



Recent Honey Adulteration Practices and Comprehensive Review on Commonly Used Analytical Approaches to Detect Honey Fraud

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Honey Fraud

Legal Basis for Honey Authenticity Judgement

A growing threat to viability of beekeeping industry and for food safety !!!



CODEX STANDARD FOR HONEY - CODEX STAN 12-1981

The Codex Alimentarius Standard and the EU Council Directive

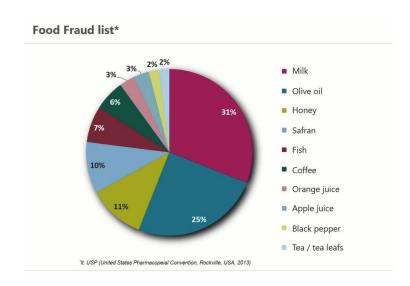
The honey standards of the Codex Alimentarius (CA) (4) and of the European Community (5) have been revised recently. The changes in the standards and the analytical methods used for their determination, following the advice of the International Honey Commission, were recently reviewed (6).

The standards of the Codex Alimentarius and the EU are very similar. The Codex Alimentarius honey standard is more detailed, containing references to quality factors such as heavy metals, pesticides and adulteration.

The definition of honey in both standards is the same:

"Honey is the natural sweet substance, produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own,

According to the CA standard the essential composition and quality factors are:
"3.1 Honey sold as such shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. Honey shall not have any objectionable matter, flavour, aroma, or taint absorbed from foreign matter during its processing and storage. The honey shall not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter.



Honey is the 3rd most Adulterated Foodstuff!!!







Honey Fraud

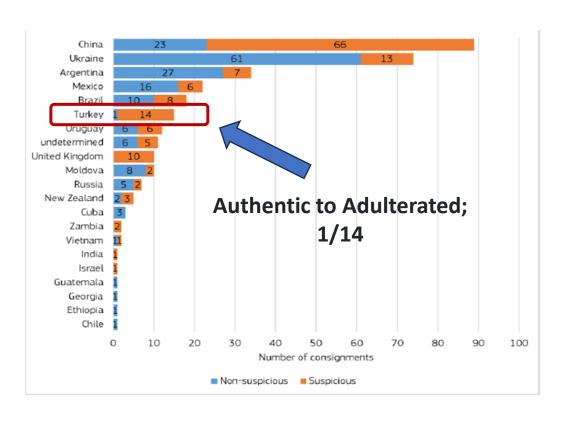
2023, EU JRC Annual Honey Adulteration Technical Report

Why Do We Need to Be Concerned?

2023; 46% of honeys (*n*=320) imported into the EU are suspicous of not complying with the provision of the 'EU Honey Directive 2001/110/EC'

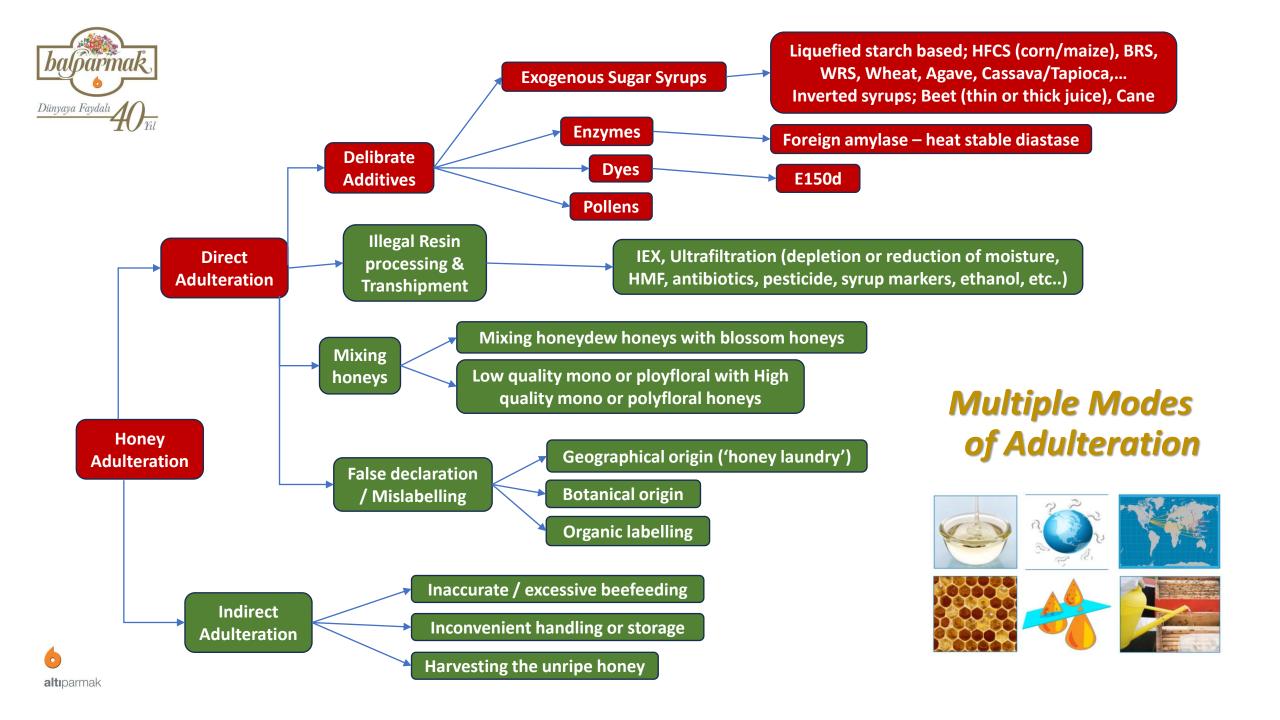
Previous Report; 14%













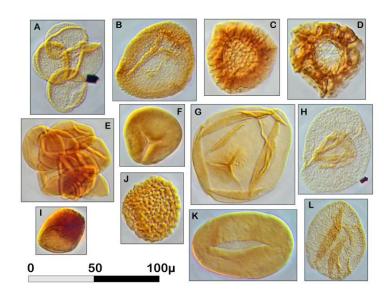


Organoleptic Analysis

It is based on testing of parameters such as **color, transparency, taste, odor, and viscosity** by experienced people.

Highly depends on personal interpretations and biased results are inevitable.

Discriminative laboratory tests are mandatory!!!



Palynologic Investigation

Botanical origins OK but not satisfying in terms of adulteration identification, pollen composition could be manipulated exogenously !!!







Even if they indicate the abnormalities, different botanical and geographical origins, climate changes, and seasonal variations impact results and false positives may be reported.

Adulteration-focused assays are necessary to provide accurate assessment !!!

Physicochemical Assays Physical - Chemical Parameters

description	technology	LOD
citric acid a)	enzymatic	20 mg/Kg
colour a)	hanna	1-150 mm
conductivity/pH-value a)	electrode	
diastase (activity) a)	enzymatic	3 U/Kg
ethanol a)	enzymatic	30 mg/Kg
F/G ratio a)	enzymatic, calculated	1%
formic acid a)	enzymatic	20 mg/Kg
free acids	titration	1 mmol/Kg
glycerine a)	enzymatic	30 mg/Kg
HMF a)	LC	1 mg/Kg
methylglyoxal (manuka honey) a)	LC	30 mg/Kg
methylanthranilate (orange blossom honey) ^{a)}	LC	0,1 mg/Kg
moisture a)	refractometry	13%-24%
saccharase (activity) ^{a)}	enzymatic	5 U/Kg
pollen analysis a) see packages code 200-204	microscopy	
proline	photometric	10 mg/kg
starch a)	microscopy	
sugar spectrum (5) a) fructose, glucose, sucrose, maltose, melezitose	LC	1%
thixotrophy (heather honey) a)	Thixotrophy (Louveaux)	pos./neg.
water-insoluble content	gravimetry	0,04 g/100g
yeast a)	microscopy	







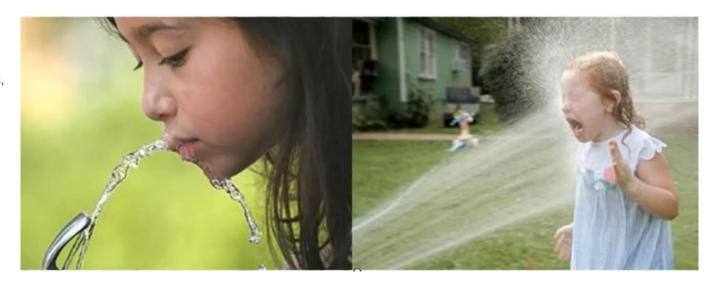
Trending Instrumental Analysis Techniques Targeted vs Untargeted

A Blessing or Curse Vacillation ???

TARGETED ANALYSIS



Looks at specific molecular masses and fragments (single substances, e.g. residue analysis, structure elucidation)



Scans an entire mass range (high resolution fingerprinting of compound mixture)



NON-TARGETED







Targeted Analyses (Marker Tests) Identification of **Honey Adulteration**

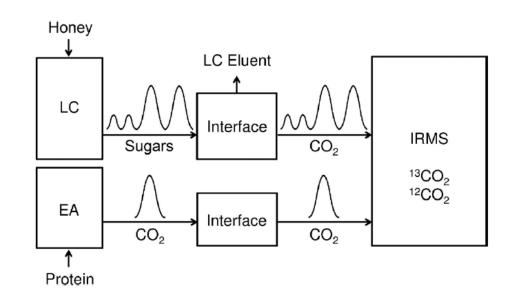






C4 & C3 Sugars by LC-EA-IRMS and EA-IRMS







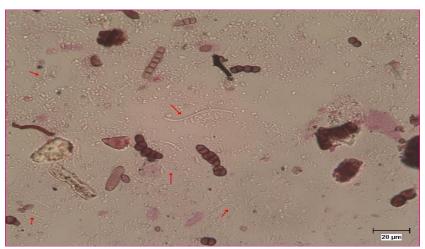
Plant Origin	Example s	Range of δ¹³C values	Method
C4-Plants	Corn Sugar Cane	-8 to -13 ‰	EA-IRMS (AOAC 998.12)
C3-Plants	Beet Rice Wheat Cichory	-22 to -30 ‰	LC-IRMS (new)

- If C4 sugars are admixed to honey, the carbon isotopic value of the bulk honey will shift to positive value while the corresponding δ^{13} C protein value will remain same.
- Inresponsive to well refined (tailored C3 syrups),
- Both method produces false positives for some botanical origins, (unique isotopic fingerprints)
- LC-EA-IRMS method is not sensitive. Detects roughly 30 %, w/w depending on honey type and adulterant.
- Detection of oligosaccharides are deceptive for honeydew honey in LC-EA-IRMS method !!!

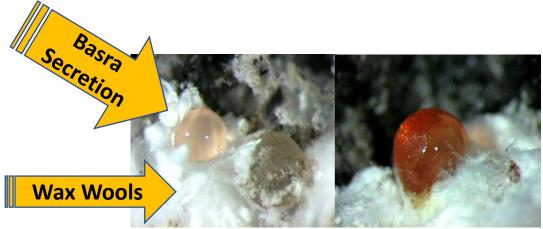




What is the Problem at Pine Honey C4% Measurements?







Pine honey is generally characterized by small-sized insoluble wax wools and honeydew elements (fungal spores, hyphae, pine spores, pollen grains).

These substances can form an appreciable amount of precipitate during acid/ heat induced protein flocculation at C4 assay.

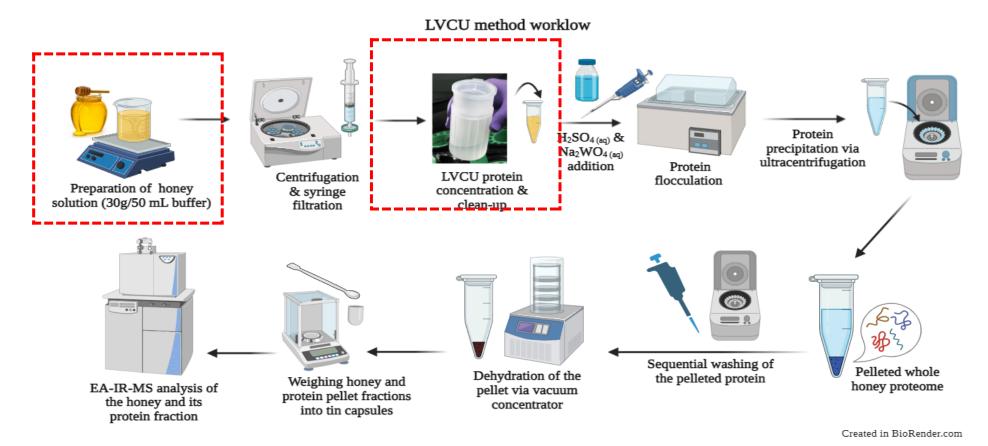
Hence, these may shift the protein-centric $\delta 13C$ values to more negative resulting higher C-4% levels at pine honeys







Developed Sample Pretreatment Protocol



LVCU = Large Volume Centrifugal Ultrafiltration Method







Feedback on Global Acceptance for New Protocol

Assessment of pine honey



Journal of Food Composition and Analysis 114 (2022) 104787



Contents lists available at ScienceDirect

Journal of Food Composition and Analysis



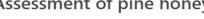


Elucidating the false positive tendency at AOAC 998.12 C-4 sugar test for pine honey samples: Modified sample preparation method for accurate δ^{13} C measurement of honey proteome



^a Chemistry Department, Marmara University, Istanbul, Turkey







← Yanıtla

Sent: Wednesday, September 28, 2022 12:31 PM To: Emel Damarlı < Emel. Damarli@balparmak.com.tr>

Tümünü yanıtla

Subject: Assessment of pine honey

Dear Emel.

I hope, this finds you well. Thank you for publishing your research on pine honey. Based on your publication, we will now change our assessment of pine honey. In case the 13C/12C isotope ratio of the protein is more negative than the value of honey, we have now the possibility to exclude the protein from assessment and can refer to your investigations. Unfortunately, we did not have the technical possibilities and capacities to carry out these investigations ourselves in the depth described.

Best wishes from Bremen

Ulrike

Ulrike Burmester Senior Lab Supervisor for Authenticity Food Services





b Department of R&D, Altiparmak Food Industry and Trade Inc., Istanbul, Turkey



Rice Syrup (White Rice Syrups & Brown Rice Syrups) Detection SM-R Method (AFGP)









Article

pubs.acs.org/JAFC

2-Acetylfuran-3-Glucopyranoside as a Novel Marker for the Detection of Honey Adulterated with Rice Syrup

Xiaofeng Xue, $^{\dagger,\ddagger,\perp}$ Qiang Wang, $^{\dagger,\parallel}$ Yi Li, $^{\ddagger,\parallel}$ Liming Wu, ‡ Lanzhen Chen, $^{\$}$ Jing Zhao, $^{\$}$ and Fengmao Liu $^{\perp,*}$

ABSTRACT: The determination of honey authenticity is of importance to ensure its quality and safety. There is an urgent need of effective methods to detect adulterated honey. A simple, rapid, and effective HPLC—DAD method was developed to detect honey adulteration by rice syrup, using a characteristic compound from rice syrup, which is presently difficult to detect by current analytical methods. The characteristic compound was identified as 2-acetylfuran-3-glucopyranoside (AFGP) by MS and NMR. Based on HPLC analyses, the average concentration of AFGP was $92 \pm 60 \text{ mg/kg}$ in rice syrup. However, AFGP was not detected in any of the natural honey samples, so it could be used as a marker for the detection of honey adulteration by rice syrup. The developed method enabled a rapid detection of honey samples adulterated with 10% rice syrup. Using the developed method, 16 out of 186 honey samples from some markets were found to be adulterated with rice syrup.

KEYWORDS: 2-acetylfuran-3-glucopyranoside, rice syrup, HPLC-DAD, adulterated honey







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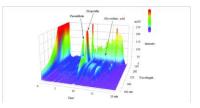
¹Department of Applied Chemistry, College of Science, China Agricultural University, Beijing, 100193, China



Marker Determination & Identification Workflow

UHPLC-DAD molecular screening (untargeted approach)

Syrup samples / Authentic Honeys from different origins / Adultered Honeys



Fractionation & Isolation the compound of interest – Analytical Scale

Sample enrcihment- extraction solvent/ratio optimization (MSweEt)

SALLE - Swedish Ext.
Sample prep. development



Scale up – semi preparative fractionation



LC-MS & GC-MS mass identification and database research







Targeted Analysis of identified molecule along with AFGP





Publication



Contents lists available at ScienceDirect

LWT







Identification of the rice syrup adulterated honey by introducing a candidate marker compound for Brown rice syrups

İsmail Emir Akyıldız ^{a,b,*}, Dilek Uzunöner ^b, Sinem Raday ^b, Sezer Acar ^b, Özge Erdem ^b, Emel Damarlı ^b

ARTICLE INFO

Keywords: Authenticity Honey Rice syrup

Sorbic acid

ABSTRACT

Identification of honey adulteration is an important area to ensure product safety and quality. White rice syrups (WRS) or brown rice syrups (BRS) can be used for honey adulteration. Up to date, qualitative analysis of 2-ace-tylfuran-3-glucopyranoside (AFGP) and the quantification of the arsenic residue are the commonly preferred methods to detect rice syrups (RS). We have figured out the BRS may have very low amount of AFGP. Therefore, it was estimated that AFGP alone may not be a very reliable marker for BRS identification. We aimed at identifying a new marker compound for BRS and to develop a novel analytical method that allows simultaneous monitoring of this compound and AFGP to highlight the addition of RS from different origins. The characteristic molecule in BRS was identified as sorbic acid. A UHPLC-MS/MS method was developed by combining dilute & shoot sample pretreatment and 107 samples were analyzed. While 21 of the samples were found adulterated with BRS, 3 samples were found to contain WRS. We suggest using sorbic acid as a marker of BRS addition to honey. Within this research, it was hypothesized that fraudulent was mostly made with BRS and adulteration may be overlooked applying the existing methodology.





^a Chemistry Department, Marmara University, Istanbul, Turkey

^b Department of R&D, Altiparmak Food Industry and Trade Inc., Istanbul, Turkey

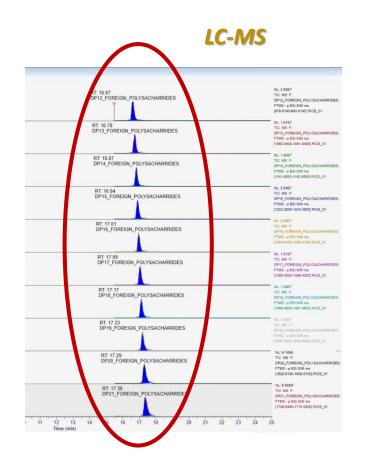


Foreign Oligosaccharides Monitoring

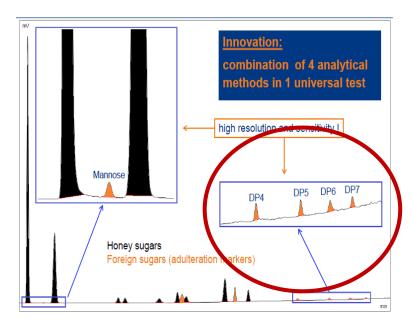
False Positives for Honeydew Honeys such as Pine, Oak, Metcalfa, Quillay !!!

LC-ELSD/CAD **Authentic honey** Adulterated honey Fig. 3: 1C-ELSD chromatogram of authentic and adulterated ple shows honey-foreign oligosocchand. (DP 5 to 27 10) which serve as marker sub-

stances to detect adulteration.



HPAE-PAD





Specific Markers for E150d Adulterants E150d Food Dyes (Caramel Sulfide Coloring)

Methyl-imidazole derivates does not seem Reliable. Residues may also be observed due to the different botanical origins of honey !!!

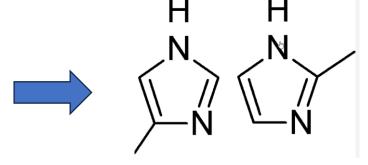












2-Methylimidazole / 4-Methylimidazole











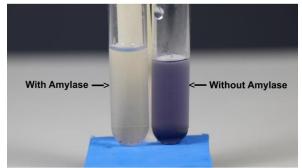


Foreign Diastase

Typically, total diastase assays use pure or dye-conjugated starch as substrate and measurements are made colorimetrically after enzymatic hydrolysis.

These assays are not specific in terms of *Apis Mellifera* L. (honey bee) amylase and can produce false positives if amylases from other organisms are added to honey.







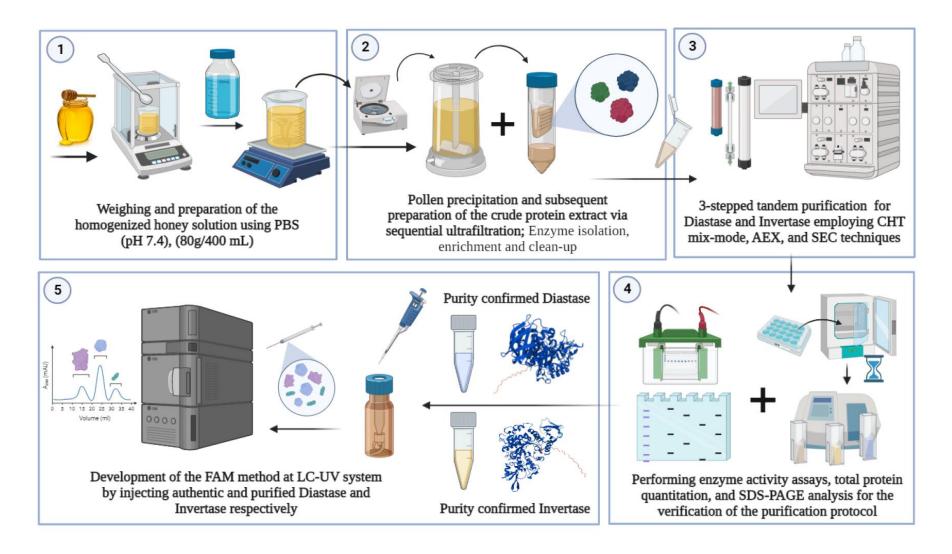






FAM (Foreign Amylase Monitoring)

(Enzyme extraction / Enrichment and Clean-up / Purification / LC-UV Analysis)













Contents lists available at ScienceDirect

Microchemical Journal





Straightforward monitoring of honey with foreign diastase by leveraging the differentiation in LC-UV proteome profiles of authentic and fraudulent samples

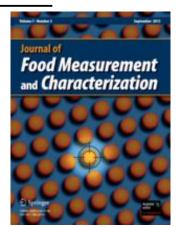
İsmail Emir Akyıldız ^{a,b,*,1}, Özge Erdem ^{b,2}, Sinem Raday ^{b,3}, Sezer Acar ^{a,b,4}, Dilek Uzunöner ^{b,5}, Emel Damarlı ^{b,6}, Ece Kök Yetimoğlu ^{a,7}

Journal of Food Measurement and Characterization https://doi.org/10.1007/s11694-022-01380-8

ORIGINAL PAPER

Development of a novel pretreatment protocol for the efficient isolation and enrichment of honey proteome using pine honey and the hypopharyngeal glands of *Apis mellifera* L.

Ismail Emir Akyıldız^{1,2} • Ece Kök Yetimoğlu¹ • Sinem Raday² • Özge Erdem² • Sezer Acar^{1,2} • Özlem Yilmaz² • Dilek Uzunöner² • Gamze Düz² • Emel Damarli²









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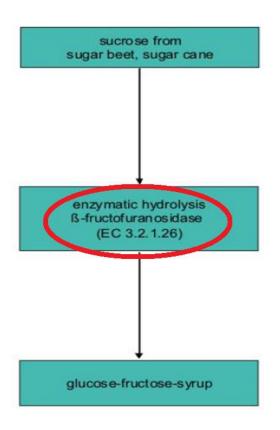


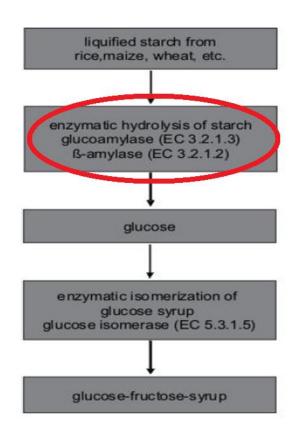
β-Fructofuranosidase and β/γ Amylase Assays

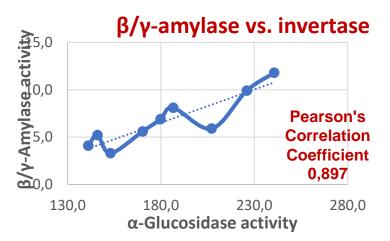




Beet and Cane sucrose are inverted mostly using other techniques and/or BFF residue is removed !!!







Equal to or greater than 5 U/kg is considered as an indicator of the use of sugar syrup.

Pine honey has high invertase values and this creates false positives !!!







Trending Techniques

Non-Targeted Metabolomic (Foodomic) Based Adulteration Identification Methods

Conventional Single marker methods (targeted only)



(Targeted and Untargeted Approaches)



As frauds become more sophisticated and deceive conventional techniques, there is a need for advanced multi-marker methods employing modern instrumentation !!!







Snippets From Our Novel Targeted & Un-Targeted Metabolomic Based HR-MS Method

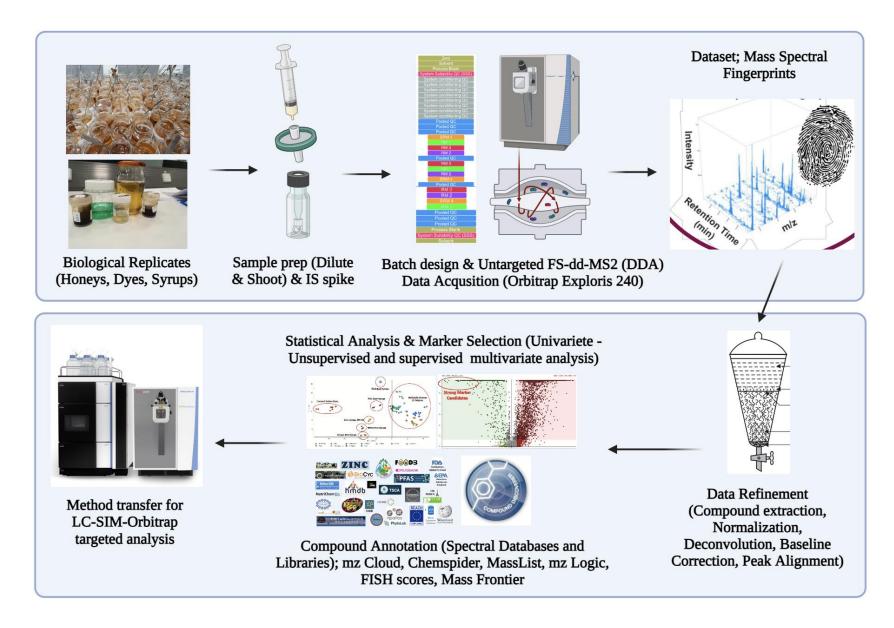








Workflow – Untargeted Metabolomic Survey - Overview

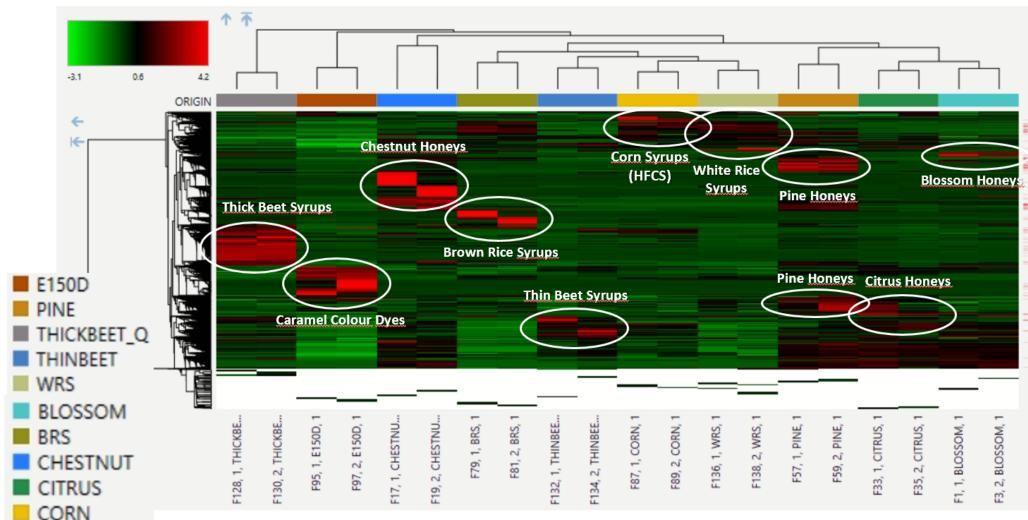








PCA Plots & Hierarchical Cluster Heatmaps (Shotgun Metabolomic – Adulterants & Honeys)





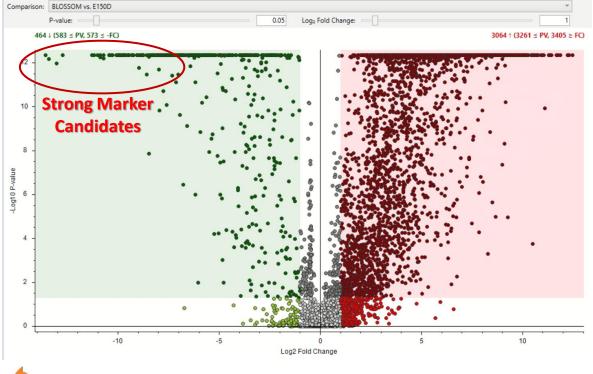




Volcano Plots

(Shotgun Metabolomic – Adulterants & Honeys)

VS E150d Dyes



Blossom Honey vs Corn Syrups (HFCS)









Targeted LC-SIM-MS Metabolomic

Multiplexed LC-SIM Assay; (E150d=10, BRS+WRS=5, CORN=9, BEET=11, Polysaccharides=10, Botanical Markers=4, Natural Honey Markers=2)

	Compound	Forn	Adduct	Precursor (m/z)	Precursor Charge (z)	t start (min)	t stop (min)	Scan Width (m/z)	RF Lens (%)	Polarity
1	BRS_M1		(no adduct)	483.2729	1	2.9	6	1	70	Negative
2	BRS_M2		(no adduct)	520.3394	1	5.4	8	1	70	Positive
3	BRS_M3		(no adduct)	468.3082	1	5.3	8.5	1	70	Positive
4	E150D_M1		(no adduct)	188.9864	1	1.5	4.5	1	70	Negative
5	BEET+CORN_M1 (Inversion and/or isomerization marker)		(no adduct)	524.1495	1	8.5	13	1	70	Positive
6	BEET+CORN_M2 (Inversion and/or isomerization marker)		(no adduct)	344.1163	1	8.5	13	1	70	Positive
7	BEET_M1		(no adduct)	683.2248	1	11.5	13.5	1	70	Negative
8	BEET_M2		(no adduct)	127.0391	1	12.2	13.7	1	70	Positive
9	BEET_M3		(no adduct)	408.1113	1	11.1	13.2	1	70	Positive
10	BRS_M4		(no adduct)	554.3463	1	5.5	8.6	1	70	Negative
11	WRS_M1		(no adduct)	305.0878	1	5	10	1	70	Negative
12	BEET_M4		(no adduct)	504.1923	1	10	15.5	1	70	Positive
13	CORN_M1		(no adduct)	239.0229	1	4	6	1	70	Negative
14	CORN_M2		(no adduct)	299.9931	1	4	6	1	70	Negative
15	CORN_M3		(no adduct)	376.9669	1	4	6	1	70	Negative
16	BEET+CORN_M3		(no adduct)	298.989	1	4	6	1	70	Negative
17	CORN_M4		(no adduct)	221.0667	1	4	6	1	70	Negative
18	CORN_M5		(no adduct)	728.2101	1	14	15.5	1	70	Negative
19	CORN_M6		(no adduct)	695.4301	1	1.5	4	1	70	Negative
20	BEET_M5		(no adduct)	307.1023	1	10	14	1	70	Positive

E150D_M2									
L130D_MZ		(no adduct)	188.0023	1	1	4	1	70	Negative
E150D_M3		(no adduct)	194.9969	1	2	5	1	70	Negative
E150D_M4		(no adduct)	164.9863	1	2	5	1	70	Negative
E150D_M5		(no adduct)	203.9973	1	2 ,	5	1	70	Negative
E150D_M6		(no adduct)	225.0075	1	5	8	1	70	Negative
E150D_M7		(no adduct)	247.0395	1	7	10	1	70	Negative
E150D_M8		(no adduct)	213.0075	1	11	14	1	70	Negative
E150D_M9		(no adduct)	182.9968	1	11	14	1	70	Negative
E150D_M10		(no adduct)	115.0411	1	7	10	1	70	Negative
Pine Marker_AcetylMaltose		(no adduct)	383.1194	1	11.5	13.5	1	50	Negative
(IS)_Glutaric Acid_neg		(no adduct)	131.0005	1	12	14	1	50	Negative
Citrus Marker_1_AnthranosideA		(no adduct)	314.1233	1	1	5	1	70	Positive
Citrus Marker_2_Caffeine		(no adduct)	195.0876	1	1	5	1	70	Positive
Chestnut Marker_KynurenicAcid		(no adduct)	188.0353	1	1	5	1	70	Negative
Manuka Marker_Rhamnopyranoside		(no adduct)	346.1497	1	1	7	1	70	Positive
Dilution Indicator_Gluconic Acid		(no adduct)	195.0516	1	12	15	1	70	Negative
Prolin		(no adduct)	116.1311	1	11	14	1	70	Positive
	E150D_M4 E150D_M5 E150D_M6 E150D_M7 E150D_M8 E150D_M9 E150D_M10 Pine Marker_AcetyIMaltose (IS)_Glutaric Acid_neg Citrus Marker_1_AnthranosideA Citrus Marker_2_Caffeine Chestnut Marker_KynurenicAcid Manuka Marker_Rhamnopyranoside Dilution Indicator_Gluconic Acid	E150D_M4 E150D_M5 E150D_M6 E150D_M6 E150D_M7 E150D_M8 E150D_M9 E150D_M10 Pine Marker_AcetylMaltose (IS)_Glutaric Acid_neg Citrus Marker_1_AnthranosideA Citrus Marker_2_Caffeine Chestnut Marker_KynurenicAcid Manuka Marker_Rhamnopyranoside Dilution Indicator_Gluconic Acid	E150D_M4 (no adduct) E150D_M5 (no adduct) E150D_M6 (no adduct) E150D_M7 (no adduct) E150D_M8 (no adduct) E150D_M9 (no adduct) E150D_M10 (no adduct) Pine Marker_AcetylMaltose (no adduct) (IS]_Glutaric Acid_neg (no adduct) Citrus Marker_1_AnthranosideA (no adduct) Citrus Marker_2_Caffeine (no adduct) Chestnut Marker_KynurenicAcid (no adduct) Manuka Marker_Rhamnopyranoside (no adduct) Diluttion Indicator_Gluconic Acid (no adduct)	E150D_M4 (no adduct) 164.9863 E150D_M5 (no adduct) 203.9973 E150D_M6 (no adduct) 225.0075 E150D_M7 (no adduct) 247.0395 E150D_M8 (no adduct) 213.0075 E150D_M9 (no adduct) 182.9968 E150D_M10 (no adduct) 333.1194 Pine Marker_AcetylMaltose (no adduct) 333.1194 (IS)_Glutaric Acid_neg (no adduct) 311.0005 Citrus Marker_1_AnthranosideA (no adduct) 314.1233 Citrus Marker_2_Caffeine (no adduct) 195.0876 Chestnut Marker_KynurenicAcid (no adduct) 346.1497 Dilution Indicator_Gluconic Acid (no adduct) 195.0516	E150D_M4 (no adduct) 164.9863 1 E150D_M5 (no adduct) 203.9973 1 E150D_M6 (no adduct) 225.0075 1 E150D_M7 (no adduct) 225.0075 1 E150D_M8 (no adduct) 213.0075 1 E150D_M9 (no adduct) 182.9968 1 E150D_M10 (no adduct) 115.0411 1 Pine Marker_AcetylMaltose (no adduct) 383.1194 1 (IS)_Glutaric Acid_neg (no adduct) 131.0005 1 Citrus Marker_1_AnthranosideA (no adduct) 314.1233 1 Citrus Marker_2_Caffeine (no adduct) 195.0876 1 Chestnut Marker_KymurenicAcid (no adduct) 188.0353 1 Manuka Marker_Rhamnopyranoside (no adduct) 346.1497 1 Dilution Indicator_Gluconic Acid (no adduct) 195.0516 1	E150D_M4 (no adduct) 164.9863 1 2 E150D_M5 (no adduct) 203.9973 1 2 E150D_M6 (no adduct) 225.0075 1 5 E150D_M7 (no adduct) 247.0395 1 7 E150D_M8 (no adduct) 213.0075 1 11 E150D_M9 (no adduct) 182.9968 1 11 E150D_M10 (no adduct) 115.0411 1 7 Pine Marker_AcetylMaltose (no adduct) 383.1194 1 11.5 (IS)_Glutaric Acid_neg (no adduct) 131.0005 1 12 Citrus Marker_1, AnthranosideA (no adduct) 314.1233 1 1 Citrus Marker_2, Caffeine (no adduct) 195.0876 1 1 Chestnut Marker_KynurenicAcid (no adduct) 346.1497 1 1 Manuka Marker_Rhamnopyranoside (no adduct) 346.1497 1 1 Dilution Indicator_Gluconic Acid (no adduct) 195.0516 1 <td>E150D_M4 (no adduct) 164.9863 1 2 5 E150D_M5 (no adduct) 203.9973 1 2 5 E150D_M6 (no adduct) 225.0075 1 5 8 E150D_M7 (no adduct) 247.0395 1 7 10 E150D_M8 (no adduct) 213.0075 1 11 14 E150D_M9 (no adduct) 182.9968 1 11 14 E150D_M10 (no adduct) 115.0411 1 7 10 Pine Marker_AcetylMaltose (no adduct) 133.1194 1 11.5 13.5 (IS)_Glutaric Acid_neg (no adduct) 131.0005 1 12 14 Citrus Marker_1, AnthranosideA (no adduct) 314.1233 1 1 5 Citrus Marker_2, Caffeine (no adduct) 195.0876 1 1 5 Chestnut Marker_KymurenicAcid (no adduct) 188.0353 1 1 5 Manuka Marker_Rhamnopyranoside<td>E150D_M4 (no adduct) 164,9863 1 2 5 1 E150D_M5 (no adduct) 203,9973 1 2 5 1 E150D_M6 (no adduct) 225,0075 1 5 8 1 E150D_M7 (no adduct) 247,0395 1 7 10 1 E150D_M8 (no adduct) 213,0075 1 11 14 1 E150D_M9 (no adduct) 182,9968 1 11 14 1 E150D_M10 (no adduct) 115,0411 1 7 10 1 E150D_M10 (no adduct) 115,0411 1 7 10 1 Pine Marker_AcetylMaltose (no adduct) 138,01194 1 11.5 13.5 1 (IS)_Glutaric Acid_neg (no adduct) 311,0005 1 12 14 1 Citrus Marker_1, AnthranosideA (no adduct) 314,1233 1 1 5 1 Citrus Marker_2,</td><td> E150D_M4</td></td>	E150D_M4 (no adduct) 164.9863 1 2 5 E150D_M5 (no adduct) 203.9973 1 2 5 E150D_M6 (no adduct) 225.0075 1 5 8 E150D_M7 (no adduct) 247.0395 1 7 10 E150D_M8 (no adduct) 213.0075 1 11 14 E150D_M9 (no adduct) 182.9968 1 11 14 E150D_M10 (no adduct) 115.0411 1 7 10 Pine Marker_AcetylMaltose (no adduct) 133.1194 1 11.5 13.5 (IS)_Glutaric Acid_neg (no adduct) 131.0005 1 12 14 Citrus Marker_1, AnthranosideA (no adduct) 314.1233 1 1 5 Citrus Marker_2, Caffeine (no adduct) 195.0876 1 1 5 Chestnut Marker_KymurenicAcid (no adduct) 188.0353 1 1 5 Manuka Marker_Rhamnopyranoside <td>E150D_M4 (no adduct) 164,9863 1 2 5 1 E150D_M5 (no adduct) 203,9973 1 2 5 1 E150D_M6 (no adduct) 225,0075 1 5 8 1 E150D_M7 (no adduct) 247,0395 1 7 10 1 E150D_M8 (no adduct) 213,0075 1 11 14 1 E150D_M9 (no adduct) 182,9968 1 11 14 1 E150D_M10 (no adduct) 115,0411 1 7 10 1 E150D_M10 (no adduct) 115,0411 1 7 10 1 Pine Marker_AcetylMaltose (no adduct) 138,01194 1 11.5 13.5 1 (IS)_Glutaric Acid_neg (no adduct) 311,0005 1 12 14 1 Citrus Marker_1, AnthranosideA (no adduct) 314,1233 1 1 5 1 Citrus Marker_2,</td> <td> E150D_M4</td>	E150D_M4 (no adduct) 164,9863 1 2 5 1 E150D_M5 (no adduct) 203,9973 1 2 5 1 E150D_M6 (no adduct) 225,0075 1 5 8 1 E150D_M7 (no adduct) 247,0395 1 7 10 1 E150D_M8 (no adduct) 213,0075 1 11 14 1 E150D_M9 (no adduct) 182,9968 1 11 14 1 E150D_M10 (no adduct) 115,0411 1 7 10 1 E150D_M10 (no adduct) 115,0411 1 7 10 1 Pine Marker_AcetylMaltose (no adduct) 138,01194 1 11.5 13.5 1 (IS)_Glutaric Acid_neg (no adduct) 311,0005 1 12 14 1 Citrus Marker_1, AnthranosideA (no adduct) 314,1233 1 1 5 1 Citrus Marker_2,	E150D_M4

	Compound	Formula	Adduct	Precursor (m/z)	Precursor Charge (z)	t start (min)	t stop (min)
1	Psicose_Mannose_X_(MAJORITY BEET MARKERS)		(no adduct)	179.056	1	7.75	15

	Compound	Formula	Adduct	Precursor (m/z)	Precursor Charge (z)	t start (min)	t stop (min)
1	DP12_FOREIGN_POLYSACHARRIDES		(no adduct)	980.314	2	15.5	25
2	DP13_FOREIGN_POLYSACHARRIDES		(no adduct)	1061.34	2	15.5	25
3	DP14_FOREIGN_POLYSACHARRIDES		(no adduct)	1142.35	2	15.5	25
4	DP15_FOREIGN_POLYSACHARRIDES		(no adduct)	1223.88	2	15.5	25
5	DP16_FOREIGN_POLYSACHARRIDES		(no adduct)	1304.91	2	15.5	25
6	DP17_FOREIGN_POLYSACHARRIDES		(no adduct)	1385.93	2	15.5	25
7	DP18_FOREIGN_POLYSACHARRIDES		(no adduct)	1466.96	2	15.5	25
8	DP19_FOREIGN_POLYSACHARRIDES		(no adduct)	1547.98	2	15.5	25
9	DP20_FOREIGN_POLYSACHARRIDES		(no adduct)	1629.01	2	15.5	25
10	DP21_FOREIGN_POLYSACHARRIDES		(no adduct)	1710.04	2	15.5	25

DEVELOPED SIM ACQUSITIONs for 50 Markers !!!







NMR & HR-MS Analysis Reports

Thin Beet (Refined-designer syrup) Inverted Sugar Syrup Detection

40% Thin Beet



Sample name **Packaging** Sampler Number sample tubes Type of seal Amount Reception temperature Reception date time Start / End of analysis

ST40 Plastic cup Client Without seal approx.50 g (each) room temperature 26.09.2023 26.09.2023 / 30.09.2023



Assessment

The NMR profiling test did not reveal any sugar addition.

The NMR profiling test revealed the following anomalies:

- the sucrose level is higher than usual in polyfloral honeys and exceeds the maximum defined by the EC directive 2001/110 for honey in general

However without knowledge of the floral composition we have passed the sample in the benefit of the doubt.

NMR Failed !!!

Test results

Authenticity

THP01 Honey Authenticity by LC-HRMS (#) Method

ICS SOP 520-17 (2021-04), LC-Orbitrap-HRMS

Result

Exogenous sugar

Not detected

HR-MS Failed Methods Should be Advanced !!!









Proficiency / Verification BIPEA PT Result



INTERLABORATORY
COMPARISONS
FINAL
REPORT

PT: 46A-ADULTERATED HONEYS

(Test No. 9)

Matrix: Adulterated honey - 9-9

Month of the test: NOVEMBER 2023

Only 2 labs among 17 participants were able to report the beet syrup adulteration (13.2%, w/w) using novel HR-MS method (E9491 – Balparmak) !!!



CRITERION		INT46		
UNIT				
LAB.		х		
E1027				
E2560				
E2637	Steps		Detail of activitie	es
E3028		D 1 0 11 2 1 2		
E3844	Matrix	Polyfloral honey spiked wit	n beet syrup.	
E4698	Spiking	Yes (13.2%)		
E5910	Spiring	100 (10.270)		
E6009				
E6365				
E6414				
E7981	des rapports 13C du miel à miel) est éga	r analyseur élémentaire couplé isotopiques a permis de mesur à et des protéines du miel. La d le à -0.9 ce qui est supérieur à as être adultéré par un sucre ex	er la déviation isotopique du ifférence arrondie (protéines- -1.2. Par conséquent ce miel	
E8152	The measure	ed sample is likely not adulterate	ed with any C4 derived sugar	
E9043	_	larker: detected of beet sugar s rs = yes; beet sugar syrup (SM-		
E9151	Non conforme	e		
E9491	Adulterated w	vith beet sugar syrup		







Real Sample Analysis

By utilizing novel targeted LC-SIM-MS metabolomic;



Predominant types of adulterations were found as beet syrup (inverted sugar syrup) (80%) and Foreign Diastase (89.2%).

This indicates inverted beet syrup usage and dilution effect masked with artificially increased diastase is the main fraudulent approach recently..















