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Honeydew honey from *Quercus* sp. is increasingly regarded as one of the most valuable honey varieties within the beekeeping industry, and consumers highly accept it. This honey is characterized by a unique botanical origin, as it can be derived from extrafloral secretions exuded by the acorns of *Quercus pyrenaica* or/and by exudates of phloem-feeding insects that extract sap from living plant tissues. Recent climatic trends, particularly the prevalence of prolonged and arid summers, have created an impact on honeydew production. To prevent adulteration and fraudulent practices and to enhance market value, it is crucial to establish reliable authentication parameters and correlate them with botanical sources. This work aimed to monitor the production of honeydew honey from black oak and to assess its specificities, thereby enhancing its commercial value. For the study, four experimental apiaries were established, each consisting of 10 colonies, located in black oak forest areas within the Montesinho Natural Park, in the northern of Portugal. Honey production was monitored over three consecutive years, from 2021 to 2023, with samples harvested in September and characterized through melissopalynology, physicochemical parameters, sugars, minerals, phenolic compounds, and volatile profile. The melissopalynological analysis revealed a high diversity of pollen, identifying a total of 53 pollen types belonging to 33 families, with *Castanea sativa* and *Rubus* sp. being the most predominant. The physicochemical parameters aligned with those characteristic of honeydew honeys. Regarding sugar composition, the detection of erlose and melezitose confirmed the presence of honeydew components. The phenolic profile characterization identified 17 compounds, with flavonoids being the most prevalent, followed by phenolic acids. The most abundant volatiles were 1-nonanol, phenyl-acetaldehyde, and benzaldehyde. The characterization of *Quercus pyrenaica* honeydew honey was essential for its market valorization, as it highlights its unique properties and quality, increasing consumer demand and product differentiation. This, in turn, will provide a new source of income for beekeepers in black oak forest areas, promoting sustainable apiculture and supporting local economies.

**Acknowledgments:** Thanks to national funding, FCT, through the project ACORNEW (MTS/SAS/0099/2020) and the institutional scientific employment program-contract with Soraia I. Falcão.

# *Quercus pyrenaica* honeydew honey: production and characterization towards botanical origin assessment and valorization

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IMPULSO À SUSTENTABILIDADE  
DA FLORESTA DE CARVALHOS  
DO **PARQUE NATURAL  
DE MONTESINHO**  
ATRAVÉS DA INOVAÇÃO:  
**VALORIZAÇÃO  
DA BOLOTA E DA MELADA  
DO CARVALHO NEGRAL**



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# *Quercus pyrenaica* honeydew honey valorization

- Organoleptic characteristics
- Higher nutritional value
- Higher therapeutic value
- Higher interest from food industry



- Less studied honeys
- Complex differentiation (Geographical, botanical, and climatic conditions)
- Different origins (plants vs insects)
- Honeydew honey production

Higher  
quality

Consumer  
higher  
demand

Higher  
comercial  
value



# Honeydew honey: origin

## Insects



The exudates on the green parts result from the feeding of sucking insects, which transform the phloem sap and excrete honeydew.



*Aphids; Eulecanium tiliae,  
Aspidiotus nerii,  
Chrysomphalus dictyospermi  
Diaspidiotus zonatus*



## Plants



Honeydew comes from sugary exudates that bees collect from the green parts of plants.



Leaves, stems, or more often from developing fruits.



In certain regions, the production of honeydew is due to extrafloral secretions of acorns during their formation. The bees collect abundant amounts of this raw material, which is then deposited in the hives as dark honey.



Pines, firs, and oaks



Honeydew production may vary depending on weather conditions and tree species



# Honeydew honey: production

## 🐝 Climatic trend ► Long and dry Summers

(Rainy springs and hot summers favor production)

## 🐝 Distribution:

It is conditioned by the existence of a dry summer period.

It is distributed throughout the Mediterranean area of the Iberian Peninsula.

Production regions: mountainous areas (600 to 1800 m) with a certain level of humidity

## 🐝 August

- 🐝 Brown exudate during acorn formation
- 🐝 Thick discharge with bubbling formation
- 🐝 It accumulates on the edges of the acorn cap

**Quercus revelance in  
beekeeping  
production**



**Higher interest  
in the honeydew  
honey  
production**



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## How can we differentiate between nectar and honeydew honeys?

- Honey/Honeydew composition
- Quality parameters

**Discrimination between honeydew honey and nectar honey is extremely important to avoid fraud and adulteration.**

**Market valorization**

**Avoid adulteration and frauds**

Reliable parameters used for authentication of honeydew honey

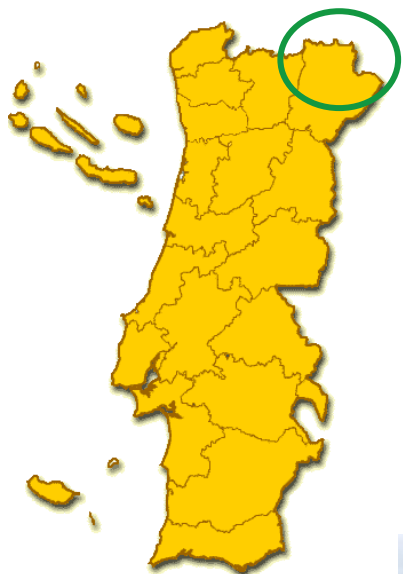
- Physicochemical parameters
- Melissopalynological analysis
- Phenolic compounds
- Antioxidant properties
- Production monitoring



# ACORNDEW project



## 4 Experimental apiaries



|                   | 2021 | 2022 | 2023 |
|-------------------|------|------|------|
| Paço: 8           |      | 7    | 6    |
| Espinhosela: 10   |      | 9    | 7    |
| Ciradilha: 9      |      | 9    | 7    |
| Rio de fornos: 10 |      | 10   | 8    |

Total  
37  
samples

Total  
35  
samples

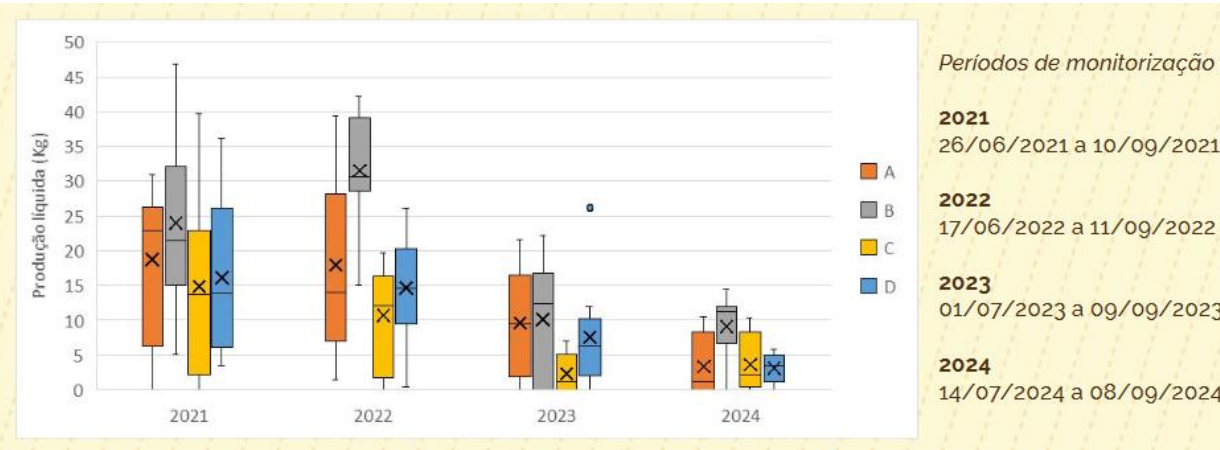
Total  
28  
samples



The honey harvest was carried out in  
September  
1 Kg sample for each beehive

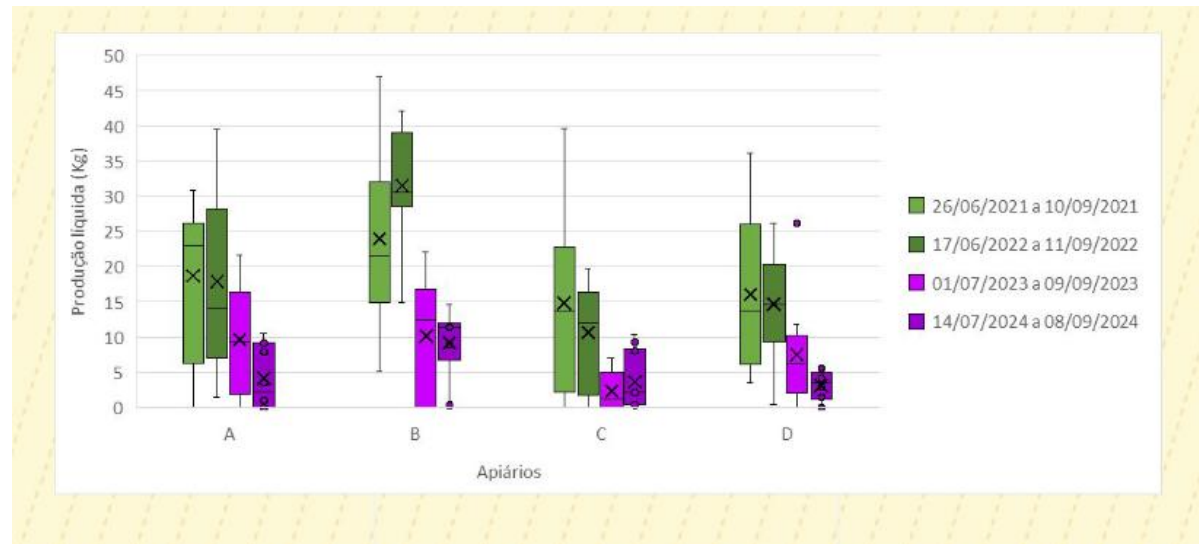


# Honeydew honey production



**Weight measurements**

**Differences between year**



**Differences between apiaries**

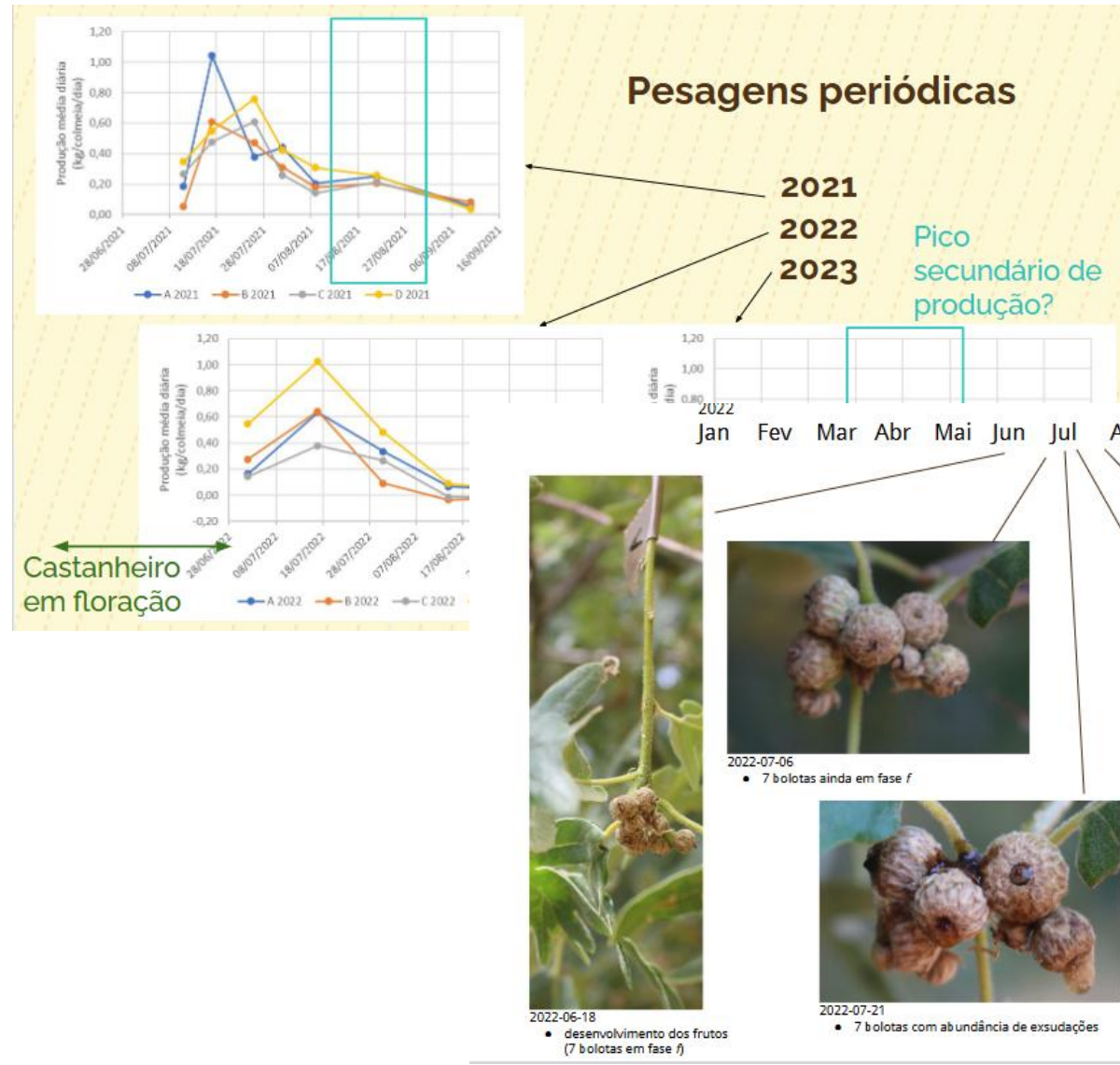


# Honeydew honey production



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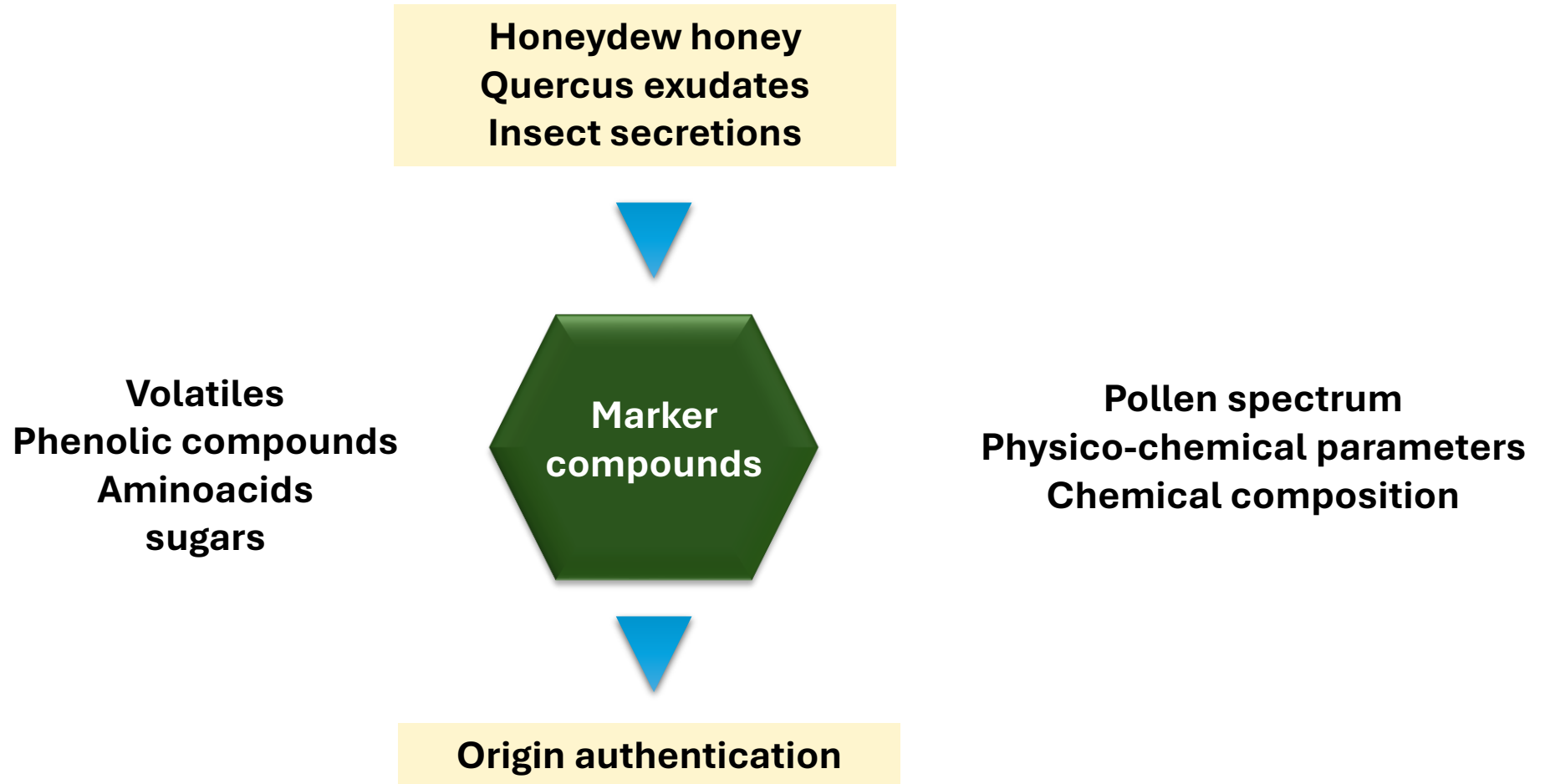
## Weight measurements



# Chemical characterization towards origin discrimination



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# Physicochemical parameters

➤ Dark ambar colour

➤ Humidity: 14.8 – 17 %; < 20%

➤ Electrical conductivity: 1.00-1.43;

Nectar honey: < 0,80 mS.cm<sup>-1</sup>

Honeydew honey: > 0,80 mS.cm<sup>-1</sup>

➤ Ash content: 0.45-0.74g/100g; < 1,2g/100g

➤ Humidity: 14.8 – 17 %; < 20%

➤ pH: 3.73-4.94; free acidity: 0.19-1.03 meq/Kg (pH 7) and 0.325-1.28 meq/Kg (pH 8.3)

➤ HMF: 0-24 mg/Kg; < 40 mg.kg<sup>-1</sup>

➤ Diastase: 9.01-32.67 DN; ≥ 8 DN

➤ Proline: 0.9-8.1 mg/g; ≥ 0,18 mg/g

## Sugars

| Apiary          | Fructose | Glucose | Turanose | Maltulose | Isomaltose | Trealose | Melibiose | Erlose | Melezitose |
|-----------------|----------|---------|----------|-----------|------------|----------|-----------|--------|------------|
| Paço            | 35,2     | 26,5    | 2,3      | 3,4       | 3,3        | 1,0      | 2,2       | 0,4    | 0,6        |
| Espinhosela     | 36,3     | 27,9    | 2,0      | 3,2       | 3,4        | 1,0      | 2,1       | 0,4    | 1,1        |
| Ciradilha       | 35,4     | 27,8    | 2,5      | 3,8       | 4,6        | 1,2      | 2,2       | 0,5    | 0,9        |
| Rio de Fornos   | 37,1     | 29,2    | 2,7      | 4,5       | 6,2        | 1,4      | 2,4       | 0,7    | 0,8        |
| Barreiros       | 40,6     | 29,2    | 2,5      | 4,7       | 6,1        | 1,1      | 2,5       | 0,6    | 0,4        |
| Lamedo          | 38,4     | 28,0    | 2,2      | 4,6       | 5,4        | 1,1      | 2,3       | 0,7    | 0,7        |
| Fonte indevacas | 38,1     | 28,5    | 2,2      | 4,3       | 5,3        | 1,2      | 2,1       | 0,6    | 0,5        |
| Fonte Indueiro  | 38,6     | 28,2    | 2,3      | 4,3       | 5,8        | 1,3      | 2,3       | 0,6    | 0,4        |
| Labanho cima    | 37,6     | 29,8    | 2,4      | 4,3       | 5,5        | 1,2      | 2,2       | 0,7    | 0,7        |

Erlose and melezitose are markers for honeydew honey

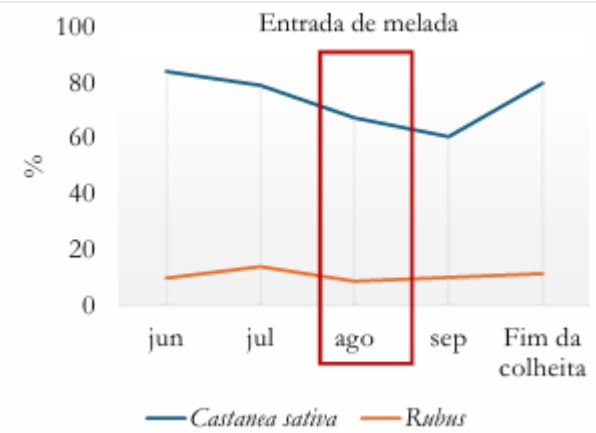


# Honeydew honey characterization

## Melissopalynological analysis

- 142 pollen types; 61 families
- Few pollen grains
- Does not cristallize

Asteraceae, Fabaceae, Ericaceae, Rosaceae, Cistaceae, Cruciferae e Labiatae

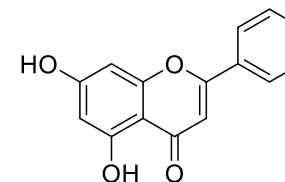
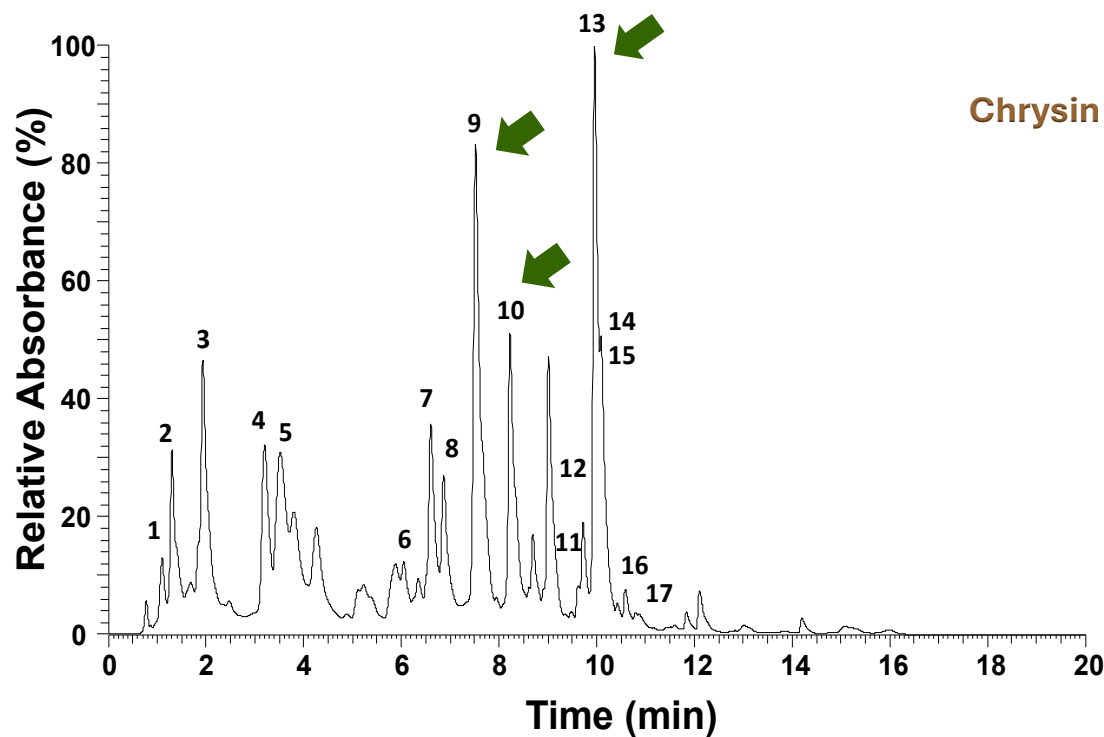


| Family         | Pollen type                    | % Presence | P (0-1) | R (1-3) | I (3-15) | A (15-45) | D >45 |
|----------------|--------------------------------|------------|---------|---------|----------|-----------|-------|
| Fagaceae       | <i>Castanea</i>                | 100,0      | 0,0     | 0,0     | 1,1      | 3,2       | 95,7  |
| Rosaceae       | <i>Rubus</i>                   | 100,0      | 5,3     | 15,0    | 57,8     | 20,9      | 1,1   |
| Fabaceae       | <i>Cytisus t.</i>              | 64,2       | 48,1    | 13,9    | 2,1      | 0,0       | 0,0   |
| Poaceae        | <i>Poaceae</i>                 | 63,6       | 44,9    | 13,9    | 4,8      | 0,0       | 0,0   |
| Boraginaceae   | <i>Echium</i>                  | 59,9       | 42,8    | 15,5    | 1,1      | 0,5       | 0,0   |
| Plantaginaceae | <i>Plantago</i>                | 59,4       | 34,8    | 15,0    | 8,0      | 0,5       | 1,1   |
| Asteraceae     | <i>Taraxacum officinale t.</i> | 53,5       | 35,3    | 11,8    | 6,4      | 0,0       | 0,0   |
| Cistaceae      | <i>Cistus</i>                  | 43,9       | 38,5    | 4,3     | 1,1      | 0,0       | 0,0   |
| Apiaceae       | <i>Foeniculum t.</i>           | 33,7       | 19,3    | 9,1     | 4,8      | 0,5       | 0,0   |
| Resedaceae     | <i>Sesamoides</i>              | 30,5       | 18,2    | 5,3     | 6,4      | 0,5       | 0,0   |
| Fabaceae       | <i>Trifolium t.</i>            | 24,6       | 19,8    | 2,7     | 1,6      | 0,5       | 0,0   |
| Ericaceae      | <i>Ericaceae</i>               | 20,9       | 20,3    | 0,5     | 0,0      | 0,0       | 0,0   |
| Labiatae       | <i>Thymus</i>                  | 20,9       | 15,0    | 3,2     | 1,1      | 1,6       | 0,0   |
| Campanulaceae  | <i>Campanula t.</i>            | 17,1       | 17,1    | 0,0     | 0,0      | 0,0       | 0,0   |
| Thymelaeaceae  | <i>Dafne</i>                   | 17,1       | 13,4    | 2,7     | 0,5      | 0,5       | 0,0   |

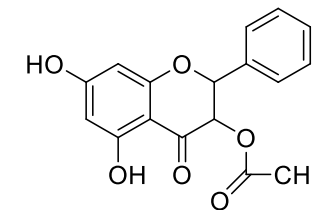


# Phenolic compounds composition

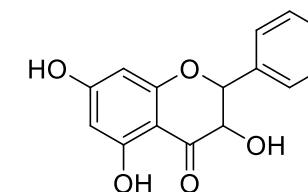
- (1) Gallic acid
- (2) Protocatechuic acid
- (3) Caffeic acid
- (4) *p*-Coumaric acid
- (5) Ellagic acid
- (6) Salycilic acid
- (7) Camphoric acid
- (8) *cis, trans*-Absciscic acid
- (9) Pinobanksin-5-methyl-ether
- (10) Pinobanksin
- (11) Quercetin-dimethyl-ether
- (12) Caffeic acid isoprenyl ester
- (13) Chrysin
- (14) Pinocembrin
- (15) Galangin
- (16) Acacetin
- (17) Caffeic acid cinnamyl ester



Chrysin



Pinobanksin-5-methyl-ether

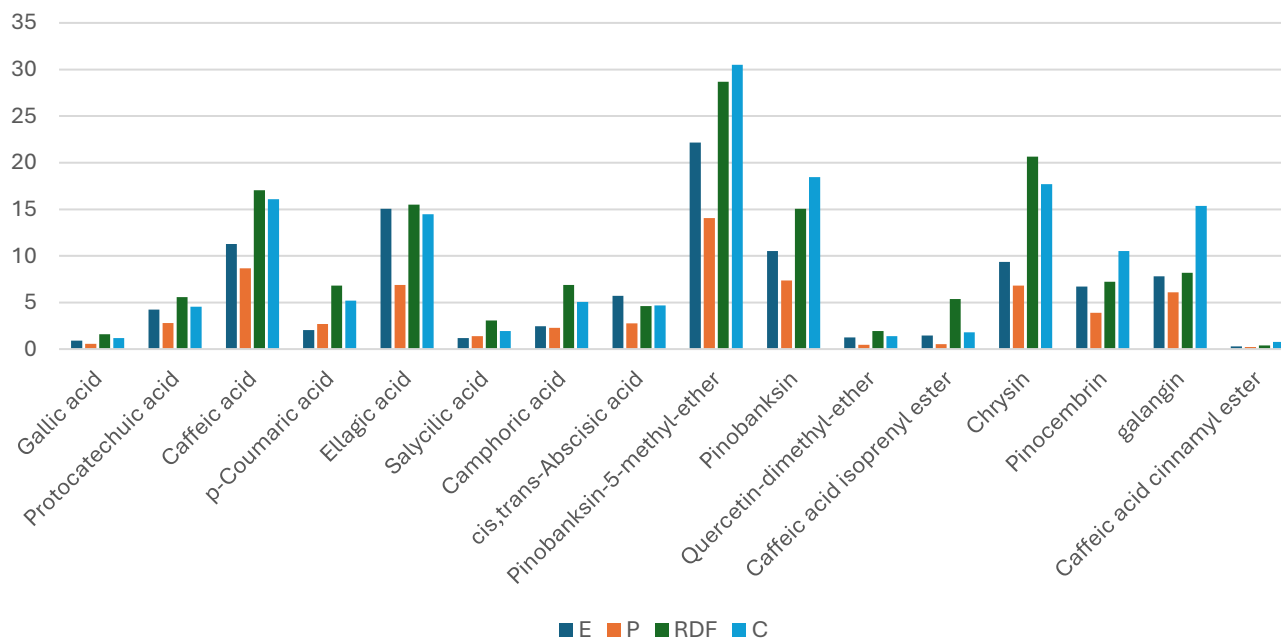


Pinobanksin



# Phenolic composition and antioxidant activity

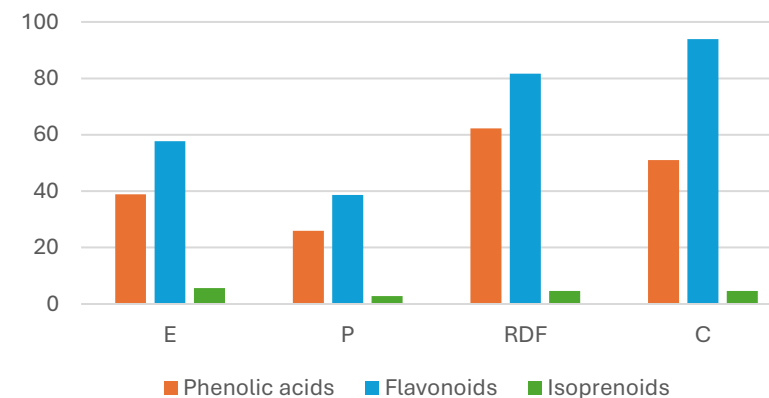
Phenolic compounds (mg/Kg honey)



Total phenolic compounds (mg/Kg honey)



Phenolic compounds (mg/Kg honey)



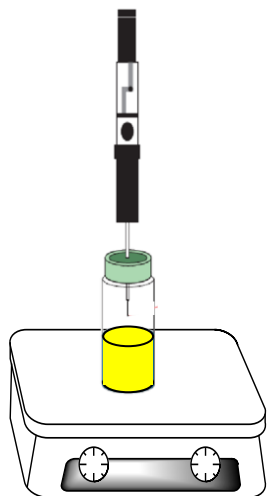


# Volatile profile

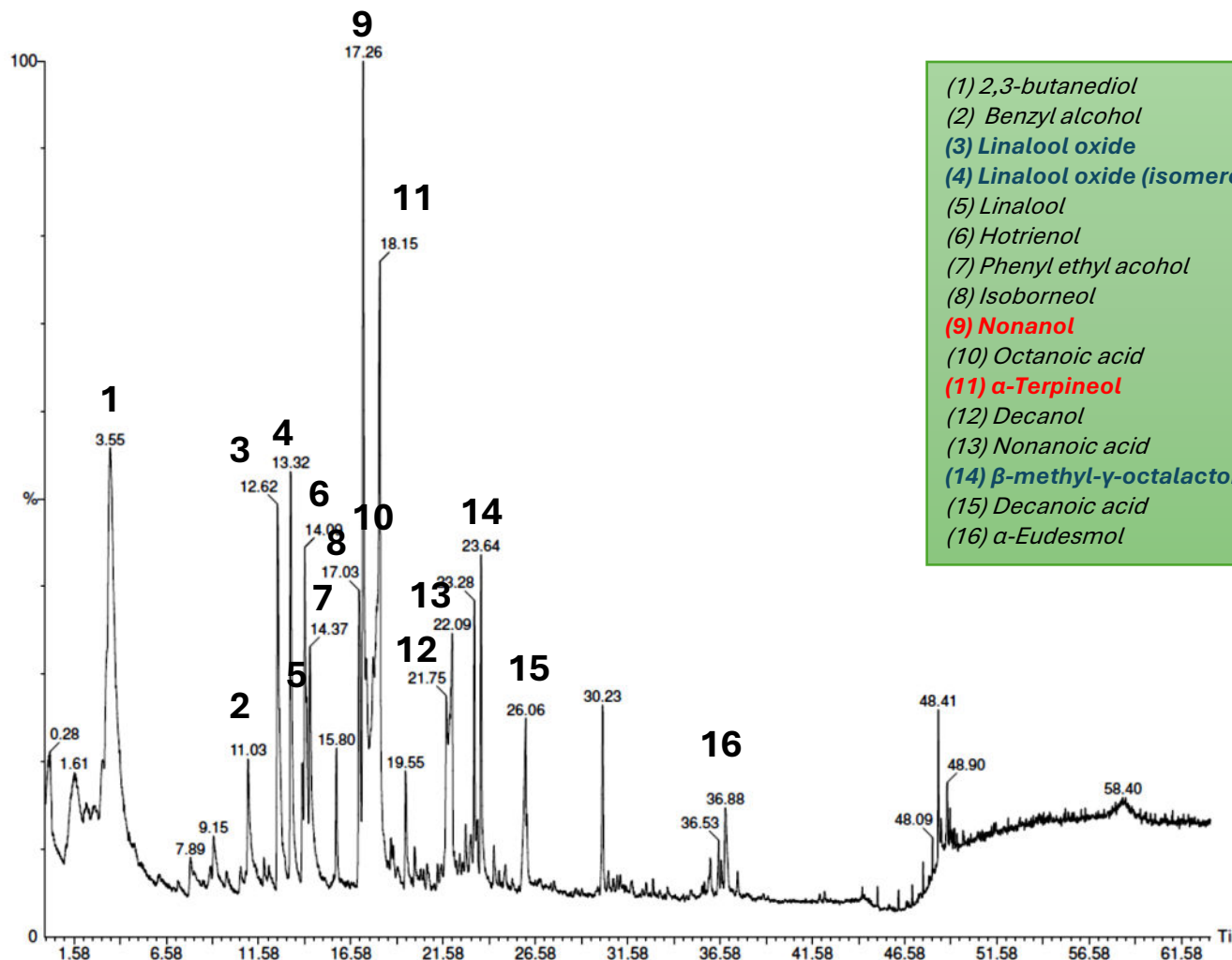


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HS-SPME



GC-MS



- (1) 2,3-butanediol
- (2) Benzyl alcohol
- (3) Linalool oxide
- (4) Linalool oxide (isomero)
- (5) Linalool
- (6) Hotrienol
- (7) Phenyl ethyl alcohol
- (8) Isoborneol
- (9) Nonanol
- (10) Octanoic acid
- (11)  $\alpha$ -Terpineol
- (12) Decanol
- (13) Nonanoic acid
- (14)  $\beta$ -methyl- $\gamma$ -octalactone
- (15) Decanoic acid
- (16)  $\alpha$ -Eudesmol



# Thank you for the attention!



[www.isa.ulisboa.pt/proj/acorndew](http://www.isa.ulisboa.pt/proj/acorndew)



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