SAFE-HONEY

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Influence of sugar syrup adulterants on bioactive properties and phenolic content of different honey types

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Summary

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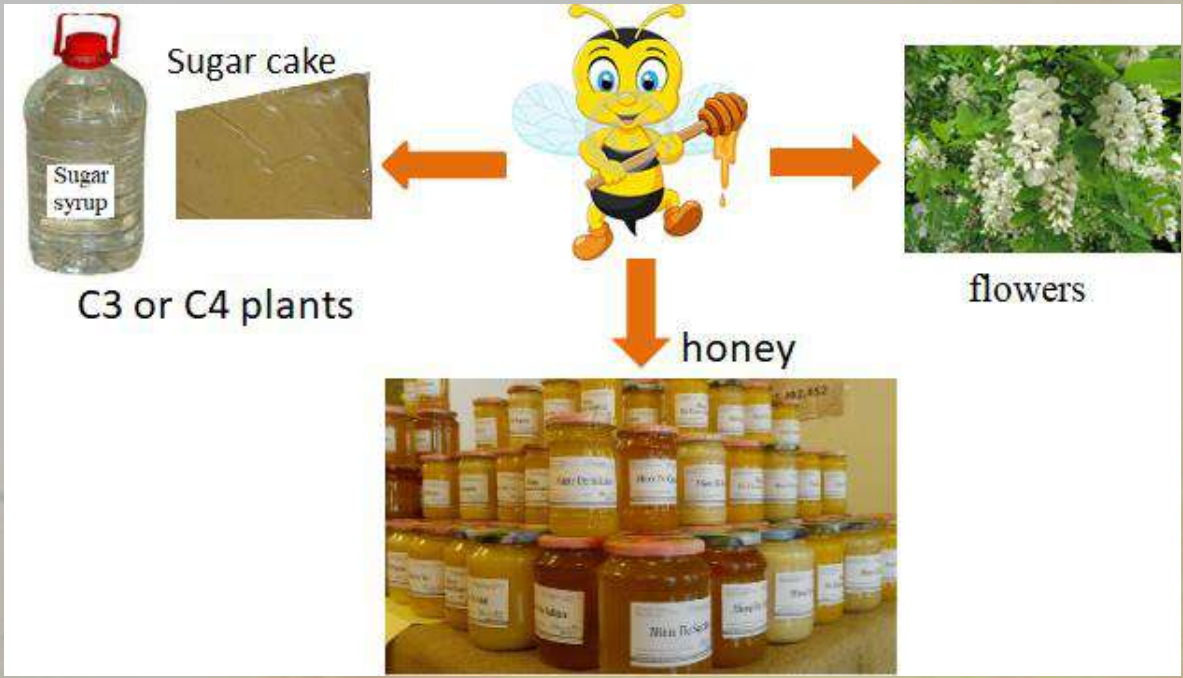
Introduction

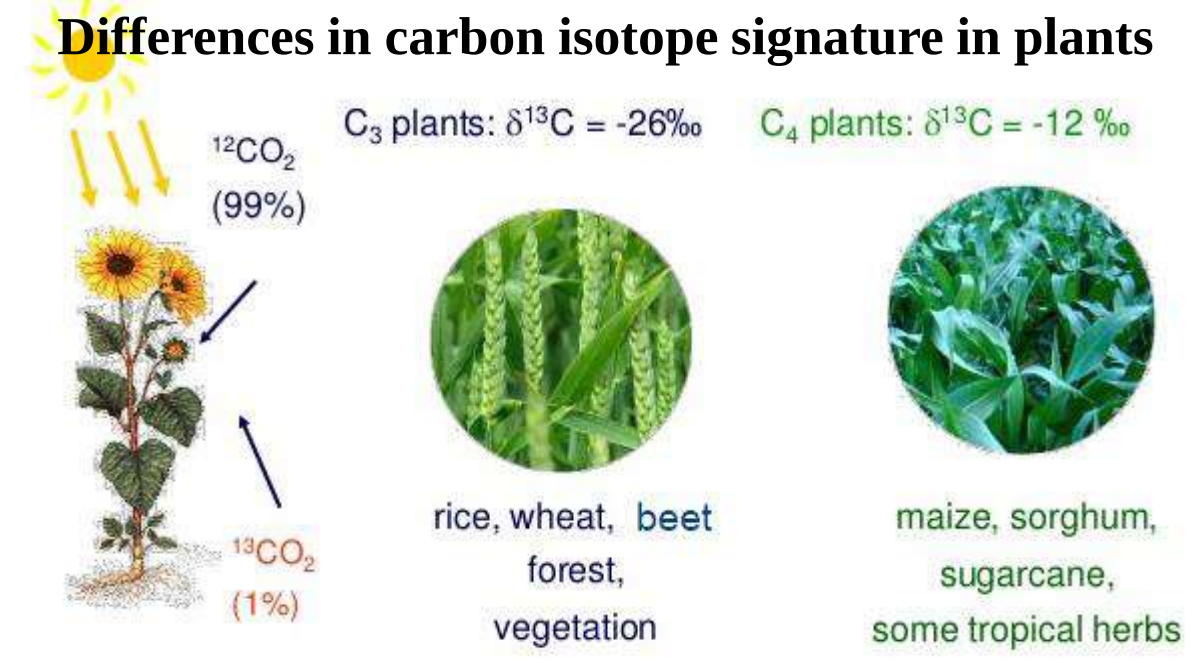
- Bee products - HONEY are natural product of high quality and medicinal properties

- Honey has been considered to have therapeutic properties since ancient times and among the factors responsible for such activity are phenolic compounds including phenolic acids and flavonoids
- Honey can be easily directly or indirectly adulterated with inexpensive sugar syrups (from C3 or C4 plants), thus negatively affecting its quality and composition.
- Evaluation of honey quality is a topical and a significant problem of the food industry.

Honey adulteration

- Direct or indirect adulteration of honey with sugar syrups represents a serious problem which affects its therapeutic effect through the modification of the biologically active compounds content.





Food and Agriculture Organization of the United Nations (FAO)
International Atomic Energy Agency (IAEA)

Current methodologies used to detect honey adulteration

			Markers	Detection
Direct adulteration	Starch syrups	High-fructose corn syrup (HFCS 42, 55, 90)	oligosaccharides, polysaccharides, difructose anhydrides (DFAs) $\delta^{13}C$ isotopic signature	HPAEC-PAD (down to 1% level) UHPLC/Q-TOF-MS (> 10% level) SCIRA (> 7% level)
		Corn syrup	oligosaccharides, polysaccharides $\delta^{13}C$ isotopic signature	HPAEC-PAD (down to 1% level) SCIRA (> 7% level)
		Rice syrup (42, 55, 90)	2-acetylfuran-3-glucopyranoside (AFGP)	HPLC-DAD (> 10% level)
	Inverted syrups	Inverted syrup from sugar cane/sugar beet	difructose anhydrides (DFAs) inulotriose $\delta^{13}C$ isotopic signature	UHPLC/Q-TOF-MS (> 10% level) GC-MS (> 5% level) SCIRA(> 7% level), SNIF-NMR (> 20% level)
	Low quality honey added to high price honey		honey bioactive constituents specific for each honey type	HPLC-DAD, HPLC-MS, GC-MS
Indirect adulteration	Starch or inverted syrups feed to bees	Honey with high level of indirect sugar	$\delta^{13}C$ isotopic signature fructosyl-fructose from HFCS Polysaccharides, DFAs and AFGP	AOAC official method detects the presence of more than 10% of HFCS in honey GC-MS identification of residual syrup in honeys produced before 3 days of bee feeding

Chemometric applications in HONEY authentication

- **Building mathematical–statistical models based on quantitative and qualitative information about the natural constituents**

- Mineral and trace elements
- Isotopic ratio
- Volatile compounds

- Amino acids
- Sugars
- Organic acids
- Phenolics

Statistical analysis

- Similarity/Disimilarity
- Honey floral origin
- Geographical location

Botanical origin

Natural organic constituents

Adulteration

Isotopic signature, impurities from syrups

Geographical origin

Mineral and isotopic ratios ($^{13}C/^{12}C$, $^{18}O/^{16}O$ and D/H) determinations

Objectives

- Investigation of honey phenolic composition and biochemical properties of different authentic honey types
- Evaluation of the effect of direct incorporation of different percent of sugar syrups in honey or indirect adulteration of honey by bee feeding with sucrose syrup on honey bioactive constituents and biochemical properties
- Discrimination between pure and adulterated honey by bee feeding with cakes and sugar syrups prior to, or within the main nectar periods
- Establish the minimal amount by which bioactive compounds profile and honey biochemical properties are definitively modified by the incorporation of syrups
- Development of appropriate methodologies for objective verification of honey authenticity

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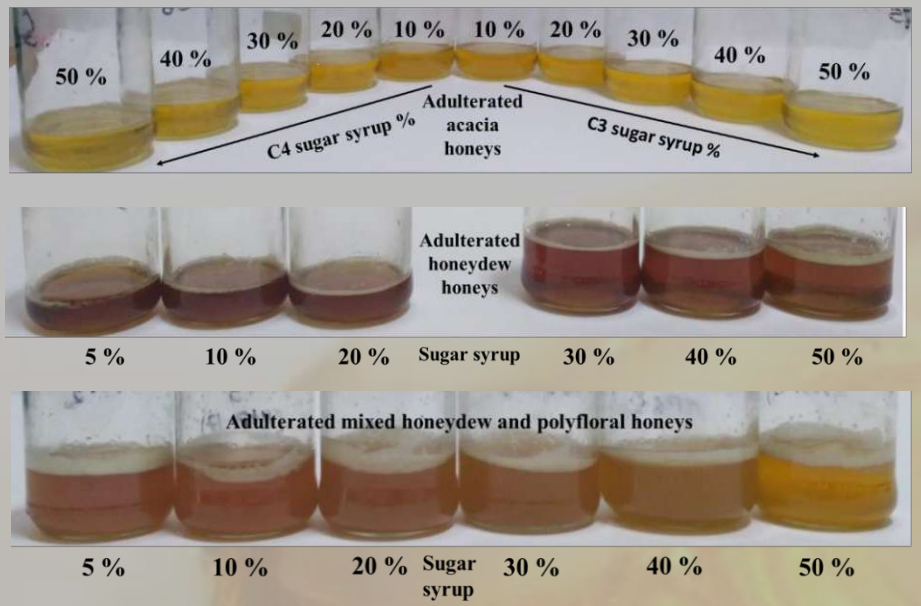
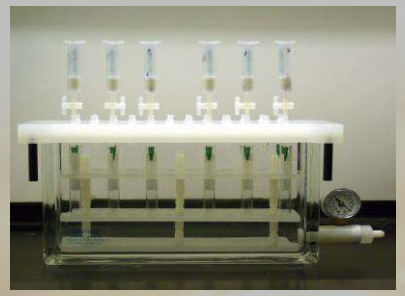
Experimental methods

Direct adulteration



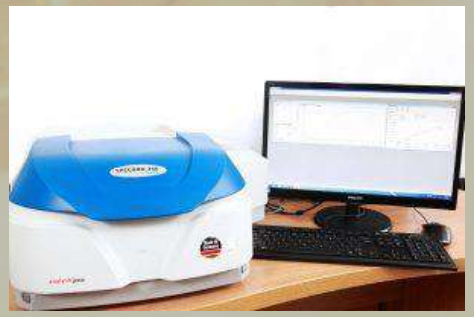
- Phenolic compounds profile: phenolic acids and flavonoids



Polymeric SPE sorbent

UHPLC-DAD-ESI/MS

- Total polyphenols (mg GAE/g), total flavonoids (mg/g rutin) and antioxidant activity (DPPH %)
- UV-Vis spectral characterization



UV-Vis spectrophotometer

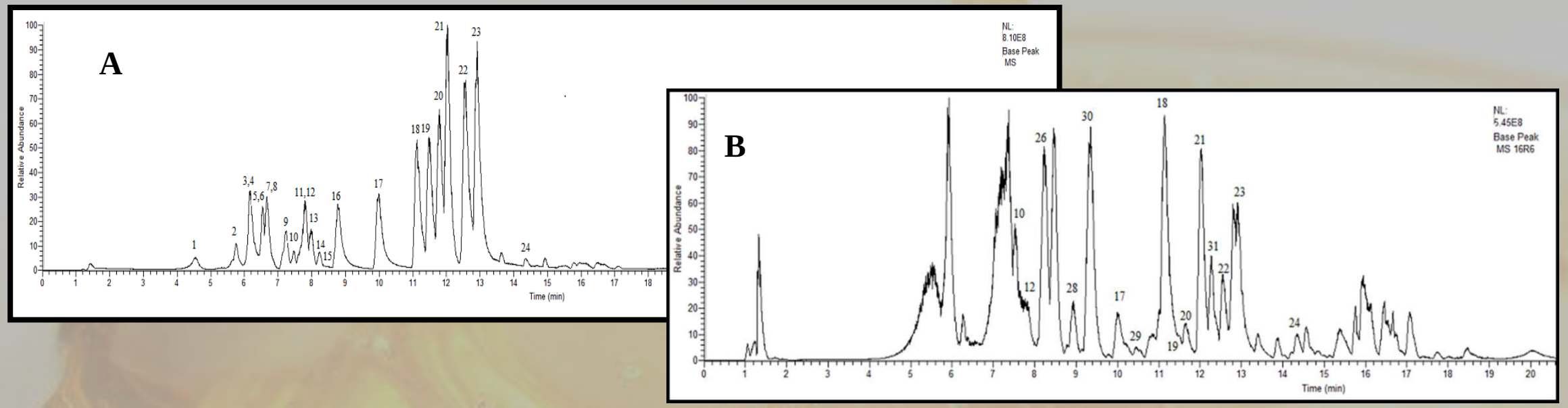
Indirect adulteration

Pure honey Adulterate honey

- determination of $\delta^{13}C$ in honey, $\delta^{13}C$ in honey protein – SCIRA – IRMS in order to establish the percent of adulteration of honey with C4 plants sugar (e.g. cane, corn syrup)
- Analytical data were processed by PCA statistical analysis in order to discriminate between the pure and adulterated honeys

Results and discussions

Base peak chromatogram of phenolic compounds standards solution (A) rape honey extract (B)



1-gallic acid, 2-3,4-dihydroxibenzoic acid, 3-catechin, 4-4-hydroxibenzoic acid, 5- chlorogenic acid, 6-epicatechin, 7-caffeic acid, 8-syringic acid, 9-p-coumaric acid, 10-ferulic acid, 11-naringin, 12-rutin, 13-hesperitin, 14-t-resveratrol, 15-t-cinnamic acid, 16-myricetin, 17-quercetin, 18-kaempferol, 19-isorhamnetin, 20-apigenin, 21-pinocembrin, 22-galangin, 23-chrysin, 24-pinostrobin, 26-abscisic acid, 28-sakuranetin, 29-alpinetin, 30-pinobanskin, 31-pinobanskin-3-O-acetate

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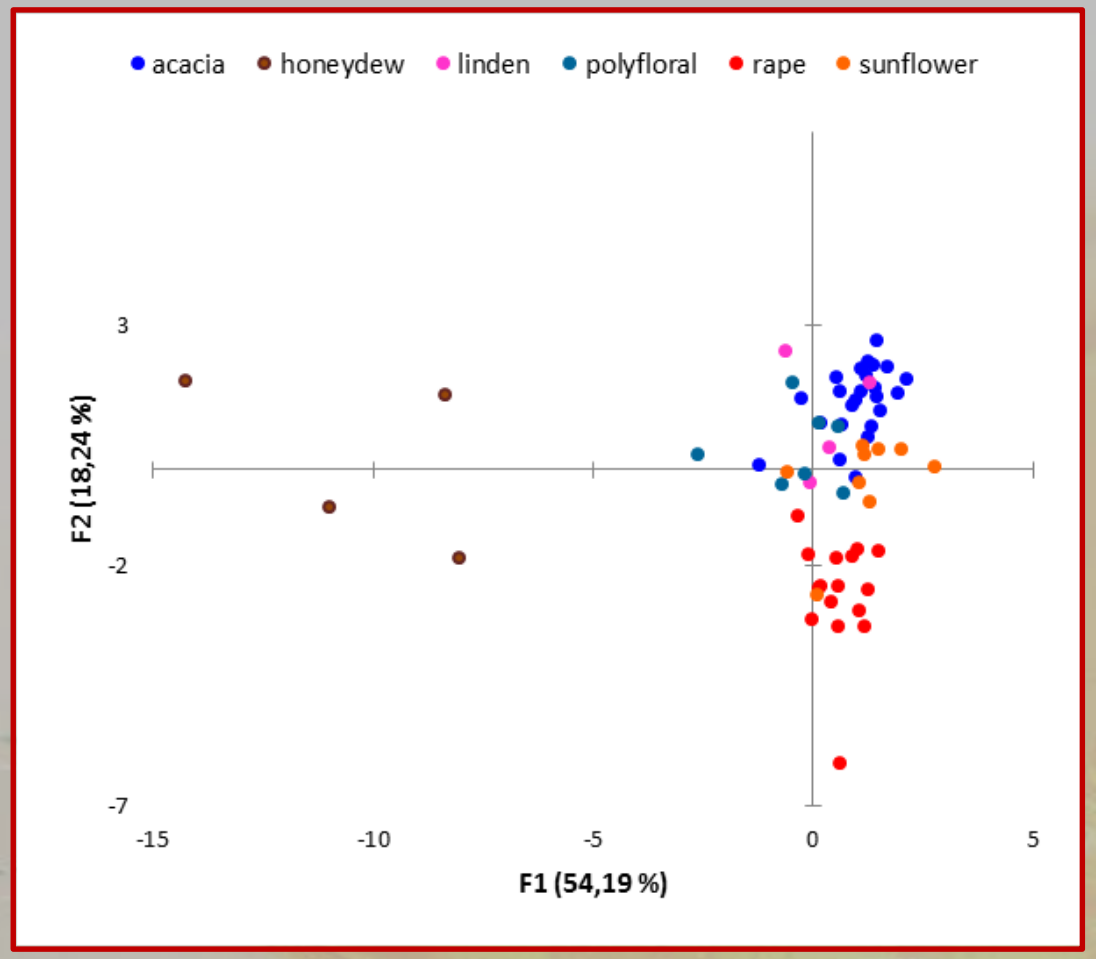
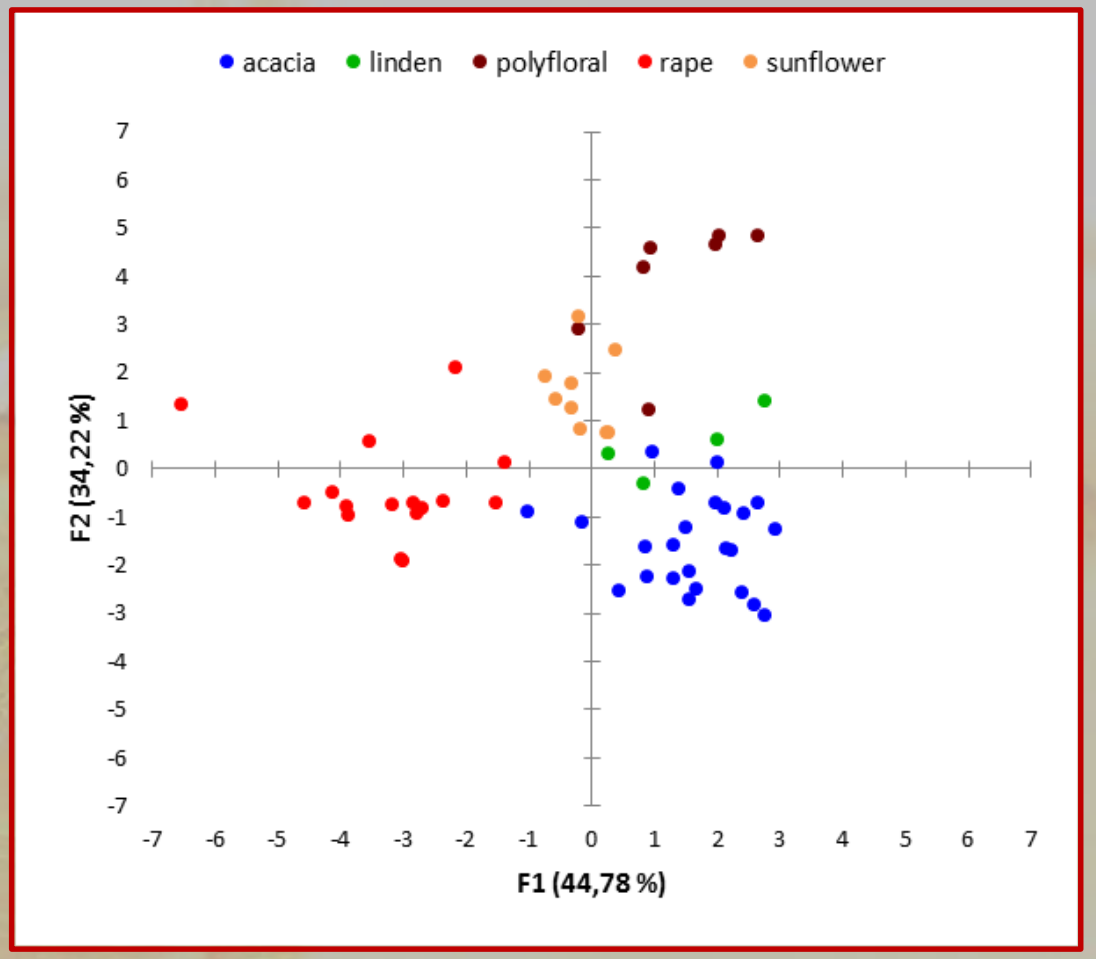
Phenolic compounds variation in pure and adulterated honeys

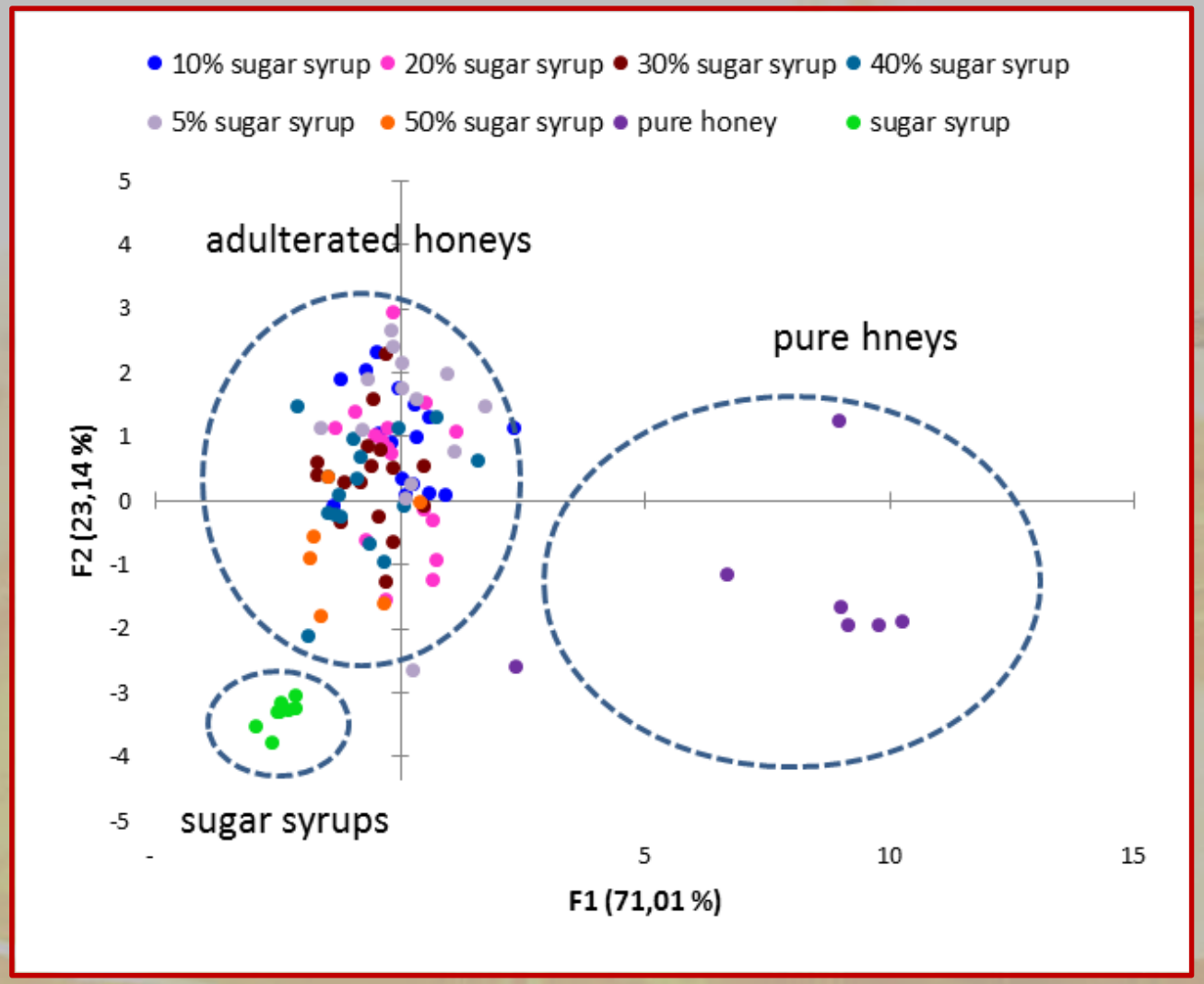
Phenolic compounds mg/kg	Sunflower		honeydew		polyfloral		rape		acacia	
	Pure honeys (n=9)	Adulterated honeys	Pure honeys (n=4)	Adulterated honeys	Pure honeys (n=7)	Adulterated honeys	Pure honeys (n=16)	Adulterated honeys	Pure honeys (n=24)	Adulterated honeys
Galic acid	0.000-0.036	0.000-0.008	0.912-4.645	0.566-1.119	0.000-0.520	0.000-0.062	0.000-0.037	0.003-0.007	0.002-0.127	0.064-0.131
3,4- dihydroxi benzoic acid	0.148-2.885	0.117-0.266	1.764-6.053	4.146-5.163	0.273-3.070	0.342-0.589	0.068-0.953	0.035-0.221	0.029-1.747	0.552-1.024
4-hydroxibenzoic acid	0.567-1.601	0.323-0.702	0.474-1.515	0.601-0.943	0.904-3.116	0.788-1.480	0.288-4.702	0.009-0.791	0.272-1.802	0.159-0.593
cafeic acid	0.353-1.884	0.128-0.334	0.235-0.550	0.181-0.279	0.431-1.261	0.354-0.674	0.052-1.473	0.025-0.103	0.082-1.057	0.075-0.169
syringic acid	0.006-0.101	0.006-0.05	0.106-1.113	0.068-0.173	0.032-0.237	0.016-0.048	0.014-0.625	0.025-0.380	0.004-0.330	0.013-0.060
p-coumaric acid	0.917-2.702	0.580-1.238	0.817-2.105	0.977-1.621	0.403-2.499	0.449-0.779	0.285-2.302	0.003-0.697	0.149-4.113	0.000-1.288
ferulic acid	0.731-2.786	0.317-0.748	0.523-2.469	0.381-1.212	0.317-1.504	0.102-0.226	0.202-2.223	0.003-1.092	0.332-4.624	0.017-1.493
cinnamic acid	0.044-0.254	0.012-0.05	0.031-0.398	0.033-0.103	0.053-0.977	0.032-0.124	0.028-0.216	0.003-0.188	0.002-0.464	0.028-0.079
rutin	0.009-0.171	0.002-0.006	0.006-0.664	0.127-0.262	0.007-2.131	0.330-0.687	0.003-0.674	0.001-0.005	0.001-0.196	0.043-0.151
quercetin	0.395-0.982	0.272-0.641	0.132-0.765	0.300-0.478	0.192-2.099	0.566-1.080	0.144-2.441	0.094-0.395	0.016-0.503	0.115-0.186
kaempferol	0.100-0.669	0.041-0.097	0.116-0.554	0.147-0.256	0.269-1.964	0.400-0.748	0.155-6.075	0.485-1.643	0.044-0.945	0.098-0.157
isorhamnetin	0.065-0.323	0.033-0.083	0.050-0.331	0.063-0.109	0.067-0.421	0.076-0.130	0.028-0.465	0.013-0.063	0.008-0.231	0.034-0.061
apigenin	0.124-1.168	0.003-0.066	0.100-1.047	0.219-0.360	0.218-3.451	0.000-0.339	0.038-0.690	0.003-0.053	0.052-0.530	0.003-0.2
pinocembrin	0.861-4.492	0.000-0.675	0.011-2.823	0.207-0.372	0.031-5.314	0.249-0.469	0.008-3.604	0.003-0.022	0.138-5.321	0.000-0.077
galangin	0.316-1.394	0.000-0.259	0.309-0.845	0.072-0.145	0.114-2.316	0.000-0.150	0.054-1.236	0.055-0.182	0.186-2.795	0.079-0.145
chrysin	0.975-3.352	0.327-0.657	0.005-2.627	0.248-0.444	0.000-4.865	0.299-0.575	0.002-1.098	0.003-0.003	0.007-2.058	0.003-0.006
pinostrobin	0.000-1.628	0.000-0.072	0.000-1.107	0.005-0.042	0.000-1.913	0.000-0.084	0.000-1.030	0.003-0.003	0.002-2.537	0.001-0.003

Honey botanical origin discrimination based on phenolic compounds





LDA discrimination between pure and adulterated honeys



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Phenolic compounds profile of sugar syrups

Phenolic compounds mg/kg	Min	Max	Average
Galic acid	0.000	0.071	0.035
3.4- dihydroxibenzoic acid	0.003	0.109	0.056
4-hydroxibenzoic acid	0.000	0.304	0.152
cafeic acid	0.000	0.103	0.051
syringic acid	0.001	0.683	0.342
p-coumaric acid	0.001	1.205	0.603
ferulic acid	0.001	0.822	0.412
cinnamic acid	0.000	0.157	0.078
rutin	0.003	0.067	0.035
quercetin	0.002	0.063	0.033
kaempferol	0.002	0.026	0.014
isorhamnetin	0.000	0.024	0.012
apigenin	0.001	0.043	0.022
pinocembrin	0.002	0.084	0.043
galangin	0.003	0.059	0.031
chrysin	0.001	0.046	0.024
pinostrobin	0.001	0.003	0.002

Biochemical properties of sugar syrups

	TP (mg GAE/100g)	TF (mg QE/100g)	DPPH%
Sugar syrup	29.47	0.15	0.16
Sugar syrup	14.94	0.12	0.71
Sugar syrup	24.63	0.59	1.49
Sugar syrup	26.42	0.56	2.37
Sugar syrup	29.64	0.20	0.94

TP – total polyphenols
TF – total flavonoids

Honey biochemical properties

Biochemical properties of pure and adulterated honeys

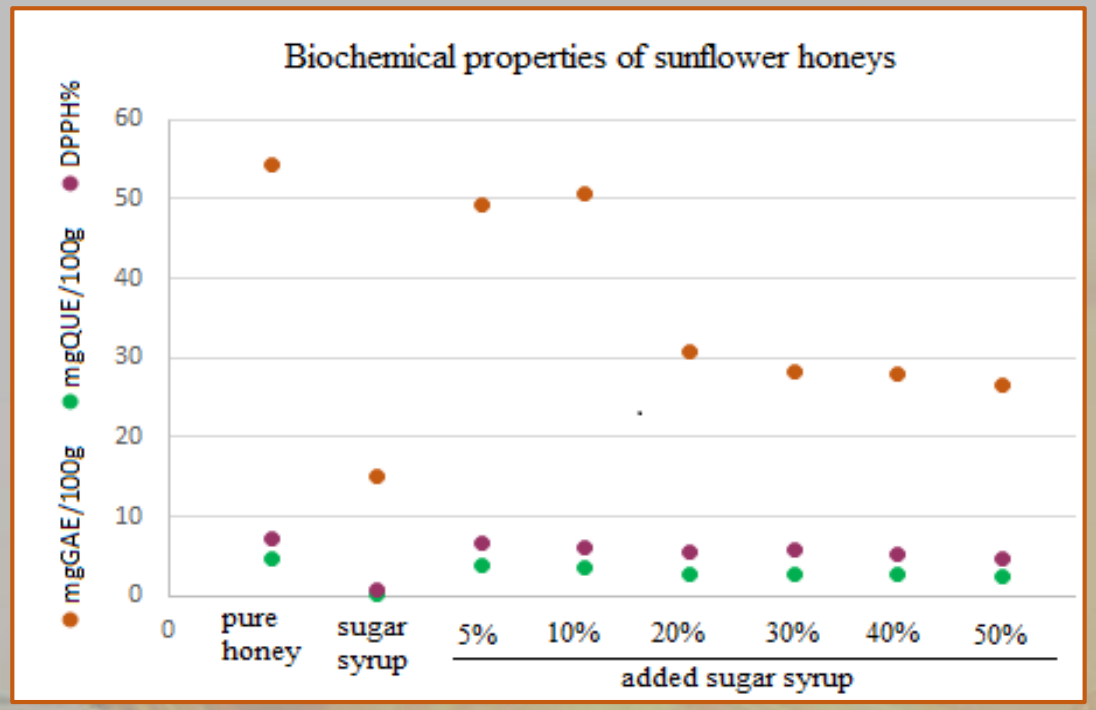
Honey type	Total polyphenols (mg GAE/100g)		Total flavonoids (mg QE/100g)		DPPH%	
	Pure honey	Adulterated honeys	Pure honey	Adulterated honeys	Pure honey	Adulterated honeys
sunflower	56.6	27.9-54.77	4.8	2.41-4.70	7.3	4.3-6.7
honeydew	128.7	65.2-122.6	8.5	5.2-8.3	29.5	14.5-27.9
linden	58.9	39.0-57.7	4.8	2.1-4.9	8.4	1.4-8.2
polyfloral	80.1	48.5-88.0	6.0	1.9-4.8	11.8	2.3-11.5
rape	41.0	32.6-46.5	4.0	1.9-4.4	2.7	1.4-3.3
acacia	50.5	37.2-55.5	2.0	1.4-2.5	7.7	4.9-7.1

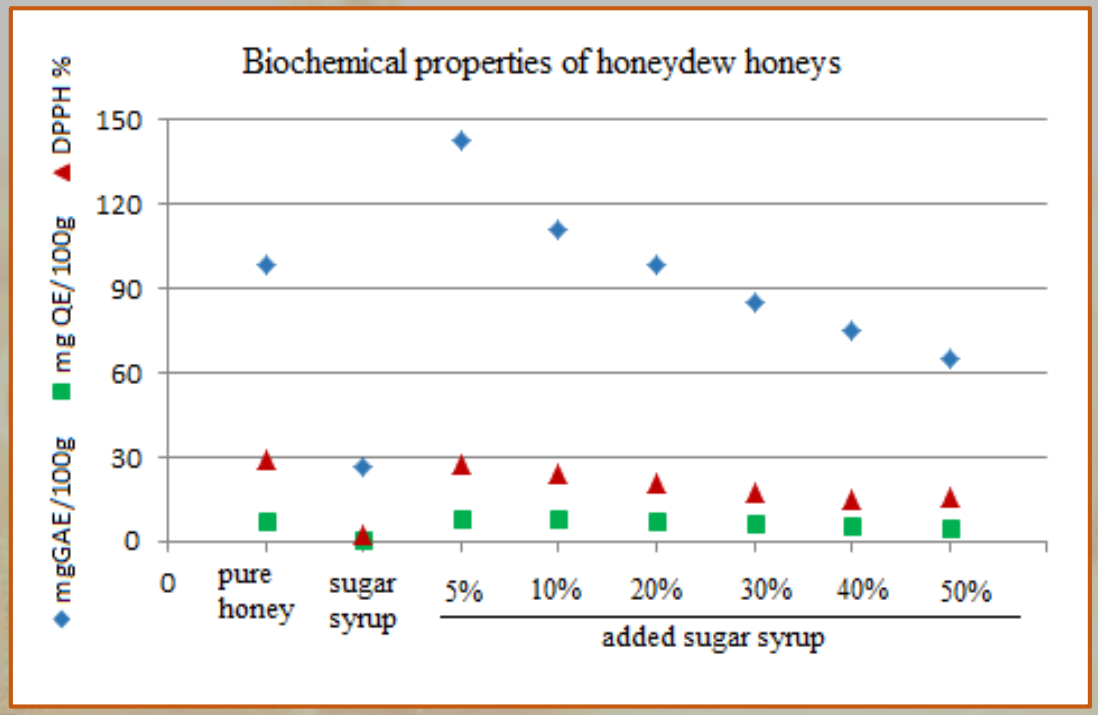
- Bioactive properties of authentic honeys were higher than in the adulterate honeys:

  56-6-128.7 mg GAE/100g, 4.8-8.5 mg QE/100g and 5.8-29.5 % DPPH in pure honeys

  27.9-122.6 mg GAE/100g, 0.05-8.3 mg QE/100g and 1.4-27.9 % DPPH in adulterated honeys

Honey biochemical properties





- Due to the fact that sugar syrups used for direct adulteration of honey shows bioactive properties, direct incorporation of sugar syrups in honey has produced an average decrease of 17.0 % for TP content, 34.4% for TF content and 27.7% for % DPPH.

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Honey isotopic signature and biochemical properties

AOAC 991.41-1996 :

- **$\delta^{13}C$ difference protein-honey (‰) < max 1**
- **C4 sugar (‰) < max 7%**

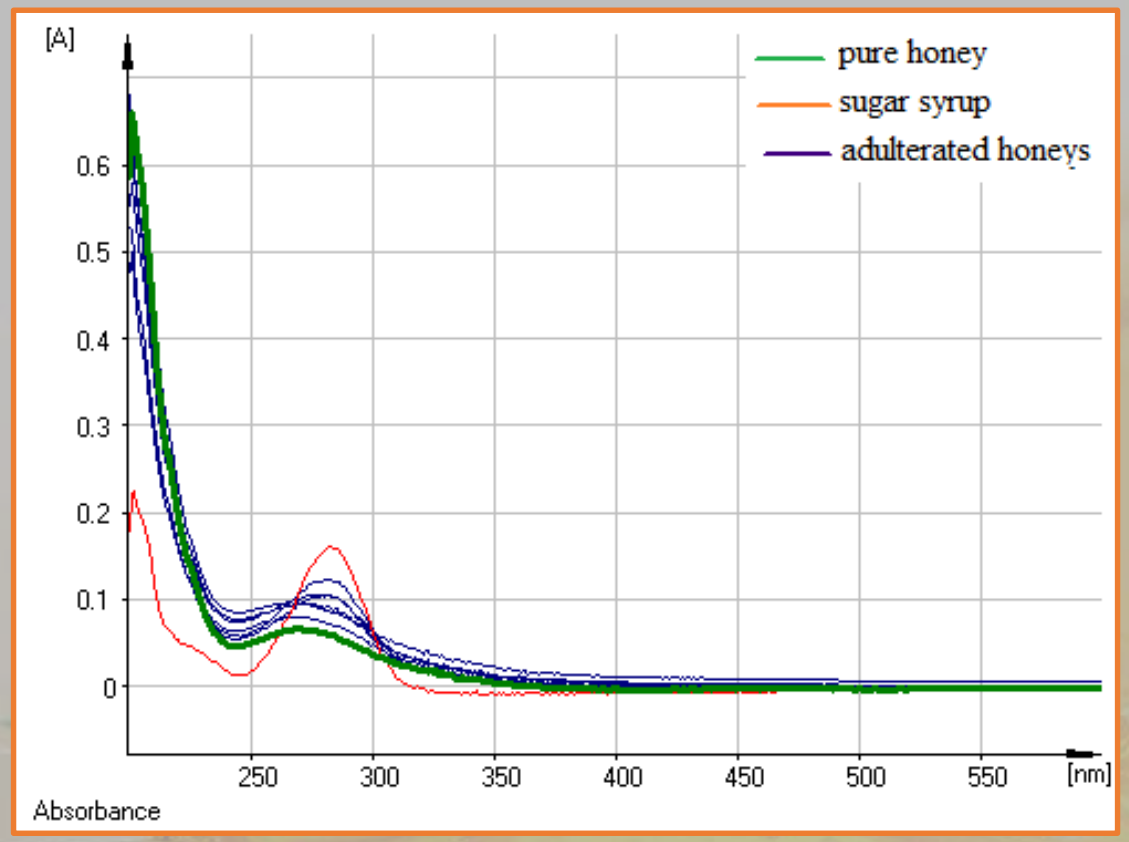
Indirect adulteration of honey

$$C4\ sugar\ (\%) = [(\delta^{13}C_{pro} - \delta^{13}C_{hon})/(\delta^{13}C_{pro} - \delta^{13}C_{sug})] \times 100$$

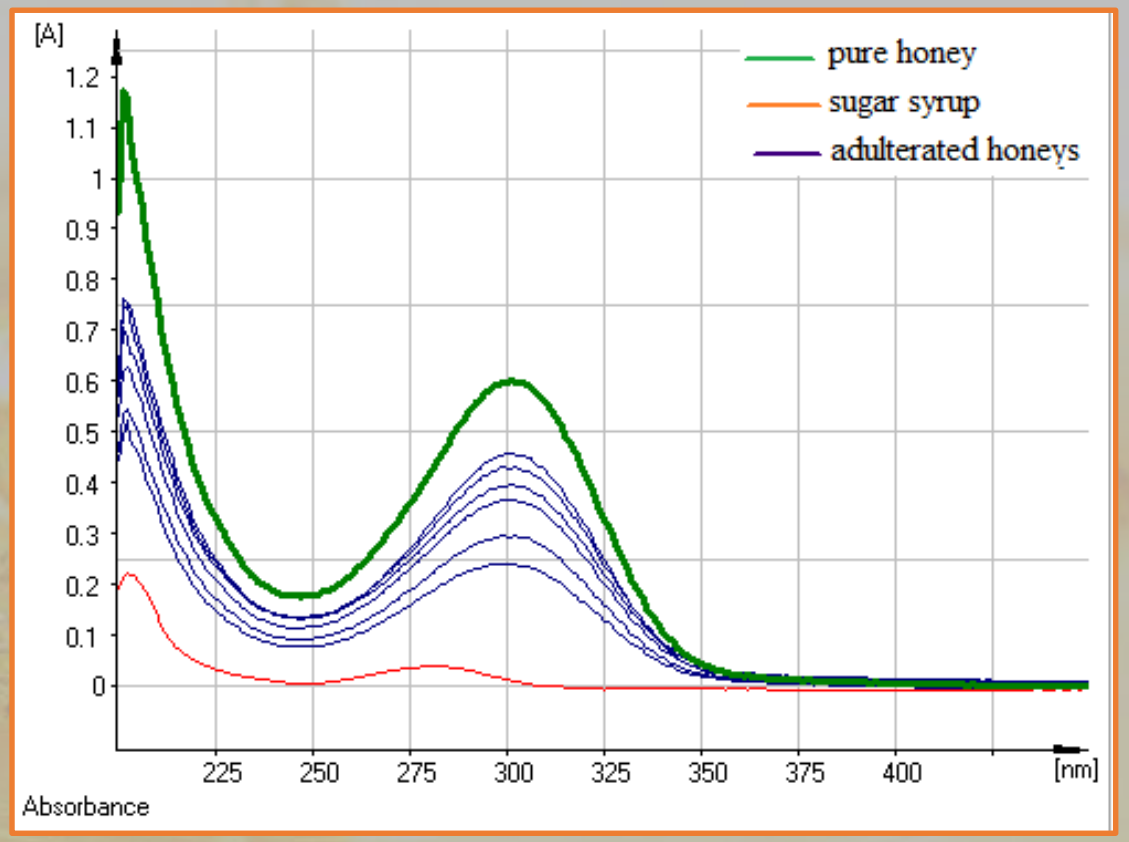
	$\delta^{13}C_{honey}$ (‰)	$\delta^{13}C_{protein}$ (‰)	C4 sugar (%)	mg GAE/100g	mg QE/100g	DPPH%
Acacia	-24,49	-24,61	0,80	50,53	2,01	7,70
Acacia	-23,3	-23,25	0,37	29,83	2,57	1,26
Acacia	-23,19	-22,34	6,72	31,36	2,53	2,69
Acacia	-23,46	-23,45	0,07	42,70	3,24	3,94
Rape	-22,12	-24,95	18,56	55,74	5,31	9,51
Rape	-26,08	-26,26	1,09	33,33	5,29	9,15
Rape	-27,09	-26,59	-2,96	40,98	4,02	2,69
Polyfloral	-22,11	-24,65	16,99	48,13	5,33	8,42
Polyfloral	-17,84	-24,49	44,96	28,70	5,90	3,45
Polyfloral	-25,5	-24,93	-3,74	70,11	6,01	11,78
Sunflower	-25,12	-25,42	1,91	46,60	4,77	7,31
Sunflower	-26,6	-25,48	7,1	59,11	8,61	9,24
Honeydew	-26,85	-25,88	-6,00	90,93	5,55	21,09
Honeydew	-25,04	-24,88	-1,05	98,68	7,07	29,45
Linden	-26,09	-25,36	-4,66	58,91	4,82	8,42

- Intensive bee feeding with sugar syrups from C4 plants conduct to a decrease of honey bioactive properties and this adulteration can be easily identified by $\delta^{13}C$ isotopic investigations.

UV-Vis fingerprinting of pure and adulterated honeys



UV-Vis spectral characterization of pure and adulterated sunflower honeys



UV-Vis spectral characterization of pure and adulterated sunflower honeys

Conclusions

- Detection of honey adulteration with sugar syrups obtained from C3 plants (beet, wheat, rice) is a challenge
- Spectroscopic techniques (UV-Vis) coupled with multivariate statistical analysis of the data can be considered as valuable fingerprinting methodologies used to detect adulteration of honey with C3 sugar syrups
- Direct incorporration of sugar syrups in honey led to the change of honey bioactive content, but within the range of variation for pure honeys
- Direct incorporation of sugar syrups in honey has produced a decrease of honey biochemical properties compared to the reference honey sample
- Intensive bee feeding during a long period of time conduct to the modification of honey chemical composition and quality similar to direct insertion of sugar into honey
- Multicomponent analysis, which involves the investigation of numerous parameters is necessary for quality control and authentication of honey

Acknowledgments

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Thank you for your attention!