARIA

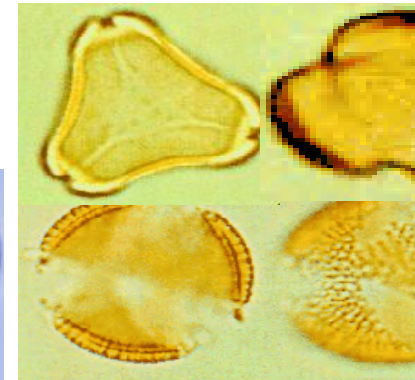
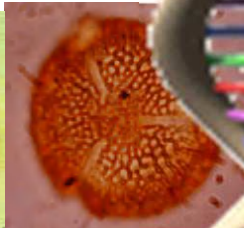
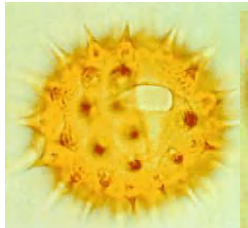
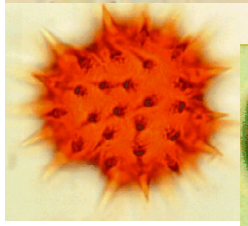


BRASIL





Phenolic and polyphenolic compounds = fingerprint



- Campos M. G.**, Webby R. F., Markham K. R., 2002. The Unique Occurrence of the Flavone Aglycone Tricetin in Myrtaceae Pollen. *Z. Naturforsch.* 57c, 944-946
- Markham K., Mitchel K., **Campos M. G.**, 1997. An unusually lipophilic flavonol glycoside from *Ranunculus sardous* Crantz. pollen - *Phytochemistry*, 45 (1), 203-204.
- Markham K., **Campos M.G.**, 1996. 7- and 8-O-methylherbacetin-3-O-sophoroside from bee pollens and some structure/activity observations. *Phytochemistry*, 43 (4) 762-767.

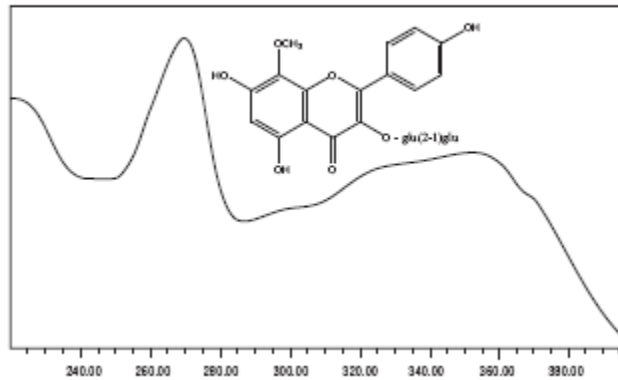
Analytical methods



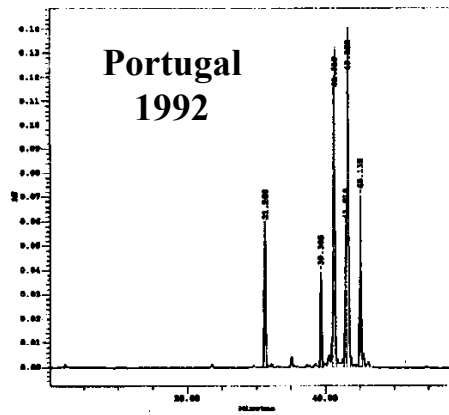
Polyphenolic profiles
Identification of the *taxon*

Ulex europaeus

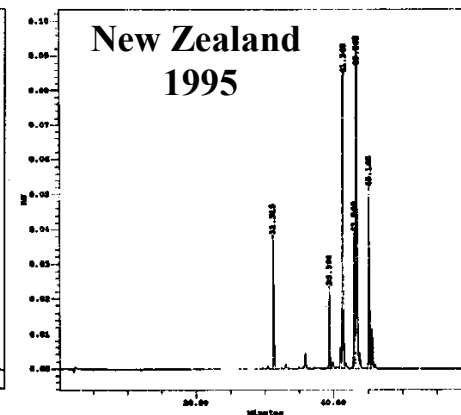
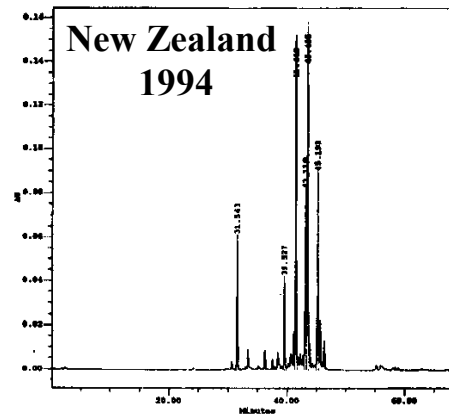
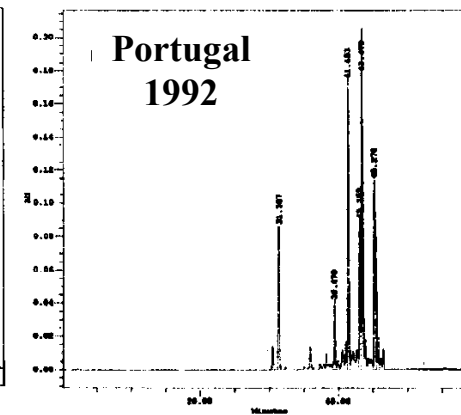
8-O-methylherbacetin-3-O-[glucosyl(1-2)glucoside]
 $\lambda_{max} = 274, 300sh, 324sh, 355sh \text{ nm}$



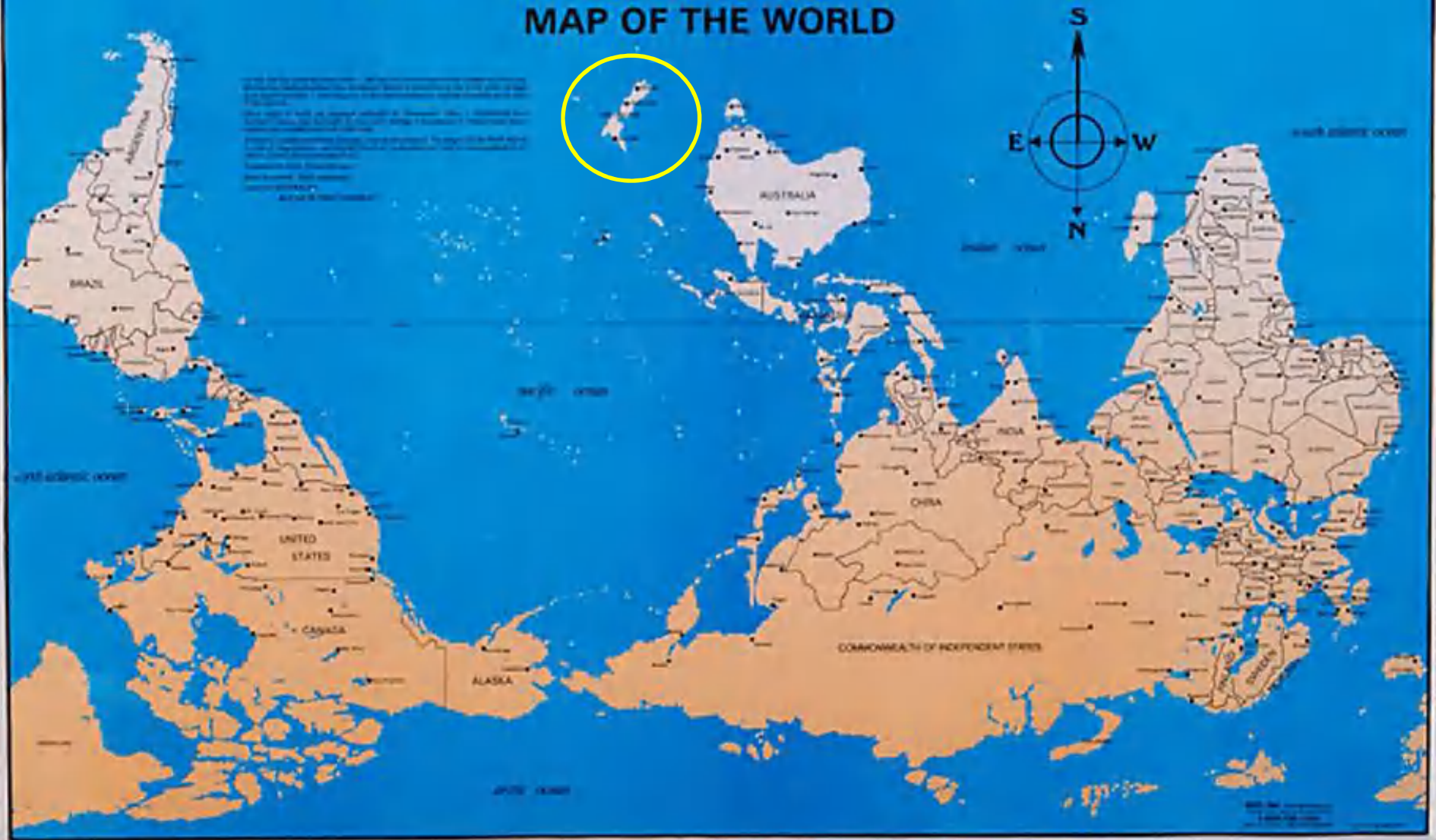
Bee pollen loads



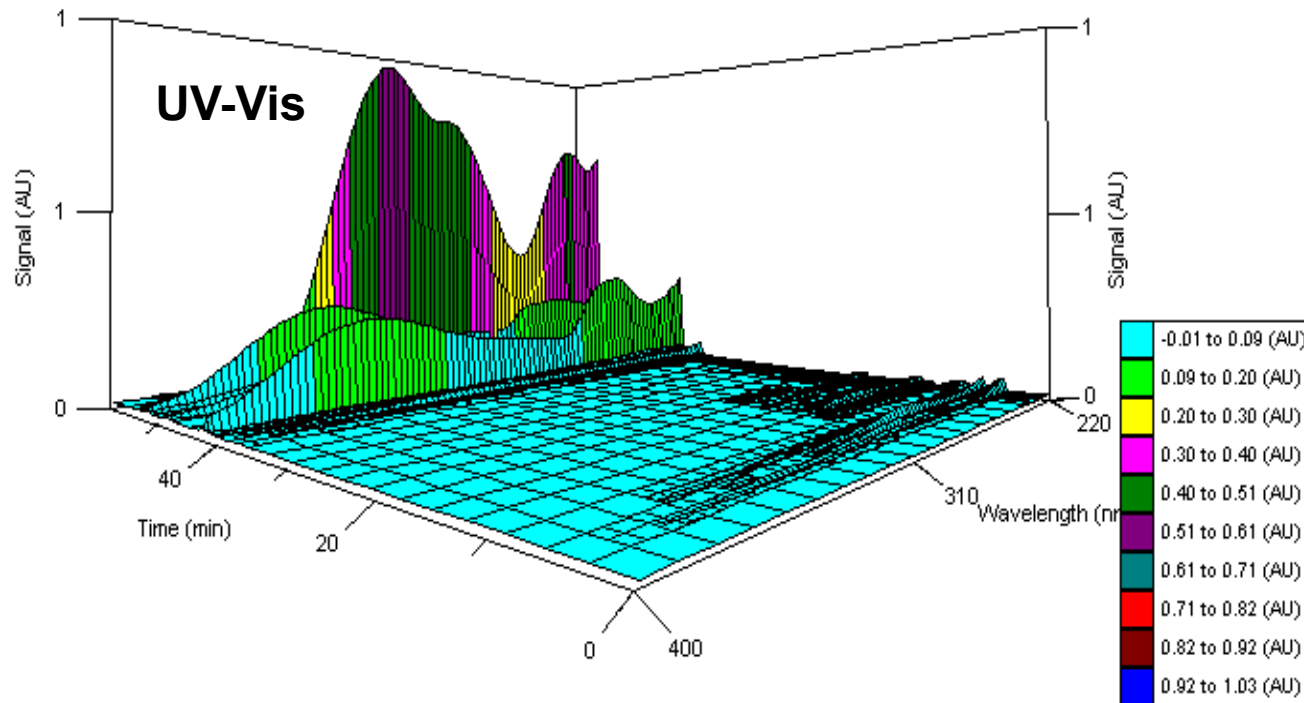
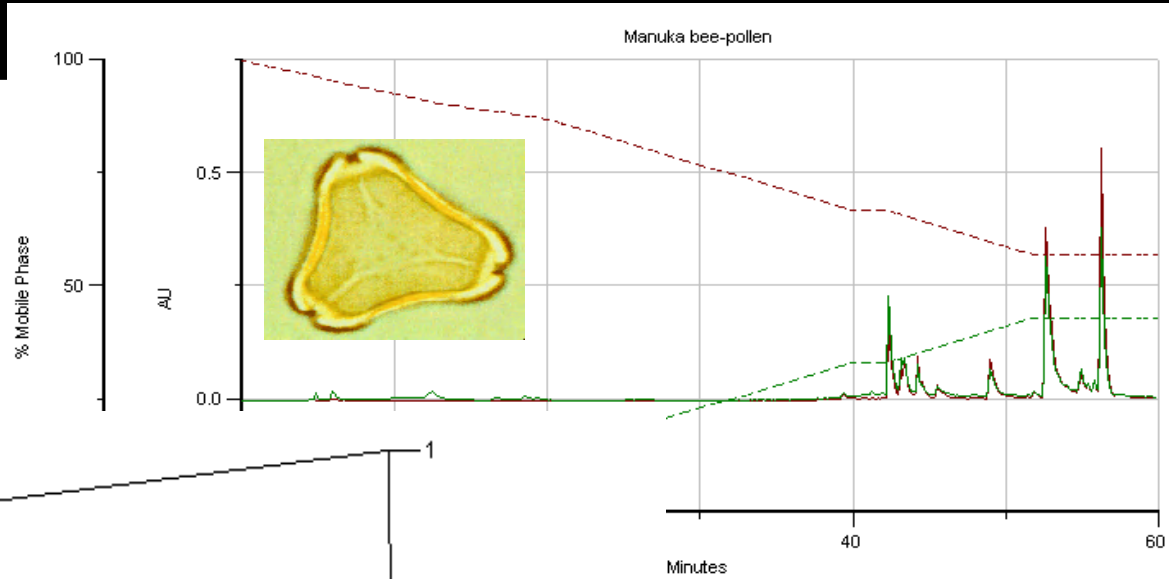
Pollen from plant



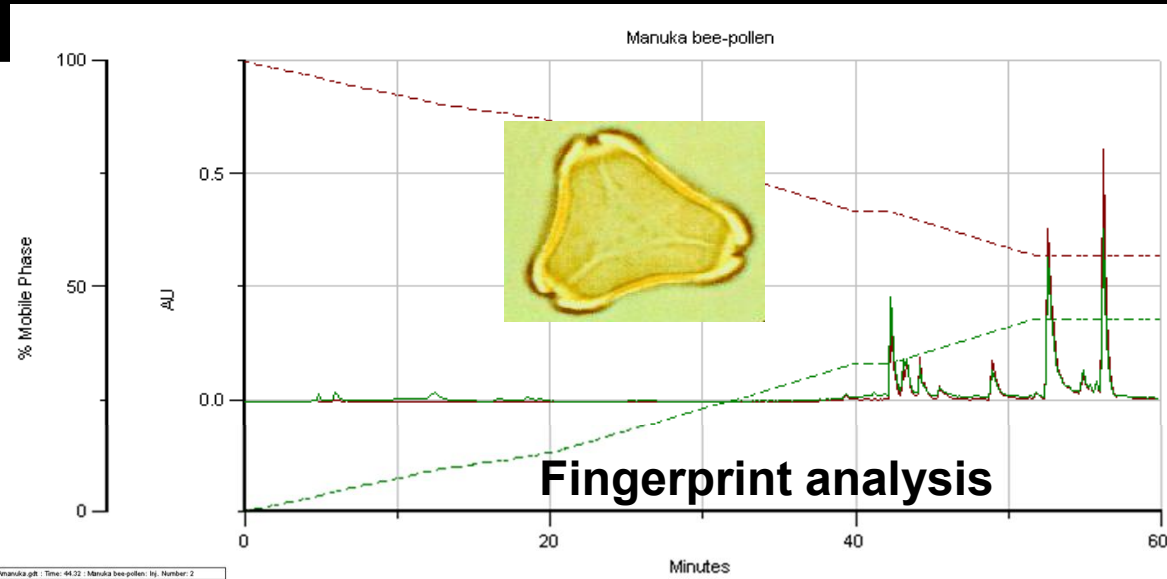
McARTHUR'S UNIVERSAL CORRECTIVE MAP OF THE WORLD



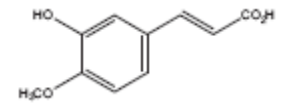
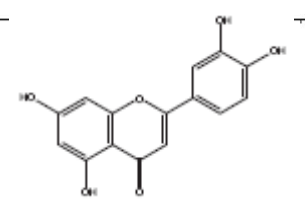
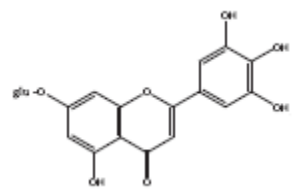
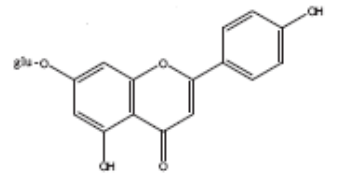
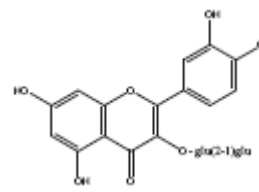
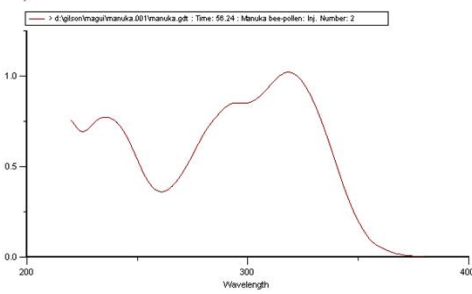
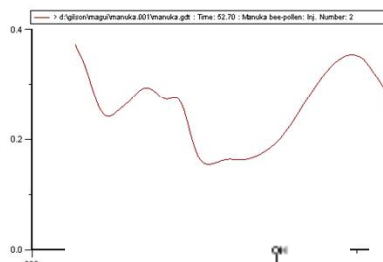
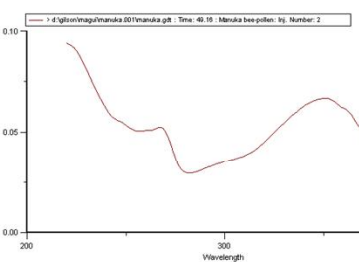
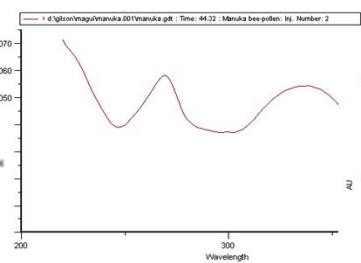
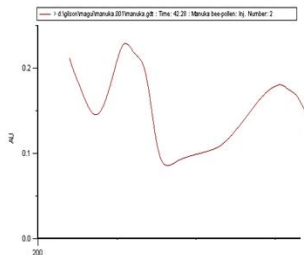
Fingerprint analysis



New Zealand



Fingerprint analysis



Maria da Graça Campos & Kenneth R. Markham

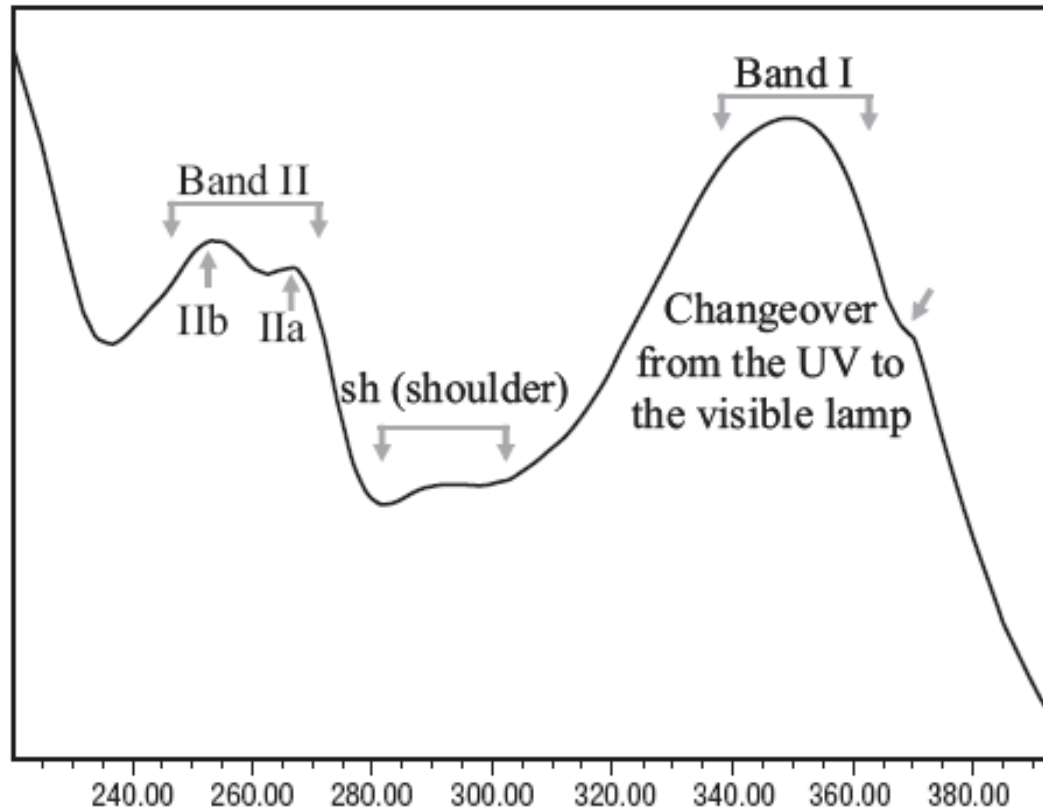
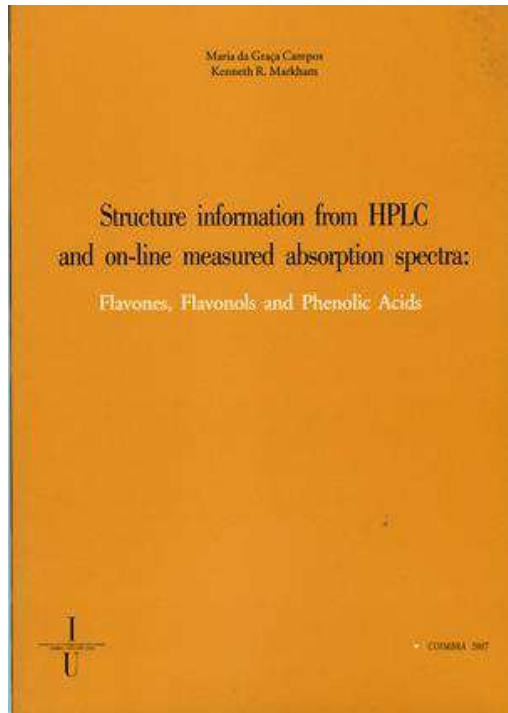
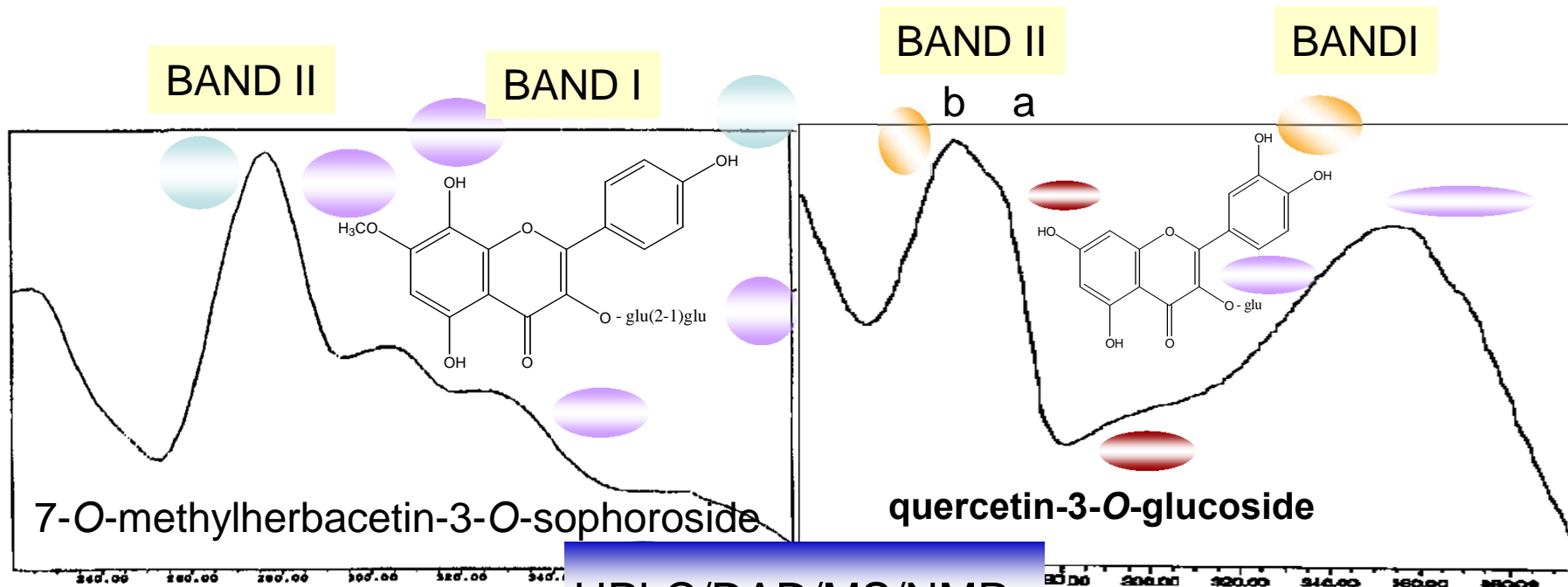
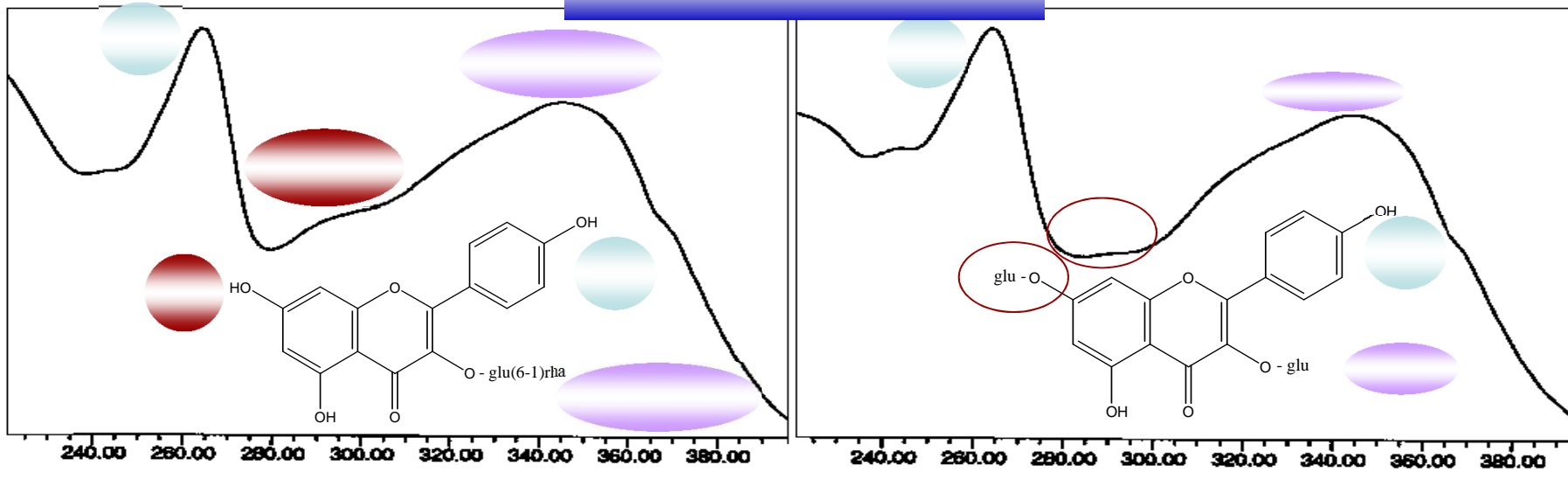


Figure 2 – Illustration of certain features of the flavonoid absorption spectrum, including the band I, IIa and IIb designations, shoulder examples including the changeover from the UV to the visible lamp.



HPLC/DAD/MS/NMR



The Unique Occurrence of the Flavone Aglycone Tricetin in Myrtaceae Pollen

Maria G. Campos^a, Rosemary F. Webby^b and Kenneth R. Markham^b

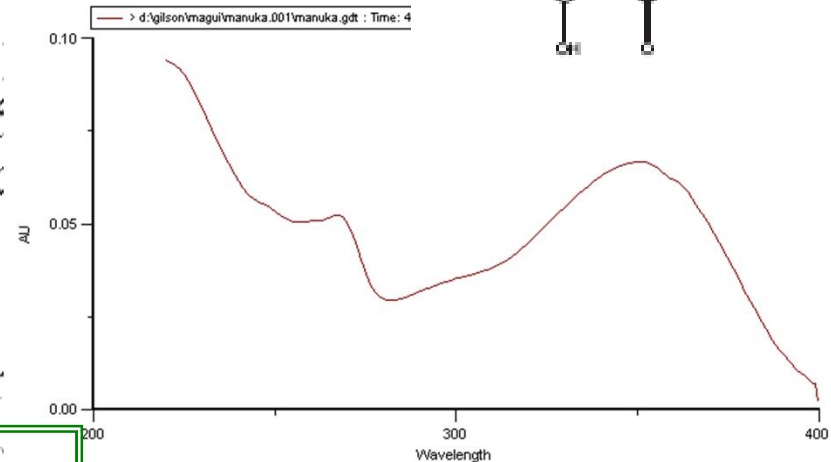
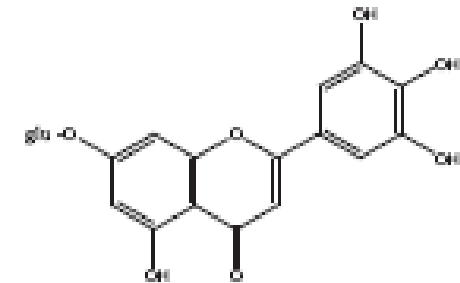
^a Laboratory of Pharmacognosy –
University of Coimbra, 3000–294
Fax: +35 1239 8271 26. Email: mgc

^b Industrial Research Ltd., P. O.
Hutt, New Zealand

* Author for correspondence and r

Z. Naturforsch **57c**, 944–946 (2002),
received June 17/July 22, 2002

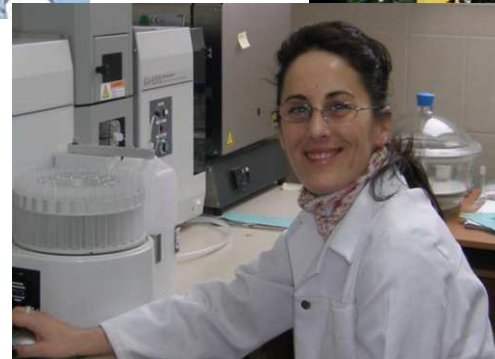
Myrtaceae Pollen, Tricetin, Flavonoid Aglycones



Rare compounds

**Portugal
New Zealand**

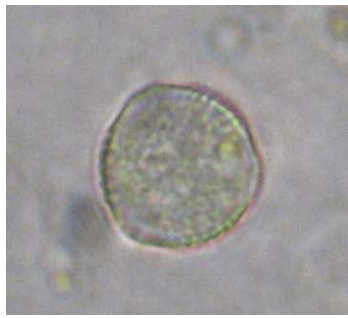
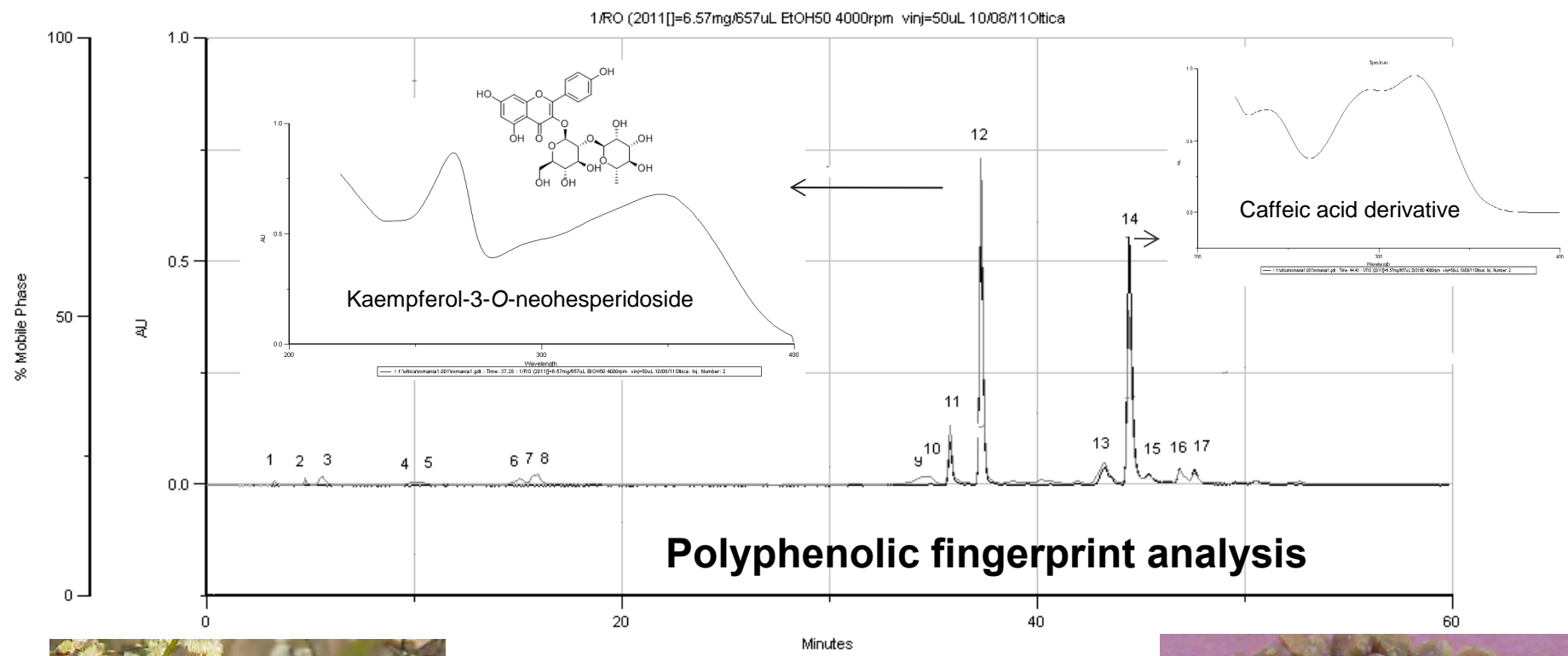
Romania



Life Sciences Institute
University of Agricultural Sciences
and Veterinary Medicine
Cluj-Napoca

Romania

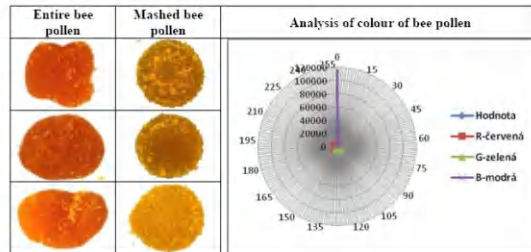
Salix sp. bee pollen



Eva Molnarova

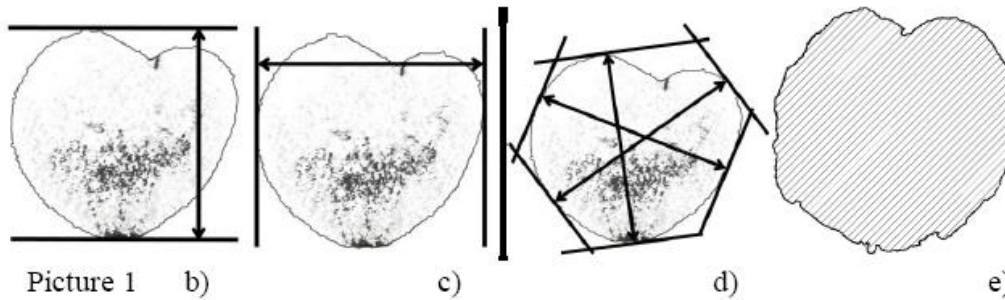
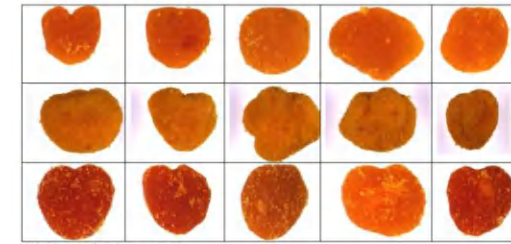
Slovakia

b) Variability in colour of bee pollen

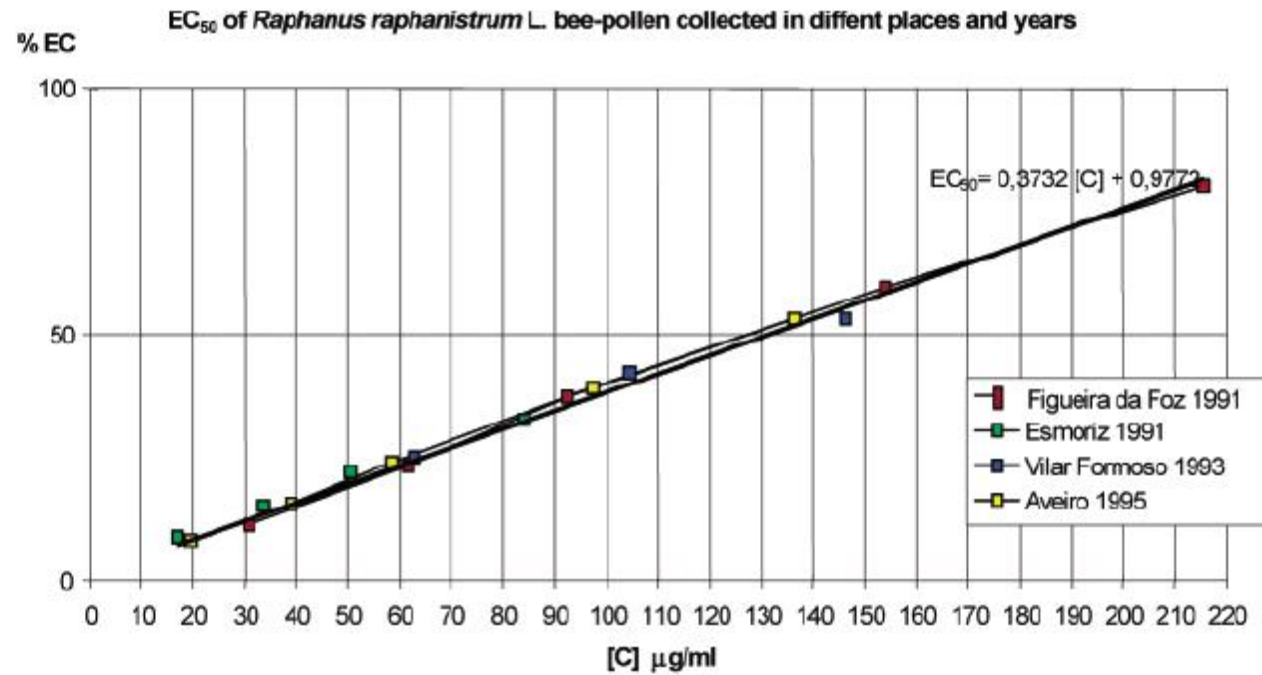


The following equipments have been used: The Macroscope Zeiss AxioVision 4.7.1. with the Software for image analysis (Zeiss AxioVision 4.7.1 Automatic Measurement Module). Digital video recordings of bee pollen have been prepared by using the fully automatic macrolupe Zeiss Discovery V12.

Picture analysis: a) Variability in shape of bee pollen



For the evaluation of these parameters, the software for image analysis (Zeiss AxioVision 4.7.1 Automatic Measurement Module) has been used. Digital video recordings of bee pollen has been prepared by using the fully automatic macrolupe Zeiss Discovery V12.





COMUNICACIONES
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**VARIABILITY OF ANTIOXIDANT ACTIVITY AMONG
HONEYBEE-COLLECTED POLLEN OF DIFFERENT
BOTANICAL ORIGIN**

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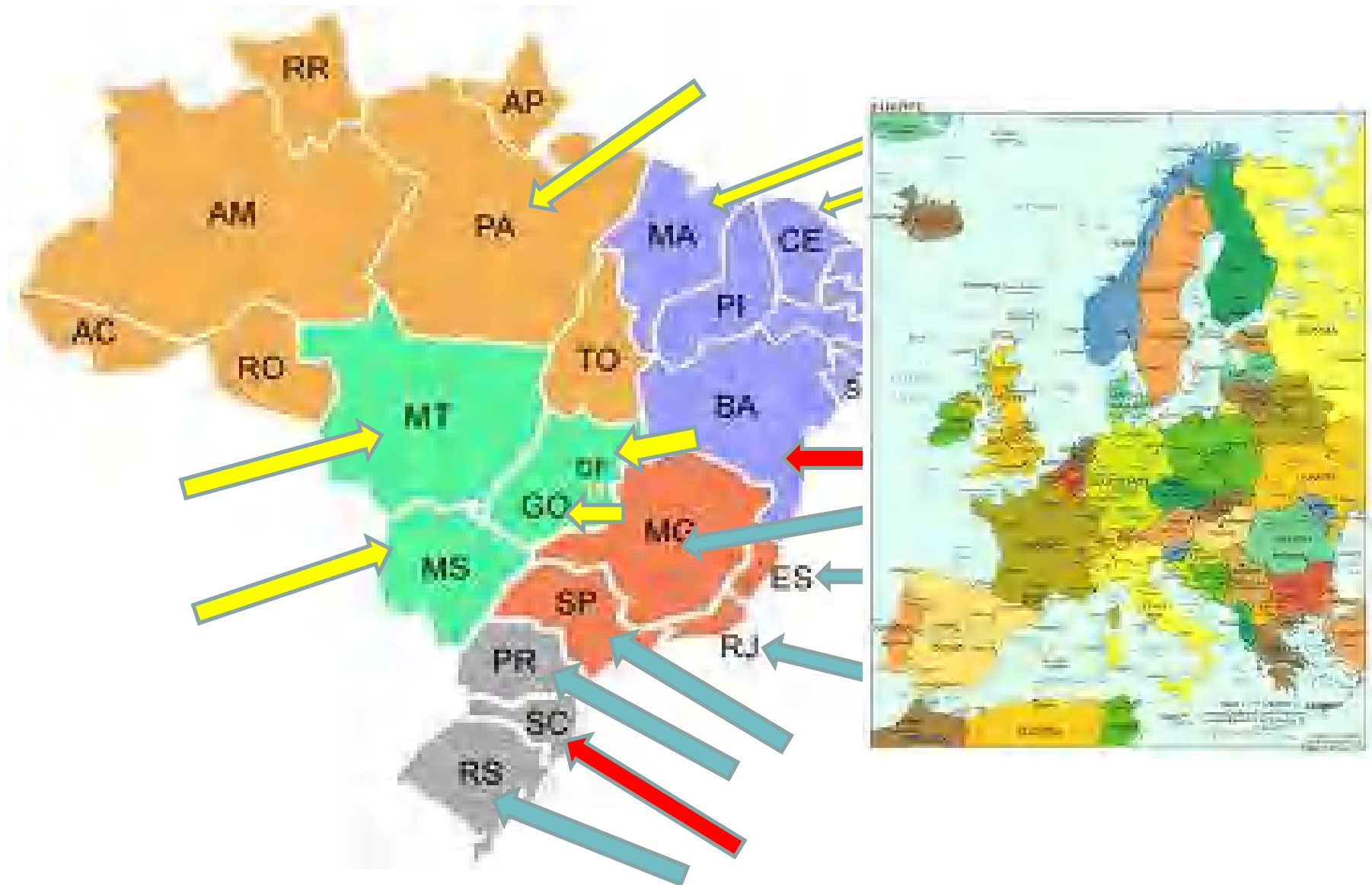


Bee pollen production in Brasil



**CENTER OF RESEARCH
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LEVANTAMENTO DE BARRETO & ORSI 2010 =
19 ESTADOS E DISTRITO FEDERAL = 20, BA+SE+SC

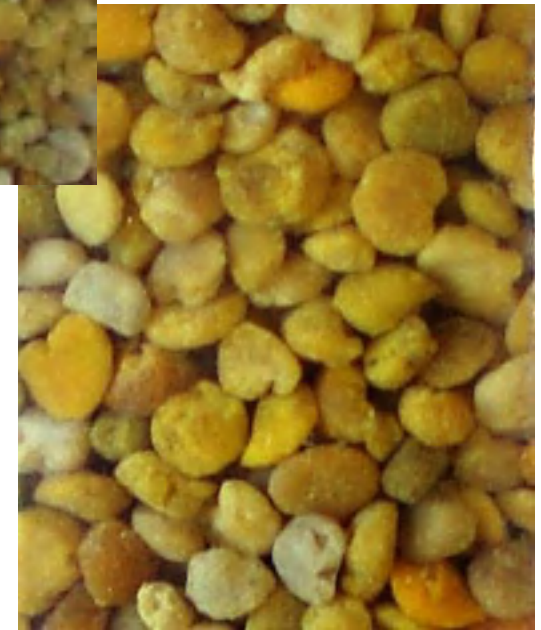




Good Practice

Bee Pollen Quality Standard Booklet

MULTIFLORAL (native flora)





Coconut Bee Pollen Identity as a Monofloral Source

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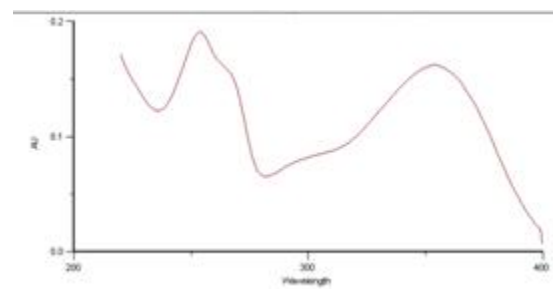
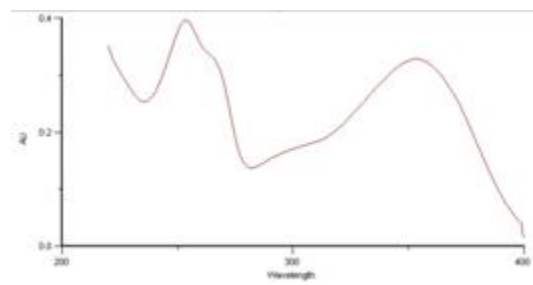
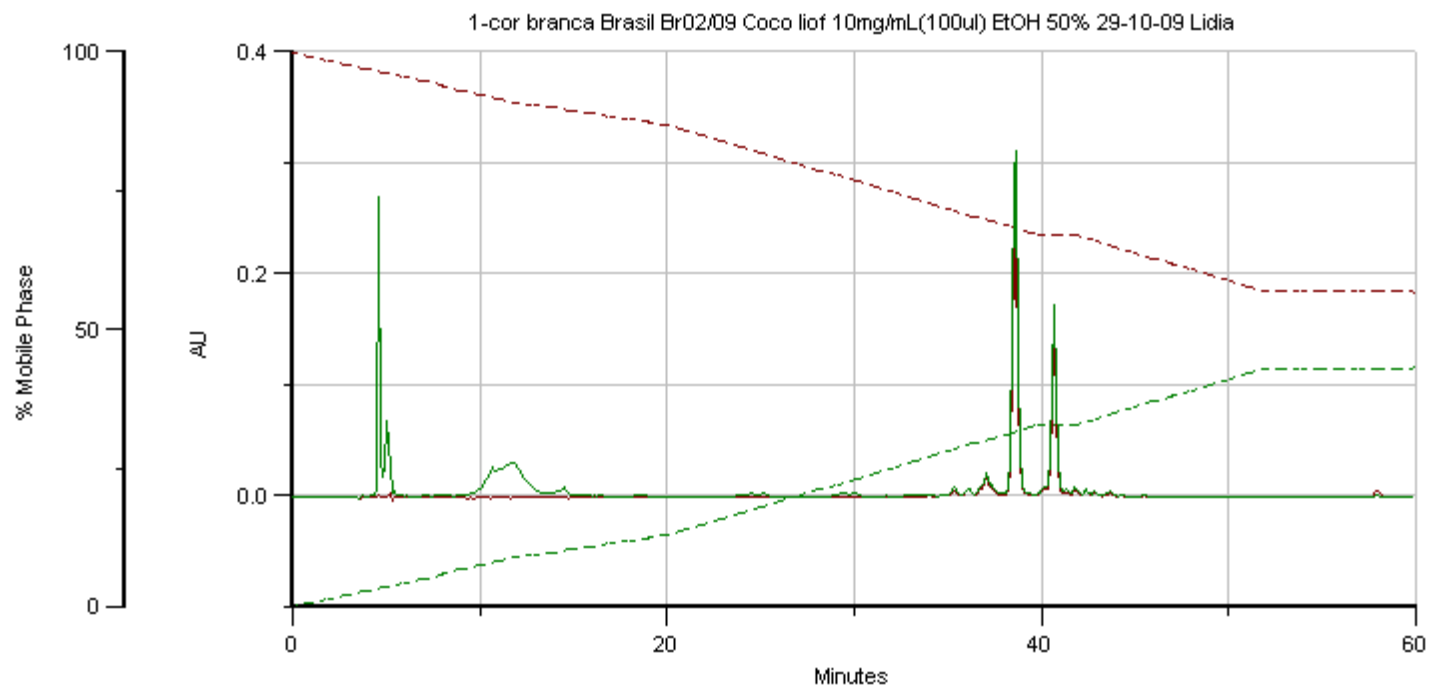
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Production area (a), Bee Pollen details (b), Drying heater (c), Packaging (d)



Bee Pollen Grains (Optic Microscopy 400x)



1 **Fisical-Chemistry map of coconut palm bee pollen produced by communities in the**
2 **coastland of the Sergipe State in Brazil**

3
4 Lídia M. Barreto ¹, Maria G. Campos ², Ângela M. Pizarro², J. S. Brito ³, M. M. Souza³,
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Table 1. Microbiological and other contaminants of bee pollen.

1.2.2. According to the flor

1.2.2.1 Monofloral bee pollen less than 80% (different taxonomic and therapeutic purposes).

1.2.2.2 Multifloral bee pollen

4. Contaminants

No organic or inorganic contaminants in quantities higher than those specified. It includes no pesticides, antibiotics (Table 1).

5. Hygiene

5.1. General Considerations

Hygiene practices related to the technical regulation of GMP (Good Manufacturing

Microbiological analysis

<i>Salmonella</i>	Absent / 10 g
<i>Staphylococcus aureus</i>	Absent / 1 g
<i>Enterobacteriaceae</i>	Max. 100/g
<i>Escherichia coli</i>	Absent./ g
Total aerobic plate count	<100 000/g
Mould and yeast	< 50 000/g
Organochlorine pesticides	< MRL*
Organophosphate pesticides	< MRL
Pyrethroids	< MRL
Alfatoxin B1	Max. 2 µg/kg
Alfatoxin B1+B2+G1+G2	Max. 4 µg/kg
Cloramphenicol (CAP)	absent
Nitrofurantoin metabolites	absent
Sulfonamides	absent
Heavy metal Pb	max 0,5 mg/kg
Heavy metal Hg	max 0,01 mg /kg
Heavy metal Cd	max 0,03 mg/kg
Radioactivity (Cs-134 and Cs-137)	<600 Bq / kg

criteria:

Significant substances except for those of natural origin from bees, wood, plants and the process of pollen by the producer. Specific tolerance criteria will be

established and correspond to the hygienic standards required for microbiological analysis and levels (Table 1).

Labels must be labelled with: denomination of the product, and, if necessary, for a specific nutrient (carbohydrates, fats) the maximum daily intake limit for consumption, and the maximum daily intake. The producer will need

	Mean ± DP
Moisture %	5.05±1.54
Ash%	3.35±0.77
Proteins %	14.64±3.67
Total sugars %	31.82±2.25
Fiber %	4.21±0.35
Free acidity mEq	246.50±41.24
pH	4.46±0.28
Lipids %	3.82±2.10
Water activity	0.32±0.01
Vitamin E mcg/g	6.05±0.37
Total Phenolics %	0.23±0.02
Total Flavonoids mg/g	4.64±0.07

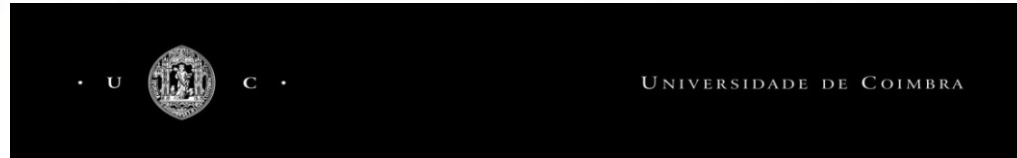
Main components	Content Min – Max g/100g dry weight
Proteins	10-40
Lipids	1-13
total carbohydrates*	13-55
Dietary fibre, pectin	0,3-20
Ash	2-6
undetermined	2-5



University of Coimbra/Faculty of Pharmacy
Health Sciences Campus - Portugal



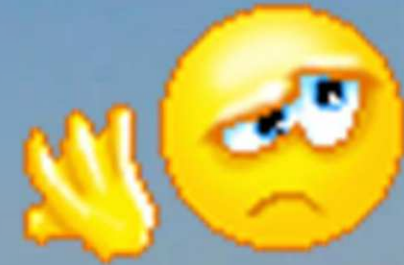
Future Challenges



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Thanks to All my Coleagues
that work with Bee Pollen



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