

# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

Živoslav Tešić<sup>1</sup>, Dušanka Milojković-Opsenica<sup>1</sup>, Jelena Trifković<sup>1</sup>, Maja Natić<sup>1</sup>,  
Branko Šikoparija<sup>2</sup>, Biljana Dojčinović<sup>3</sup>

<sup>1</sup>Faculty of Chemistry, University of Belgrade, Belgrade, Serbia;

<sup>2</sup>Faculty of Sciences, Department of Biology and ecology, Novi Sad, Serbia;

<sup>3</sup>Centre of Chemistry, IChTM, University of Belgrade, Belgrade, Serbia



# Faculty of Chemistry University of Belgrade



# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## Production of honey in Serbia



- Long tradition of beekeeping
- 4000-5000 t/year
- Utilization of the capacity: ~33%
- Great potential for apiculture

### Unifloral:

• Linden (*Tilia Cordata*):  
Srem, Braničevo, Bor



• Acacia (*Robinia pseudoacacia*):



Entire region of Serbia

• Sunflower (*Heliantus annuus*):



Vojvodina – Banat, Bačka

### Multifloral:

Entire region of Serbia



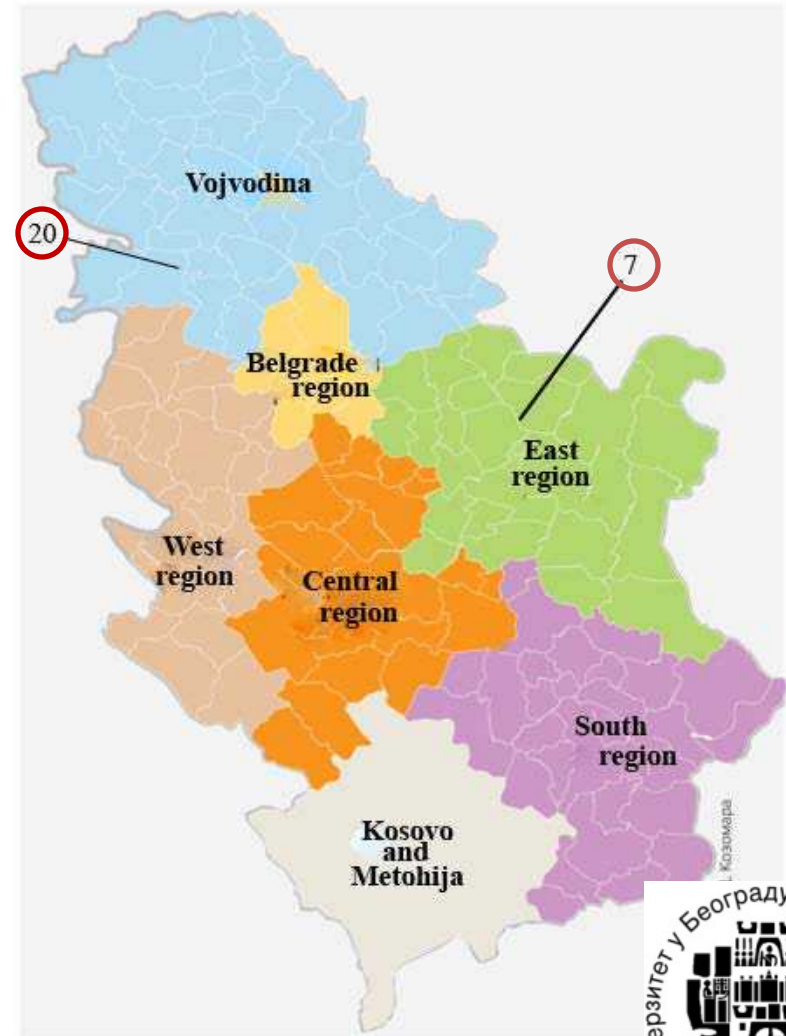
# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## Linden honey

- intense aroma
- medical function from the properties of the linden flower (linden blossoms contain flavonoids, volatile oils and mucilaginous components that soothe and reduce inflammation)

## Characterization of Serbian linden honey

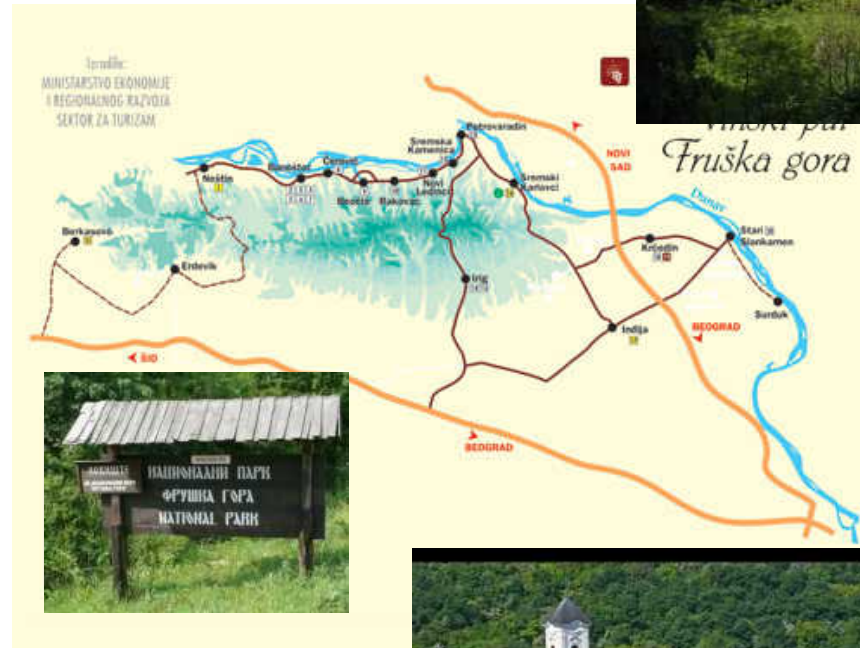
- two geographical regions: Fruška gora mountain and East Serbia
- honey produced in different seasons (2001-2012)



# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## National park Fruška gora

- a mountain in north Sremia (Srem)
- covered with meadows, pastures, cornfields, orchards and vineyards
- largest concentration of linden trees in Europe!



# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## Analytical methods

- Melissopalynological analysis - quantitative and qualitative determination of pollen and honeydew elements isolated from honey samples.

Harmonized Methods of Melissopalynology (von der Ohe et al., 2004)

- Basic physicochemical parameters (moisture, free acidity, pH)

The Harmonised Methods of the IHC

(Bogdanov, Martin, & Lullmann, 1997)

- Mineral composition - K, Na, Ca, Mg, Cu, Zn, Fe, Mn, Co, Ni, Cr, Cd

Inductively coupled plasma optical emission spectroscopy,

ICP-OES, Thermo Fisher



# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## • Sugar composition

Monosacharides (70%)	Disacharides (20%)	Higher sugars (1-3%)
➤ Glucose	➤ Sacccharose	➤ Melesitose
➤ Fructose	➤ Maltose	➤ Maltotriose
	➤ Isomaltose	➤ Isomaltotriose
	➤ Trehalose	➤ Panose
	➤ Gentiobiose	
	➤ Turanose	

Ion chromatograph, IC 3000, Dionex



# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

- Phenolic acids and flavonoids  
flavonoids (catechin, myricetin, rutin, luteolin, quercetin, apigenin, naringenin, chrysin, pinocembrin, and galangin),  
phenolic acids (gallic, chlorogenic, caffeic, and p-coumaric acid)  
*cis, trans*-abscisic acid

UHPLC

+



LTQ Orbitrap MS with HESI-II

ThermoFisher Scientific



# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

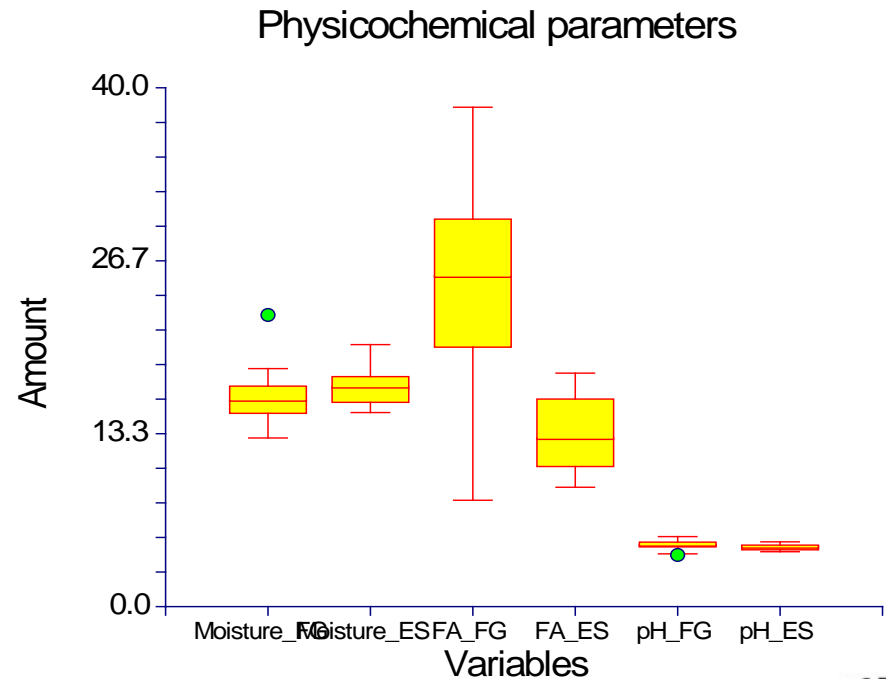
## *Melissopalynological analysis of linden honey*

- Total pollen content: in average 15374 PG/10g of honey.  
This corresponds to unifloral honeys collected on sources which pollen is underrepresented in nectar (Louveaux et al., 1978), such are *Tilia* species (Riccardelli D'Albore, 1997).
- *Tilia* pollen: in average contribution of 77% (notably higher than the maximum recorded for European lime honeys, Persano Oddo and Piro, 2004; Pelimon, 1960).
- In 90.32% samples lime tree pollen was predominant (>45%) and all the other registered pollen types contribute with <10%.
- In remained 9.68% of samples: *Tilia* pollen contribution is 43%, 34% and 25%, *Robinia pseudoacacia* (3-40%), *Brassica napus* (6-10%) or *Amorpha fruticosa* (51%) pollen.
- **No significant difference** has been recorded in mean values of the melissopalynological parameters for honey samples produced on Fruška gora and in the region of East Serbia.

# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## *Physicochemical parameters*

- The moisture content indicated a proper degree of maturity, in compliance with the international requirements with levels of humidity lower than 20% (Council Directive 2001/110/EC and Revised Codex Standard for Honey, Codex STAN 12–1981).
- All samples met the requirements set by the Regulation, which requires that the acidity should not exceed 50.0 meq/kg.
- pH values are consistent with the previously reported values of European unifloral honeys (Persano Oddo & Piro, 2004).



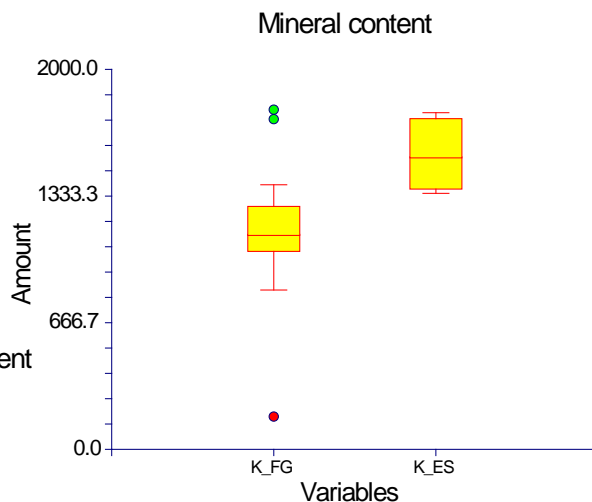
# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## Mineral content

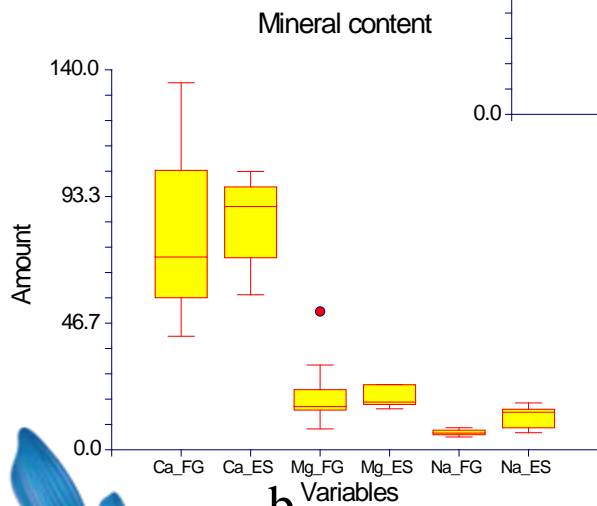
Macro elements

(mg/kg):

- a) K
- b) Ca, Mg, Na

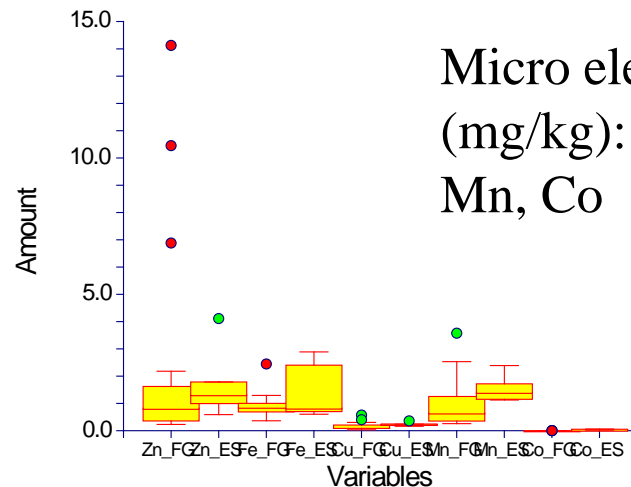


a



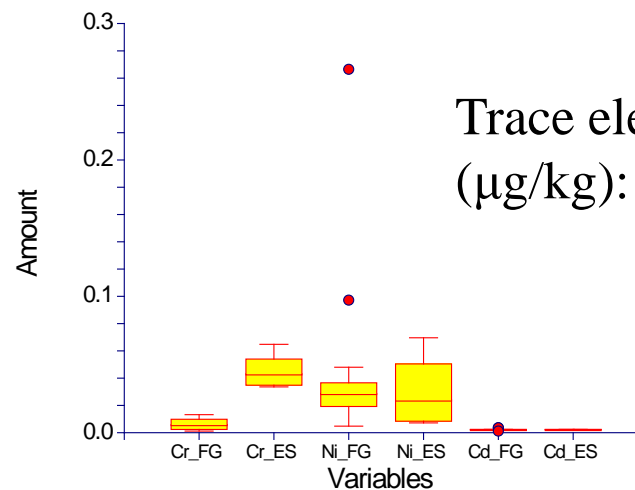
b

## Mineral content



Micro elements  
(mg/kg): Zn, Fe, Cu,  
Mn, Co

## Mineral content



Trace elements  
(µg/kg): Cr, Ni, Cd

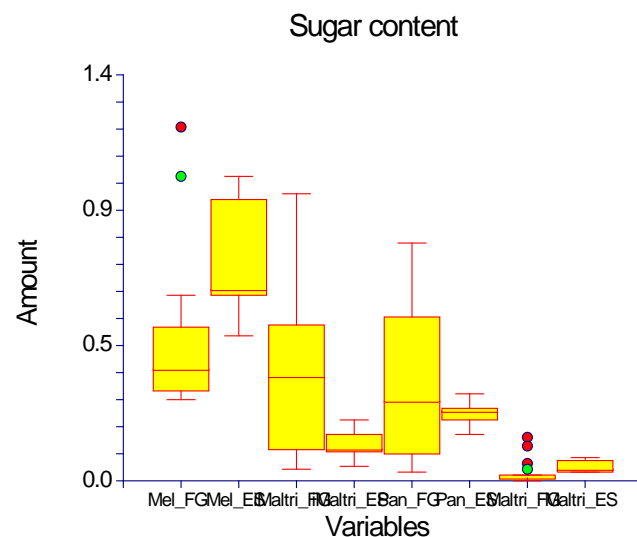
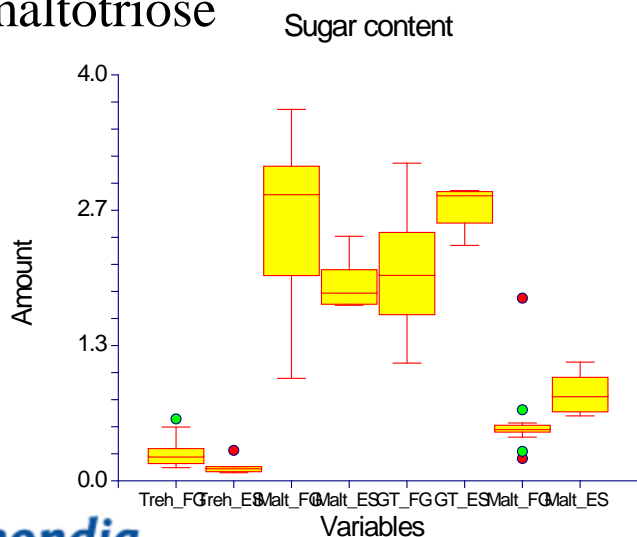
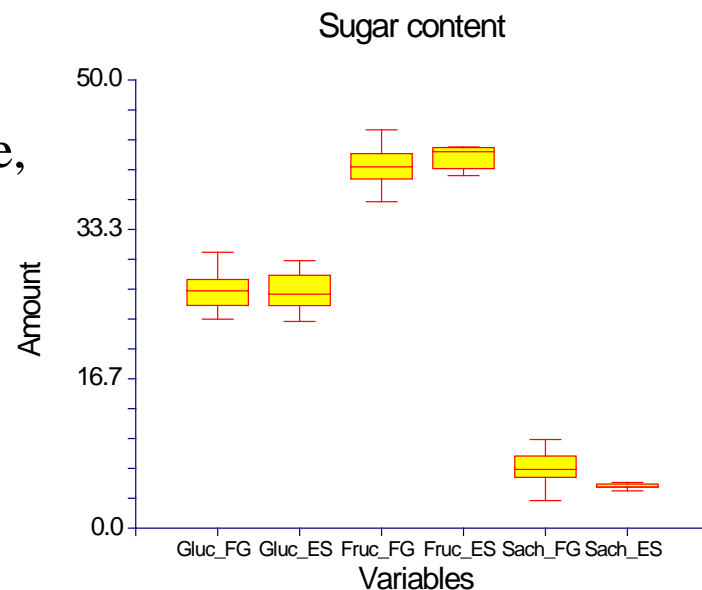
# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## *Sugar content*

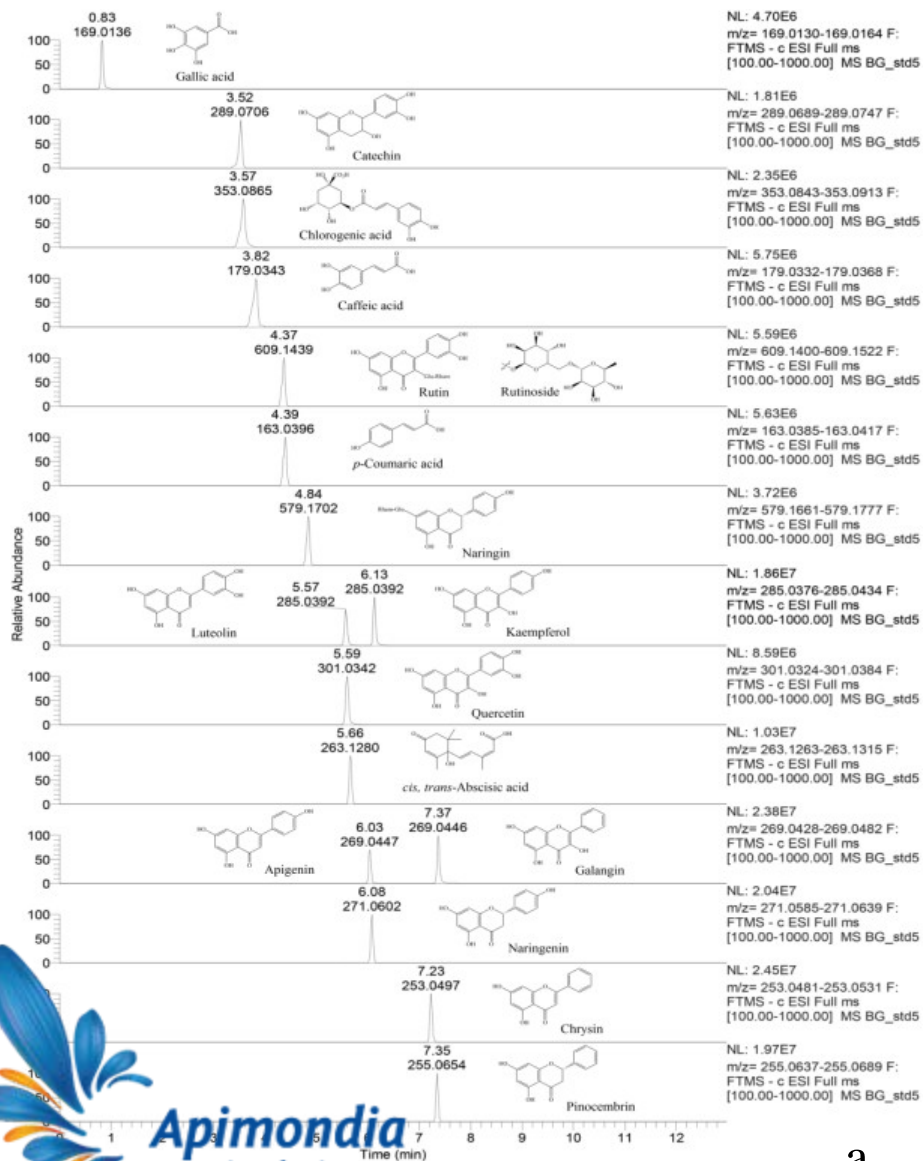
Major components: glucose, fructose and saccharose

Minor components:

- Disaccharides – trehalose, maltose, turanose and gentiobiose, isomaltose
- Trisaccharide – melesitose, isomaltotriose, panose, maltotriose

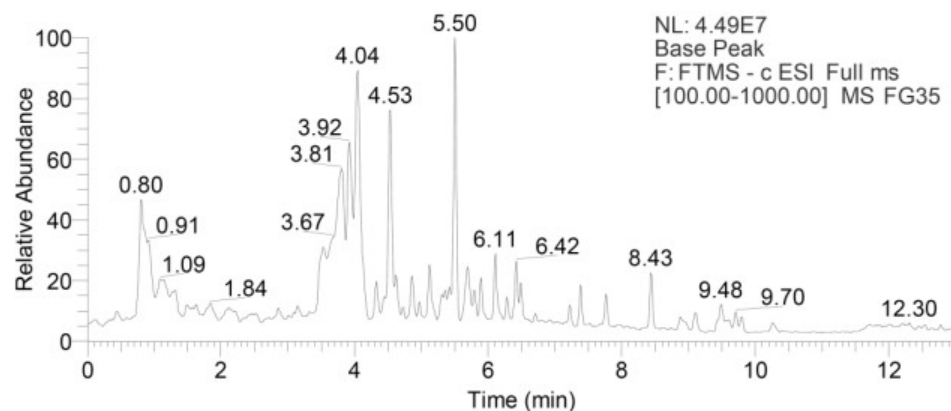


# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY



## Phenolic acids and flavonoids

- The extracted ion chromatograms of standards
- Selected base peak chromatogram of linden honey extract

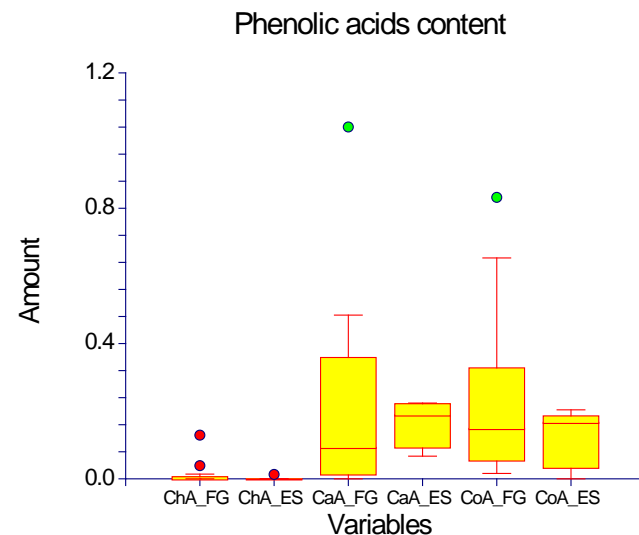
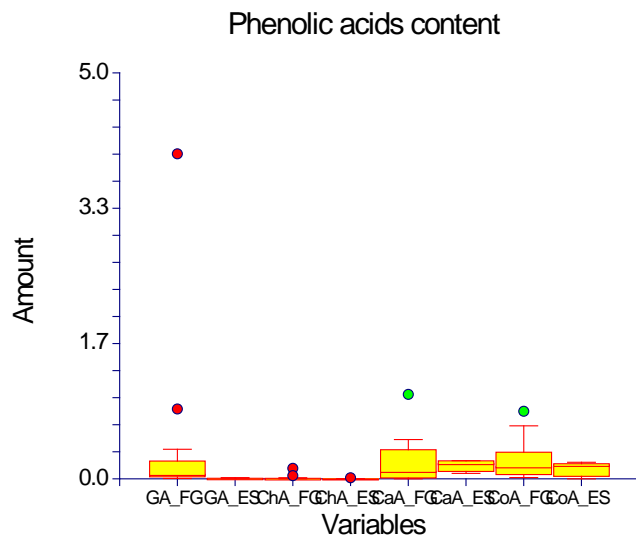


b

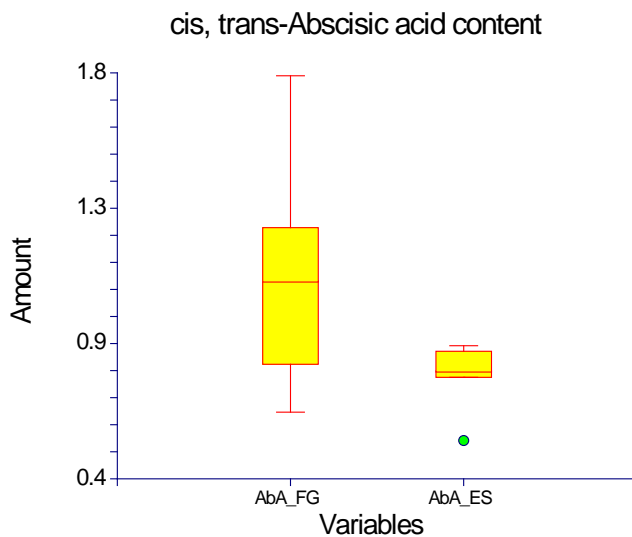
a

# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

Phenolic acids  
(mg/kg): gallic acid  
(GA), chlorogenic acid  
(ChA), caffeic acid  
(CaA), and p-coumaric  
acid (CoA)



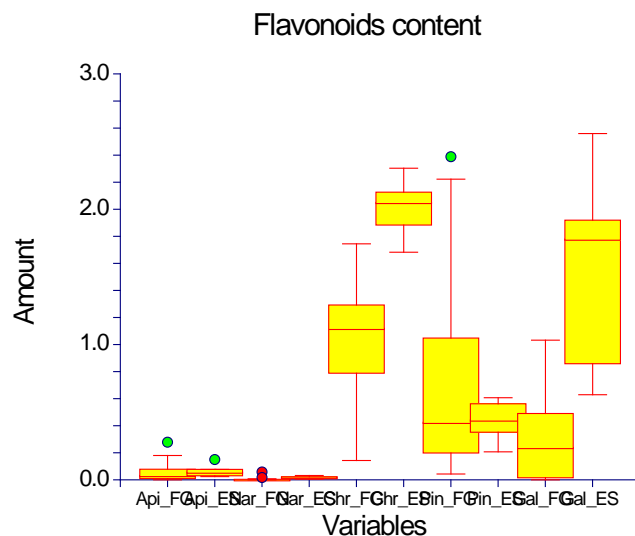
*cis, trans* – Abscisic acid  
content (mg/kg)



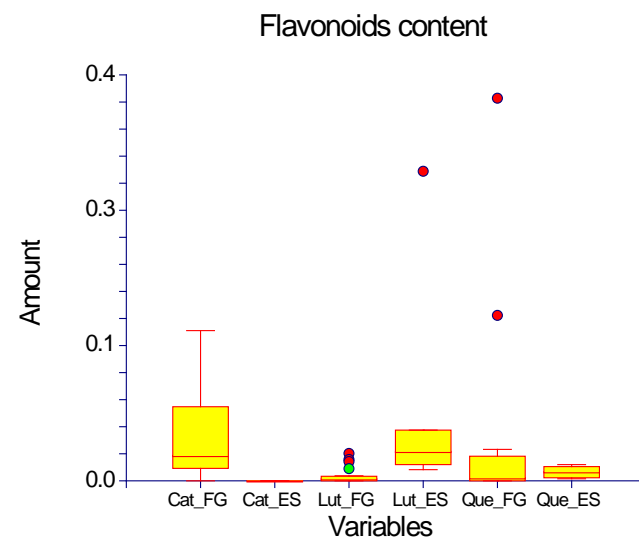
# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

Flavonoids (mg/kg):

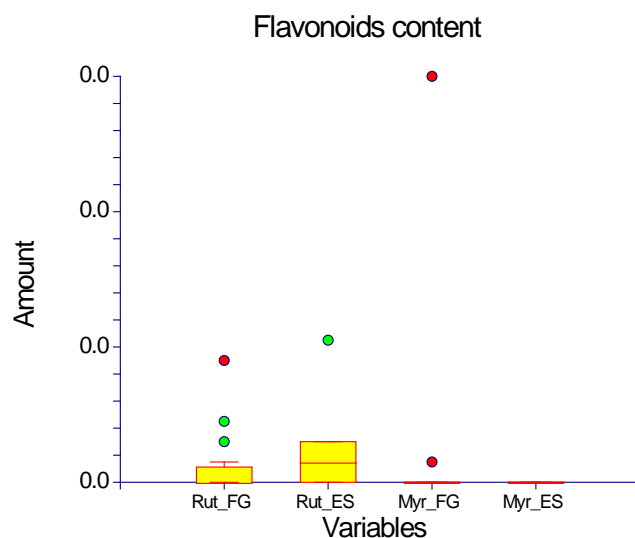
- a) apigenin (Api), naringenin (Nar), chrysin (Chr), pinocembrin (Pin), galangin (Gal)



- b) catechin (Cat), luteolin (Lut), quercetin (Que)



- c) rutin (Rut), myricetin (Myr)



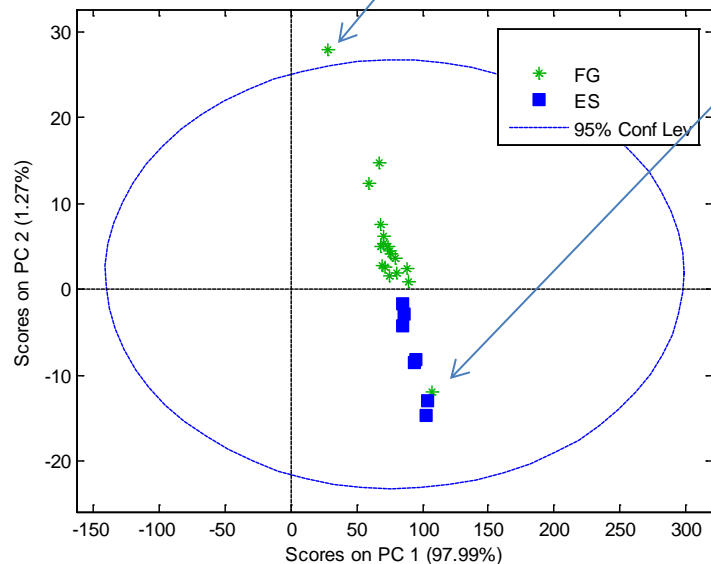
# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

Determination of geographical origin of Serbian linden honey:

- Unsupervised classification chemometrics: principal component analysis (PCA)
- Supervised chemometric method: partial least square-discriminant analysis (PLS-DA)

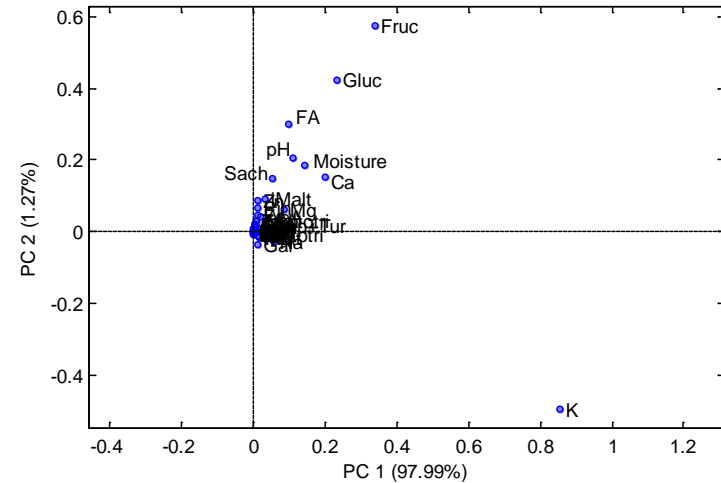
**PCA**

Sample with low present of linden pollen (compared to other samples)



PCA scores plot

Sample from FG with high content of K, similar to samples from ES



PCA loading plot

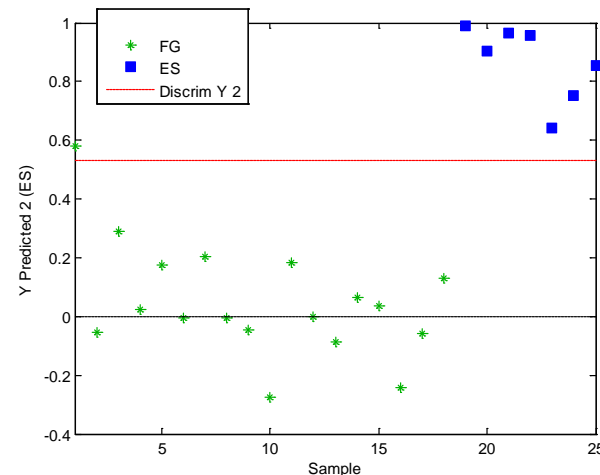
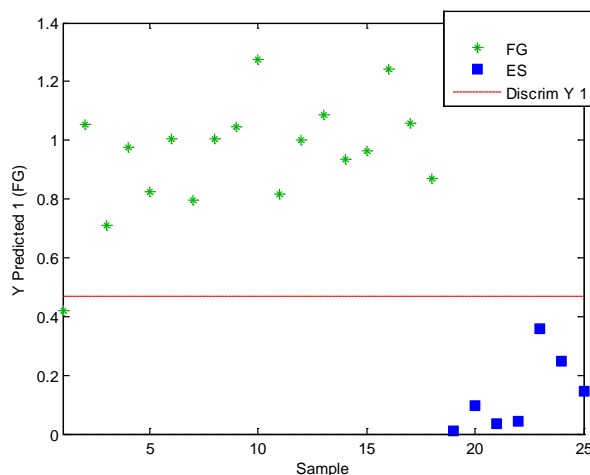


# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## PLS-DA model

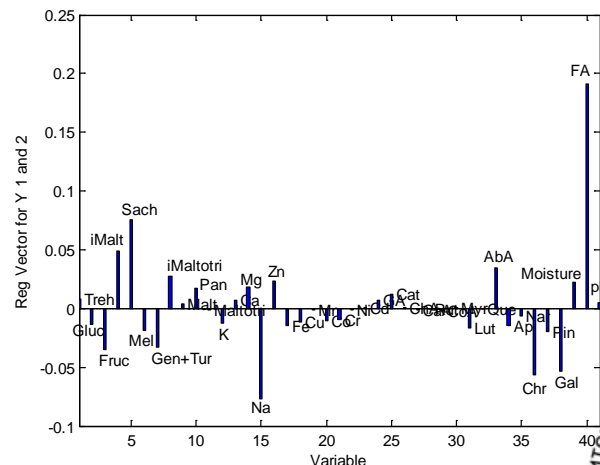
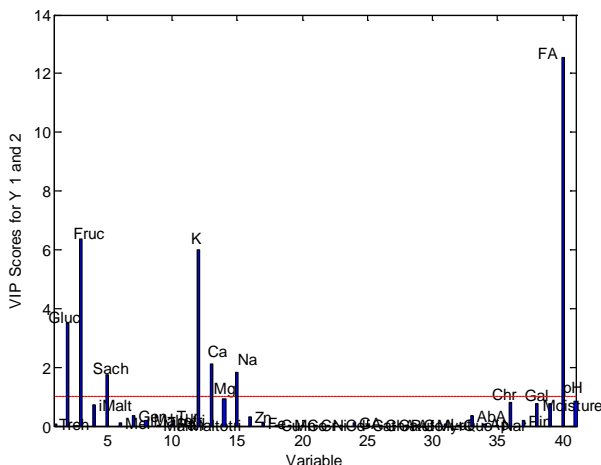
Initial model

	FG	ES
$R^2_{cal}$	0.8163	0.8163
$R^2_{CV}$	0.6409	0.6409
RMSEC	0.1924	0.1924
RMSECV	0.2754	0.2754



Scores plots of data for Fruška gora and East Serbia

Plots of the variables versus VIP scores in model for FG and ES

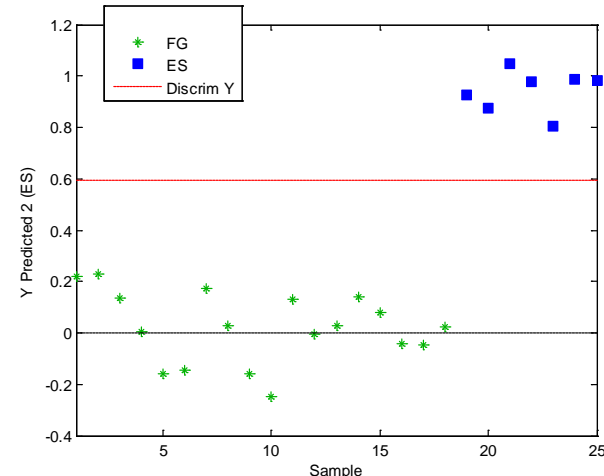
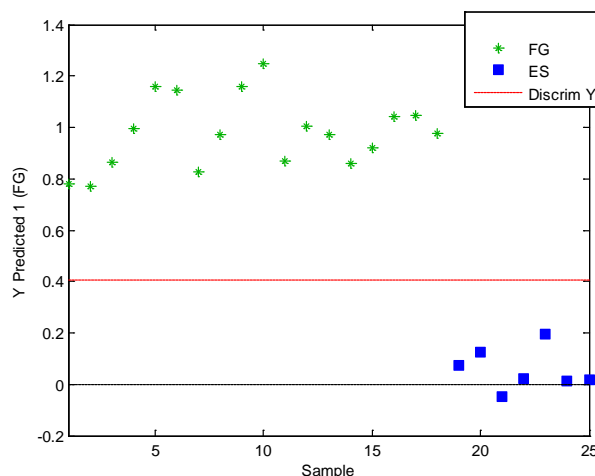


Plot of the coefficients of parameters in model for FG and ES

# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

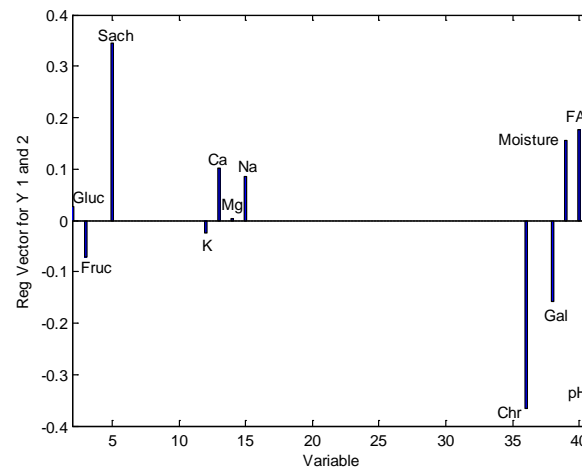
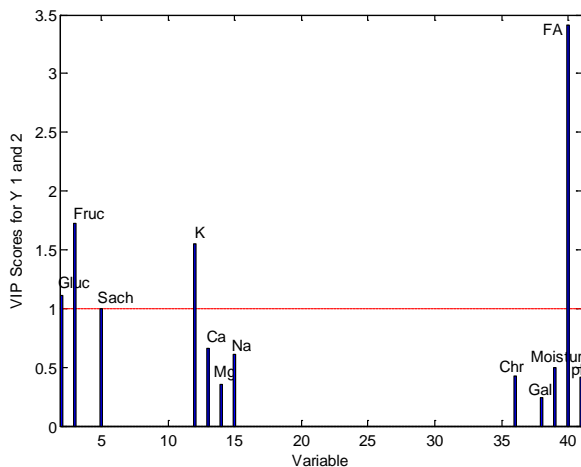
## Model with VIP variables

	FG	ES
$R^2_{cal}$	0.9215	0.9215
$R^2_{CV}$	0.6818	0.6818
$RMSEC$	0.1258	0.1258
$RMSECV$	0.2668	0.2668



Scores plots of data for Fruška gora and East Serbia

## Plots of the variables versus VIP scores in model for FG and ES



Plot of the coefficients of parameters in model for FG and ES

# MODERN ANALYTICAL METHODS IN CHARACTERIZATION OF LINDEN HONEY

## *Acknowledgment*

- Ministry of Education, Science and Technological Development of Serbia, Grants No. 172017 and 451-03-2372-IP Type 1/107
- FP7 RegPot project FCUB ERA GA No. 256716



Кристина Лазаревић, Милица Јоветић, Предраг Симић, Родољуб Живадиновић, Јелена Кечкеш, Силвио Кечкеш, Ивана Зековић, Леа Ленхардт, Мирослав Драмићанин, Зита Келемен, Биљана Марошановић, Филип Андрић, Јелена Трифковић, Маја Натић, Урош Гашић, Томислав Тости, Живослав Тешић, Душанка Милојковић-Опсеница, Драган Манојловић, Иван Анђелковић, Далибор Станковић, Петар Ристивојевић, Александра Радоичић, Срђан, Душан, Милена,