Studies for Italian Propolis Characterization

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6TH APIMEDICA & 5TH APIQUALITY

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Different plants Different «propolis types»

Poplar: *Populus spp.*
(Europe)

Green: *Baccharis spp.*
(Brazil)

Different plants Different «propolis types»

Red: *Dalbergia spp.* (Brazil, Mexico, Cuba)

Clusia: *Clusia spp.* (Cuba, Venezuela)

Pacific: *Macaranga tanarius* (Indonesia, Taiwan, Okinawa)

Mediterranean:
Cupressaceae
(Greece, Sicily, Malta)

Propolis gathering

50-100 grams per year

Uses of propolis by bees

- to create an aseptic environment
- to smooth out and coat the internal walls and parts of the beehive
- to make the entrance of the hive weathertight
- to build a protective barrier against the enemies narrowing the entrance
- to seal cracks and crevices
- to strengthen the thin borders of combs
- to embalm the carcasses of dead hive invaders

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Uses of propolis
for human health purposes
properties and activities

- Antibacterial properties
- Antifungal properties
- Antiviral properties

- Anti-inflammatory
- Antioxidant
- Antiulcer
- Hepatoprotective
- Cytotoxic
- Immunostimulating
- …

Uses of propolis
for human health purposes
uses

- Dermatology
- Otorhinolaryngology
- Dentistry
- Gastroenterology
- Gynaecology
- Oncology

- Cosmetics

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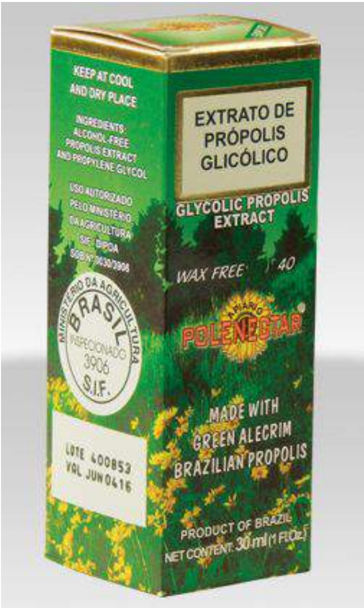
Uses of propolis
for human health purposes
uses

- Dietary supplement
  - Hydroalcoholic solution
  - Glycolic solution
  - Tablets
  - …



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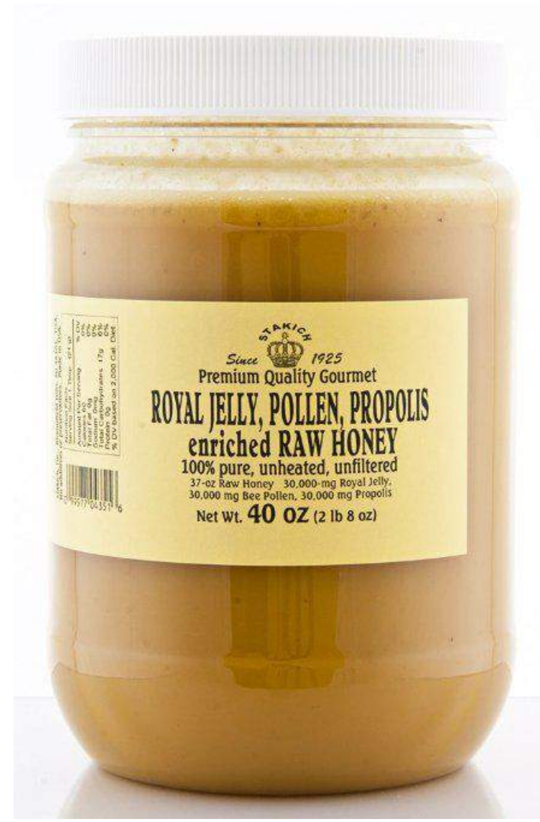
Uses of propolis
as food

- Candies
- As a food ingredient



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Increasing interest in propolis

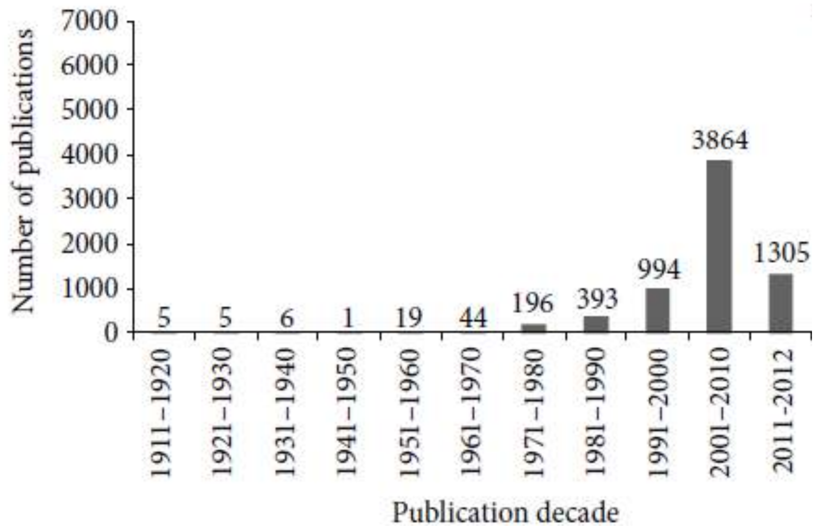

Figure 2: Scientific productivity on propolis between the decades (*Chemical Abstracts*).

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Volume 2013, Article ID 697390, 13 pages
http://dx.doi.org/10.1155/2013/697390

Review Article

Recent Progress of Propolis for Its Biological and Chemical Compositions and Its Botanical Origin

Viviane Cristina Toreti, Helia Harumi Sato, Glaucia Maria Pastore, and Yong Kun Park

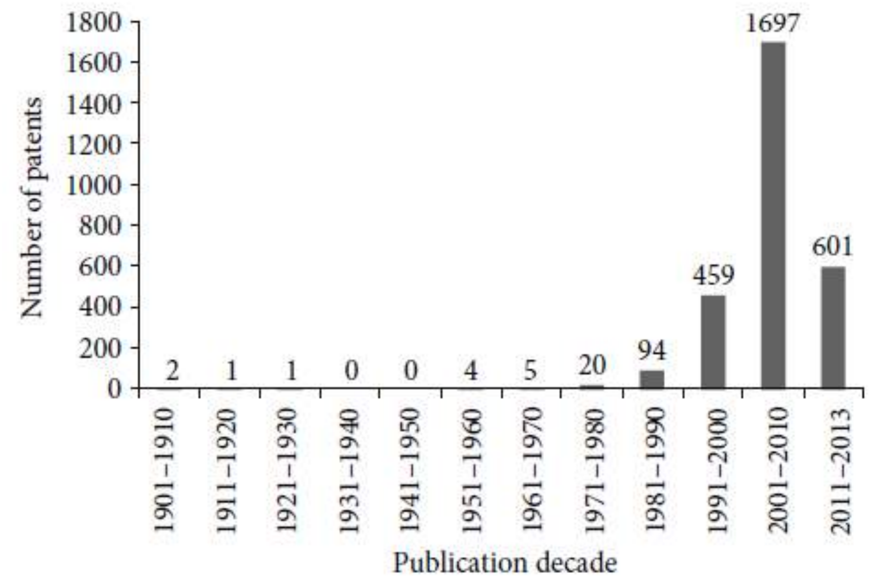
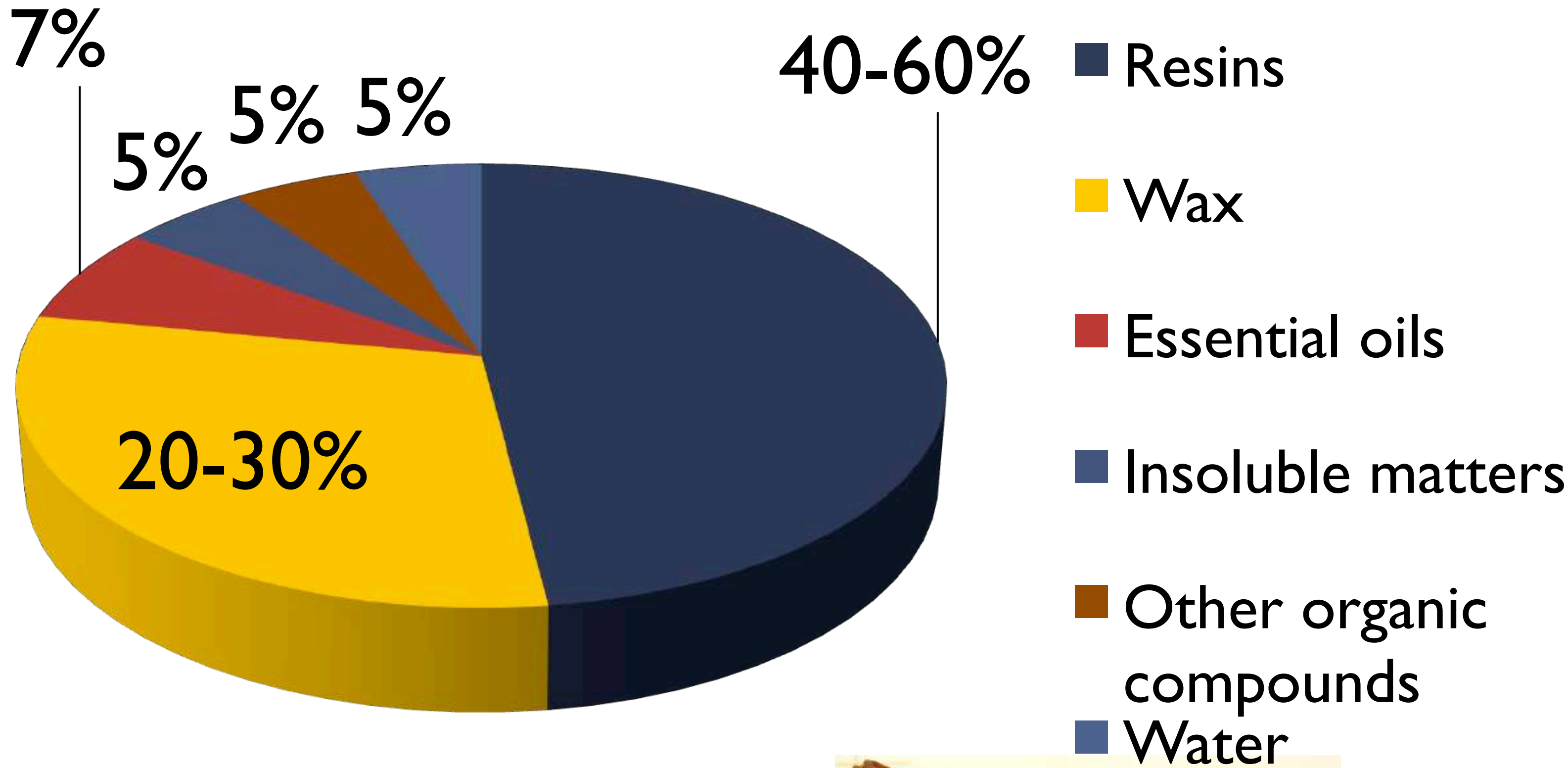


Figure 4: Scientific production on propolis by patents (*Chemical Abstracts*).

Propolis composition



Propolis standardization

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Standard methods for Apis mellifera propolis research

Vassya Bankova, Davide Bertelli, Renata Borba, Bruno José Conti, Ildenize Barbosa da Silva Cunha, Carolina Danert, Marcos Nogueira Eberlin, Soraia I Falcão, María Inés Isla, María Inés Nieva Moreno, Giulia Papotti, Milena Popova, Karina Basso Santiago, Ana Salas, Alexandra Christine Helena Frankland Sawaya, Nicolas Vilczaki Schwab, José Maurício Sforcin, Michael Simone-Finstrom, Marla Spivak, Boryana Trusheva, Miguel Vilas-Boas, Michael Wilson & Catiana Zampini

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Propolis composition

Propolis type	Geographic origin	Plant source	Main constituents
Poplar	Europe, North America, non-tropic regions of Asia, New Zealand	*Populus* spp. (*Aigeiros, P. nigra L.*)	Flavones, Flavonones, cinnamic acids and their esters
Green	Brazil	*Baccharis dracunculifolia*	Prenylated, p-coumaric acid, diterpenic acids
Birch	Russia	*Betula verrucosa*	Flavones, flavonols
Red	Brazil, Cuba, Mexico	*Dalbergia ecastophyllum*	Isoflavonoids (isoflavans and pterocarpans)
Mediterranean	Sicily, Greece, Malta, Crete	Cupressaceae	Diterpenes (mainly acids of labdane type)
Clusia	Cuba, Venezuela	*Clusia* spp.	Polyprenylated and benzophenones
Pacific	Okinawa, Taiwan, Indonesia	*Macaranga tanarius*	C-prenil-flavonones

Survey on Italian propolis

The aim was to:

- assess the main physical-chemical characteristics and the active components that characterize Italian propolis
- evaluate whether, as reported by the studies done up to today, Italian propolis belongs to the group **"Poplar type"**
- Expand the database for the characteristics of the Italian production for classification models of propolis types.

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Survey on Italian propolis

Bankova V. *et* al., 2002
3 Italian samples (10 total)

Popova M. *et* al., 2007
26 Italian samples (114 total)

Gardana C. *et* al., 2007
9 Italian samples (107 total)

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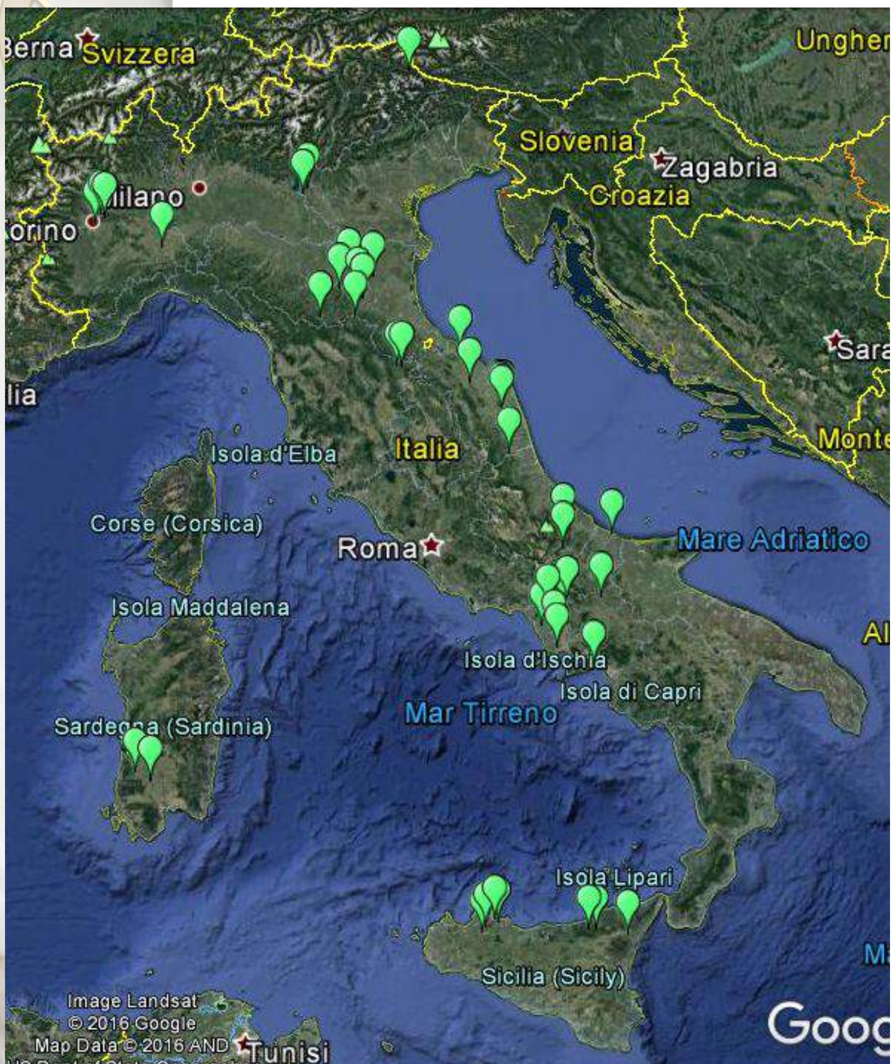
Survey on Italian propolis



2013

43 samples

from all over the country, islands included

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Survey on Italian propolis

Samples were assessed for their sensory characteristics:

colour, texture and odour

Survey on Italian propolis

Wax, resin and mechanical impurities evaluation

Determined according to the ***Norma*** IRAM-***INTA***, 2004 (Soxhlet)

	Wax g/100g	Resin g/100g	Mechanical impurities g/100g
Mean	30,7	57,9	11,3
Min	10,0	19,9	4,3
Max	82,2	82,8	26,5

Survey on Italian propolis

Phenolic compounds evaluation

Total phenolics content: Folin-Ciocalteu method
Reference gallic acid (IRAM-INTA method)

Flavone and flavonol content: $AlCl_3$ coloration
Reference quercetin (IRAM-INTA method)

Flavanones and dihydroflavonols content:
2,4-dinitrophenylhydrazine coloration
Reference pinocembrin (Popova et al. , 2004)

Survey on Italian propolis

Phenolic compounds evaluation

Characteristic (%)	Mean (n=38)	Mean (Popova 2007)	Min. – Max (n=38)	Min. - Max (Popova 2007)
Resins	57.9 ± 16.5	57	19.9-82.8	18 – 82
Phenolics	22.5 ± 4.5	28	10.8- 29.5	7.9 – 46
Flavones and flavonols	7.0 ± 2.9	8	1.1- 15.0	1.3 – 17.9
Flavanones and dihydroflavonols	7.5 ± 3.0	6	1.2 – 11.9	1.5 – 15.2

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Survey on Italian propolis

Phenolic compounds evaluation

Table 5. Specific criteria and standard values for the content of bioactive constituents in propolis.

Propolis type		Minimum % by weight in raw propolis	Reference
Poplar propolis	Total phenolics	21	(Popova et al., 2004)
	Total flavones and flavonols	4	(Popova et al., 2004)
	Total flavanones and dihydroflavonols	4	(Popova et al., 2004)
Brazilian green propolis	Total phenolics	5	(Sawaya et al., 2011)
	Total flavonoids	0.5	(Sawaya et al., 2011)

Standard methods for Apis mellifera propolis research

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Survey on Italian propolis

DPPH· Free Radical-Scavenging Activity

The scavenging activity of DPPH• was assayed according to the method of Brand-Williams et al.

Desoxyribose assay metodo Halliwell et al.

Reducing Power

The reducing power (RP) was determined according to the method described by Oyaizu.

Chelating activity metodo Dinis et al.

Survey on Italian propolis

DPPH• Free Radical-Scavenging Activity (TEs/g)	Desoxyribose assay (K)	Reducing Power (TEs/g)	Chelating activity (%)
2,63 ± 0,7	1,06 ± 0,2	0,74 ± 0,2	24,67 ± 4,8

Survey on Italian propolis

Phenolic evaluation by LC-MS/MS

Quercetin (µg/ml)	Sakuranetin (µg/ml)	Kaempferide (µg/ml)	Pinostrobin (µg/ml)	Apigenin (µg/ml)
58.7± 29.0	67.9 ± 43.5	13.6 ± 9.8	134.8 ± 97.1	43.3 ± 24.6

Kaempferol (µg/ml)	Chrysin (µg/ml)	Acacetin (µg/ml)	Pinocembrin (µg/ml)	Galangin (µg/ml)
59.7 ± 26.7	5390.1 ± 2432.5	4.7 ± 2.8	2725.0 ± 2085.8	3137.7 ± 1517.3

p-Coumaric acid (µg/ml)	Caffeic acid (µg/ml)	trans-Cinnamic (µg/ml)	Ferulic acid (µg/ml)	Isoferulic acid (µg/ml)
26.1 ± 28,8	62.6 ± 45.7	81.0 ± 72.5	689.0±1127.9	1331.3±2288.2

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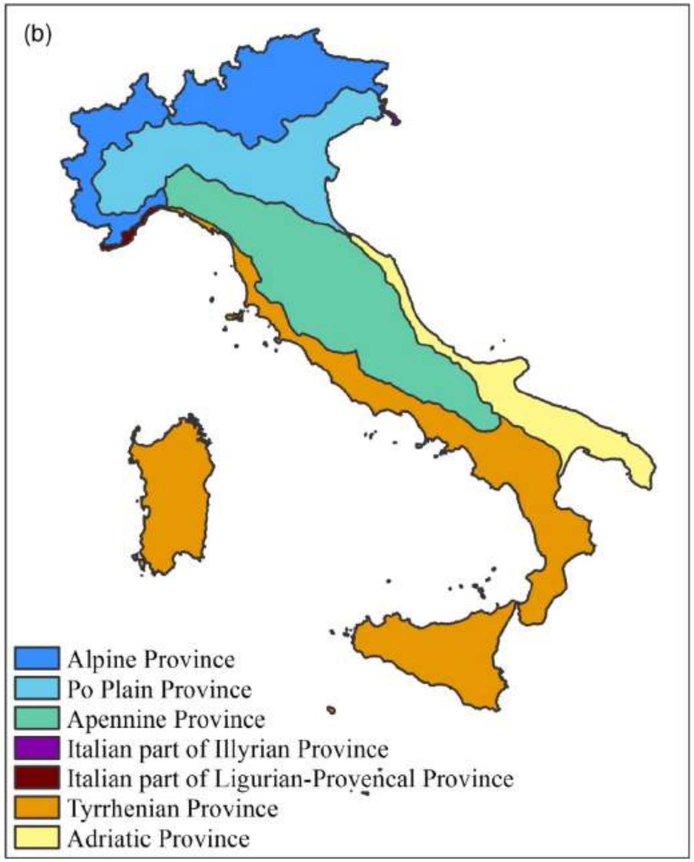
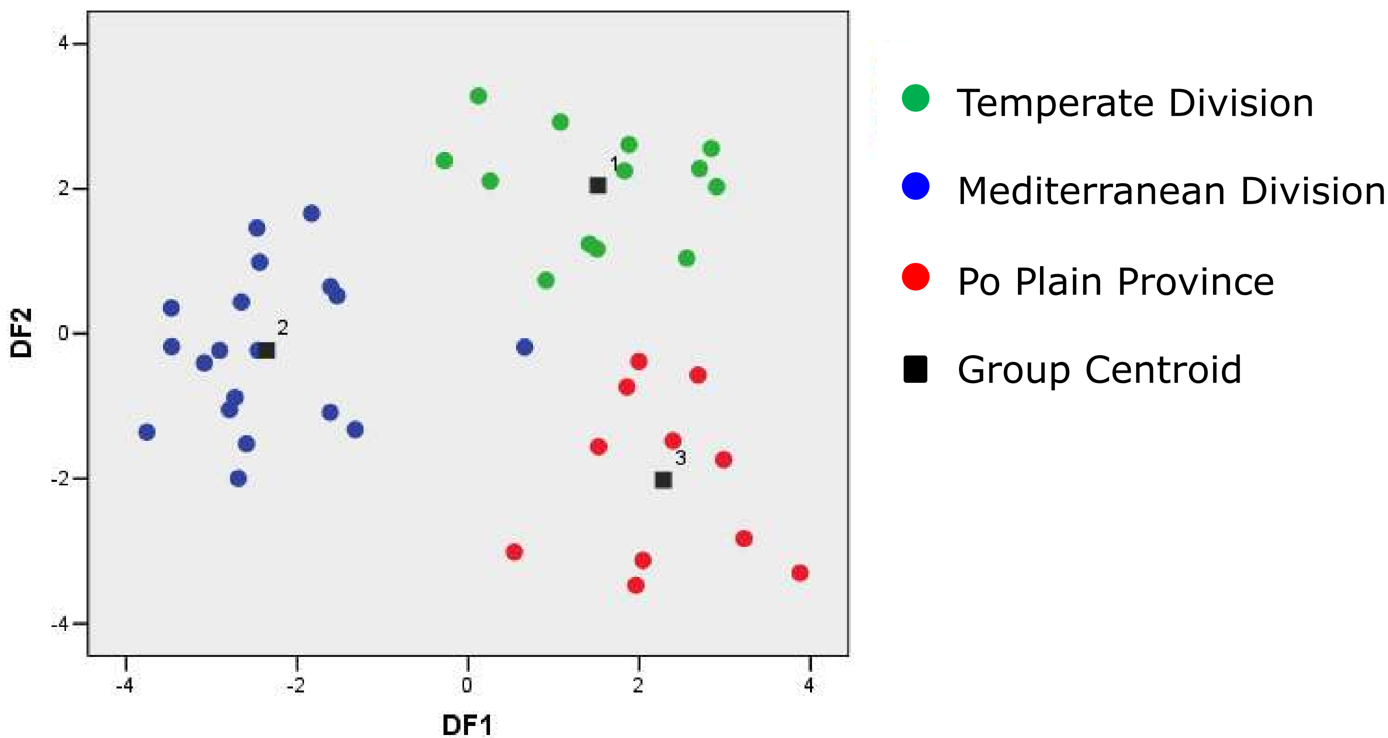
Survey on Italian propolis



Published in: C. Blasi; G. Capotorti; R. Copiz; D. Guida; B. Mollo; D. Smiraglia; L. Zavattero; *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* **2014,** 148, 1255-1345.
DOI: 10.1080/11263504.2014.985756

Survey on Italian propolis

GDA analysis – supervised multivariate analysis



The DF1 explains 65.8% of the total variance.
The cross-validation shows a predictive capacity of 79.8%.
The DFs are particularly correlated with the signals of kaempferol, apigenin and caffeic acid for DF1 and chrysin, galangin and ferulic acid for DF2.

Survey on Italian propolis

Conclusion

- Most of the analysed Italian propolis samples satisfied the proposed quality requirements for the “poplar type” proposed by Popova et al. (2007)…
- … but some of the samples (from Sicily and Sardinia) have shown a low or very low value in flavonoid content and do not comply with “poplar type”. Mediterranean type?
- A more detailed and careful statistical analysis comprehensive of all Italian samples showed that it was possible to distinguish three different groups corresponding to the mapped ecoregions of Italy as was reported recently by Blasi et al.(2014).
- Standardization of Italian propolis is needed to ensure a real evaluation of the product.


Thank you for your attention