50 Years of transforming lives through dairying

Present Scenario in Quality Testing of Honey

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Coverage of Presentation

- Overview of Honey Regulation
- Codex Standard / EU standard
- Comparison between Domestic and Export quality honey
- International trends in Honey
- Status of Current testing facilities in India and required improvement
- Collaboration with National Bee Board (NBB) for setting up facility at NDDB for testing

CALF

Established in 2009 as a single window service to cater the testing needs of dairy and food industry





Area of Service

- Milk & Milk Products, Food and Agricultural products, Fruits and vegetables
- Cattle Feed and Feed Ingredient
- Genetic analysis in blood and semen
- Training to quality control professionals





Accreditation & Recognition

- CALF is accredited by National Accreditation Board for Testing and Calibration Laboratories (NABL), DST, Govt of India as per ISO 17025 requirements, vide certificate nos. **T-2678 and T-2679** for chemical, microbiological and genetics analysis valid till October 2017.
- Bureau of Indian Standards (BIS) recognition for dairy products, cattle feed and mineral mixture.
- Food Safety Standards Authority of India (FSSAI) has granted Referral Food lab status for dairy products.

What is Honey ?

Attribute	Definition
General	It is a super saturated solution of sugar with high viscosity, high density and hygroscopic with granulation tendencies.
FSSAI/ AGMARK	Honey means the natural sweet substance produced by honey bees from the nectar of blossoms or from the secretions of plants which honey bees collect, transform and store in honey comb for ripening.
Codex & EU-Council Directive 2001/110/EC	It is a natural sweet substance produced by *Honey bees* (*Apis mellifera*) from the nectar of plants or secretions of living parts of plants or **excretions of plant sucking insects on the living parts of plant** , which bees collect, transform by combining specific substances of their own, deposits, **dehydrate**, store and leave in honeycombs to ripen and mature.

Types of Honey as per EU Council Directive

Types of Honey	Name of Honey	Definition
a) According to origin	i) Blossom honey or nectar honey	Honey obtained from the nectar of plants
	ii) Honeydew honey	Honey obtained mainly from excretions of plant sucking insects (*Hemiptera*) on the living part of plants or secretions of living parts of plants
b) According to mode of production and/or presentation	iii) Comb honey	Honey stored by bees in the cells of freshly built broodless combs or thin comb foundation sheets made solely of beeswax and sold in sealed whole combs or sections of such combs
	iv) Chunk honey or cut comb in honey	Honey which contains one or more pieces of comb honey
	v) Drained honey	Honey obtained by draining decapped broodless combs
	vi) Extracted honey	Honey obtained by centrifuging decapped broodless combs
	vii) Pressed honey	Honey obtained by pressing broodless combs with or without the application of moderate heat not exceeding 45°C
	viii) Filtered honey	Honey obtained by removing foreign inorganic or organic matter in such a way as to result in the significant removal of pollen.

Chemical constituents of Honey

Moisture **pH**	13.4 to 22.9 % 3.42 to 6.10
Carbohydrates	A. Monosaccharides: Fructose (27.25 to 44.26 %) and Glucose (22.03 to 40.75%); B. Disaccharides : Sucrose (0.25 to 7.57 %), Maltose (2.74 to 15.98 %) other sugar- isomaltose, maltulose, turanose and kojibiose. C. Higher sugars (0.13 to 8.49 %)
Proteins and Amino Acids	Nitrogen : 0 to 0.13 % which is in the form of number of enzymes & amino acids A. Invertase, amylase, glucose oxidase, catalase, acid phosphorylase B. Honey also contains eighteen free amino acids, of which the most abundant is proline.
Vitamins, Minerals and Antioxidants	A. Trace amounts of the B vitamins like riboflavin, niacin, folic acid, pantothenic acid, vitamin B6 and ascorbic acid (vitamin C), B. Minerals calcium, iron, zinc, potassium, phosphorous, magnesium, selenium, chromium and manganese. C. The main group of antioxidants are the flavonoids, (sp. pinocembrin, is unique to honey and bee propolis), catalase and selenium are also antioxidants.
Organic Acids	< 0.5 % of solid mass Organic acids such as acetic, butanoic, formic, citric, succinic, lactic, malic, pyroglutamic and gluconic acids and a number of aromatic acids.

Bee keeping in the United states Agri. Hand book No. 335 on the basis of analysis of 490 samples of floral honey & chemistry of Bees

Overview of Regulation

Various Regulation for Common Quality parameters

Quality Parameters Requirements for Honey										
Sr. No.	Characteristics	FSSAI	BIS			AGMARK			Codex	EU
			Special grade	A Grade	Standard grade	Special grade	A Grade	Standard grade		
1	Specific gravity at 27°C, Min	1.35	1.37	1.37	1.37	1.4	1.37	1.35	NA	NA
2	Moisture, % by mass, Max	25	20	22	25	20	22	25	20	20
	i) for Heather honey	NA	NA	NA	NA				23	
3	Total reducing sugars, % by mass, Min	65	70	65	65	70	65	65	60 F&G	60 F&G
	i) for Carbia colossa and Honey dew	60	NA	NA	NA	60	60	60	NA	
4	Sucrose, % by mass, Max	5	5	5	5	5	5	5	5	5
	i) for Carbia colossa and Honey dew	10	NA	NA	NA	10	10	10	NA	
5	Sucrose content, max, g/100g	NA	NA	NA	NA				5	
	i) for Alfalfa, Citrus spp, false Acacia, French. Honey suckle,	NA	NA	NA	NA				10	
	Menzies banksia, Red Gum, Leatherwood,									
	Eucryphia milligani, max, g/100g									
	ii) Lavender, Borage	NA	NA	NA	NA				15	
6	Fructose-glucose ratio, Min	0.95	1	1	1	1	0.95	0.95	NA	NA
7	Fructose and Glucose content (sum of both), g/100g	NA	NA	NA	NA				60	60
	i) Honeydew honey,Blends of	NA	NA	NA	NA				45	

Various Regulation for Common Quality parameters (contd..)

Quality Parameters Requirements for Honey										
Sr. No.	Characteristics	FSSAI	BIS			AGMARK			Codex	EU
			Special grade	A Grade	Standard grade	Special grade	A Grade	Standard grade		
8	Ash, % by mass, Max	0.5	0.5	0.5	0.5	0.5	0.5	0.5	NA	0.5
9	Acidity (expressed as formic acid), % by mass, Max	0.2	0.2	0.2	0.2	0.2	0.2	0.2	NA	
10	Fiehe's test	Negative	Negative	Negative	Negative	Negative	Negative	Negative	NA	
11	Hydroxy methyl furfural (HMF), mg/kg, Max	80	80	80	80	80	80	80	NA	
	i) after processing/blending	NA	NA	NA	NA				40	40
	ii) honeys originated from region with tropical ambient	NA	NA	NA	NA				80	80
	temerature and blends of these									
12	Total count of pollens and plant elements/g of honey, Max	NA	50000	50000	50000	50000	50000	50000		
13	Optical density, at 660 nm, %, Max	NA	0.3	0.3	0.3	0.3	0.3	0.3		
14	Water insoluble solids content, max, g/100g	NA	NA	NA	NA	0.5	0.5	0.5	0.1	0.1
	i) pressed honey, g/100g	NA	NA	NA	NA				0.5	0.5
15	Free acidity, max, milliequivalents acid per 1000g	NA	NA	NA	NA	40	40	40	50	50
16	Diastase activity, min, Schade units	NA	NA	NA	NA	3	3	3	8	8
	i) honeys with a low natural enzyme content, min, Schade units	NA	NA	NA	NA				3	3
17	Electrical conductivity, max, mS/cm	NA	NA	NA	NA				0.8	0.8
	i) Honeydew, Chestnut honey and blends of these except	NA	NA	NA	NA				0.8	0.8
	Strawberry tree, Bell heather, eucalyptus, Lime, Ling Heather,	NA	NA	NA	NA					
	Manuka or jelly bush, Tea tree, min, mS/cm	NA	NA	NA	NA					
18	Aniline chloride test	NA	NA	NA	NA	Negative	Negative	Negative	NA	

Domestic Regulation

FSSAI

AGMARK

Honey Regulation- Comparison for Contaminants

Sr. No.	Category of Tests	FSSAI	BIS	AGMARK	EIC RMP for EU	CODEX
1	Pesticides	NA	NA	NA	22	Yes
2	Heavy metals	8	NA	As per FSSAI	2	Yes
3	Antibiotics	10	NA	As per FSSAI	12	Yes
Agmark outlines to follow Codex Alementarious commission or buyers requirement for heavy metals, pesticides and other food safety requirments for export.						

List of Antibiotics as per FSSAI

Sl. No.	Name of Antibiotics	Tolerance Limit (µg/kg)
1	Chloramphenicol	0.3
2	Nitrofurans and its metabolites	0.5 (either individually or collectively)
3	Sulphonamides and its emtabolites	5.0 (either individually or collectively)
4	Streptomycin	5.0
5	Tetracycline	5.0
	(a) Oxytetracycline	5.0
	(b) Chlortetracycline	5.0
6	Ampicillin	5.0
7	Enrofloxacin	5.0
8	Ciprofloxacin	5.0
9	Erythromycin	5.0
10	Tylosin	5.0

International Trend-Authenticity

Authenticity	Motive ?
Adulteration with cheap sugars	• To increase the volume of product
Mislabelled for **botanical origin**	• To Modify the consumer perception of quality and value of honey
Mislabelled for **geographical origin**	• To circumvent bans • To avoid tariff rules • High realization for honey changing country of origin

Authenticity – Fraud on Geographical Origin

- Anti dumping Duty of 220 % was imposed on Chinese honey by USA in 2001.
- To circumvent the anti dumping duty, honey was shipped from China to Australia via Singapore.
- The honey was declared as a product of Australia and exported to USA.
- The payment were made from USA to Australia and from Australia to Chinese by respective companies.

European Control plan (2015) For Authenticity

- To assess the prevalence of market honey for authenticity, 28 member states, Switzerland and Norway collected 2237 samples and tested for
  - **Adulteration with sugar**
  - Mislabelling with regard to **botanical** and **geographical origin**
  - Physico-chemical parameters

Authenticity Findings of European Control Plan

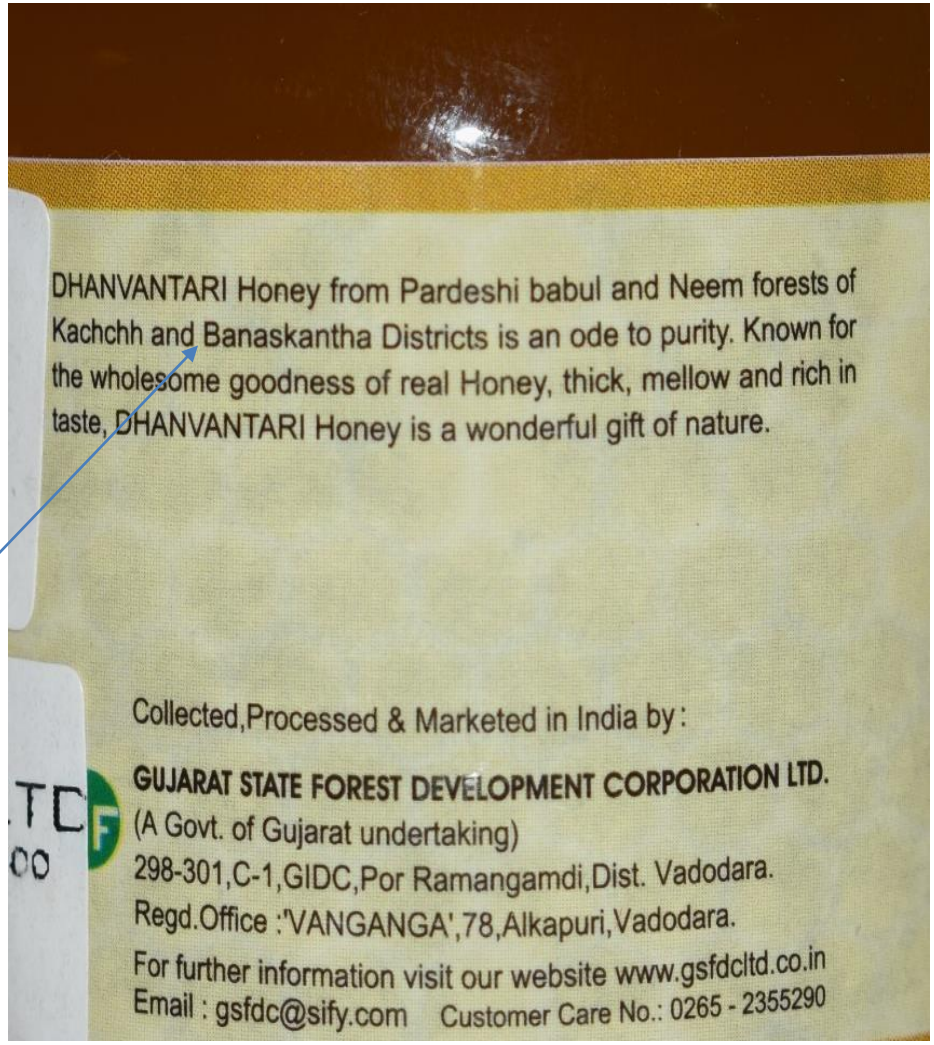
Type of Non Compliance	Non Compliance samples (%)
Physico-chemical parameters	2 %
Botanical Source	7 %
Geographical Origin	2 %
Sugar	6 %
Other Labelling	2 %
Total	19 %

Authenticity – Indian Scenario

- FSSAI & BIS does not outline requirement for Botanical & Geographical origin
- AGMARK specifies under marking and labelling :
  - Name of Geographical origin/area is Optional.
  - Honey may be designated according to floral or plant source **(Botanical)** if it comes wholly or mainly from any particular source and has the organoleptic, physico-chemical and microscopic properties corresponding with the origin.

Model-Geographical India

GEOGRAPHICAL



Authenticity - An Analytical Challenge

- It is a complex analysis, requiring high end equipment, trained and competent manpower.
- Adulteration with sugar from C4 plants (cane & corn) can be checked as per **AOAC 998.12** by Elemental Analyser hyphenated with Isotope Ratio Mass Spectrometry (EA-IRMS).
- Botanical origin are derived from the pollen spectrum of honey.
- Geographical origin are verified from pollen spectrum and traceability documents.

Authenticity- Techniques for Adulteration

- Sugar (sucrose) is already present in sample and if adulterated with exogenous sugar, so it is difficult to detect & confirm with routine laboratory techniques and equipment.
- Analysis technique is based upon Stable carbon isotope ratio analysis.
- In nature, carbon exists as ^{12}C and ^{13}C with a abundance of 98.93 % and 1.07 %.
- The distribution of these isotope in any product is know as the **isotopic signature** of product and remains constant.

Authenticity – Technique of Adulteration (contd..)

- During Photosynthesis process, plants follows C3 or C4 pathways for carbon fixation.
- C3 and C4 plants have different **Isotopic Signatures.**
- Generally, plants visited by honey bees for production of honey are C3 plants.
- The common source of adulteration for sugar (cane sugar or corn syrup) are C4 plants.
- On the basis of different isotopic signature of C3 and C4 plants, exogenous sugars of C4 plant origin can be quantified by using EA-IRMS.

Authenticity –
Analytical Challenges in Adulteration

- The available testing method have a strong limitation to detect the adulteration with exogenous sugars derived from plants with a C3 photosynthetic cycle such as beet, rice, cassava, wheat, chicory, maple tree and many fruits.
- The LC-IRMS would be used for this analysis (Non official method).

Authenticity – Analytical Technique for Adulteration

Adulteration with C4 sugars

Samples Heated at High Temp

↓

Converted to gases

↓

Gases are analysed for ^{12}C and ^{13}C

Adulteration with C3 sugars

Sugars are separated on LC

↓

Samples are dried after separation

↓

Sugars are heated at High temp and converted to gases.

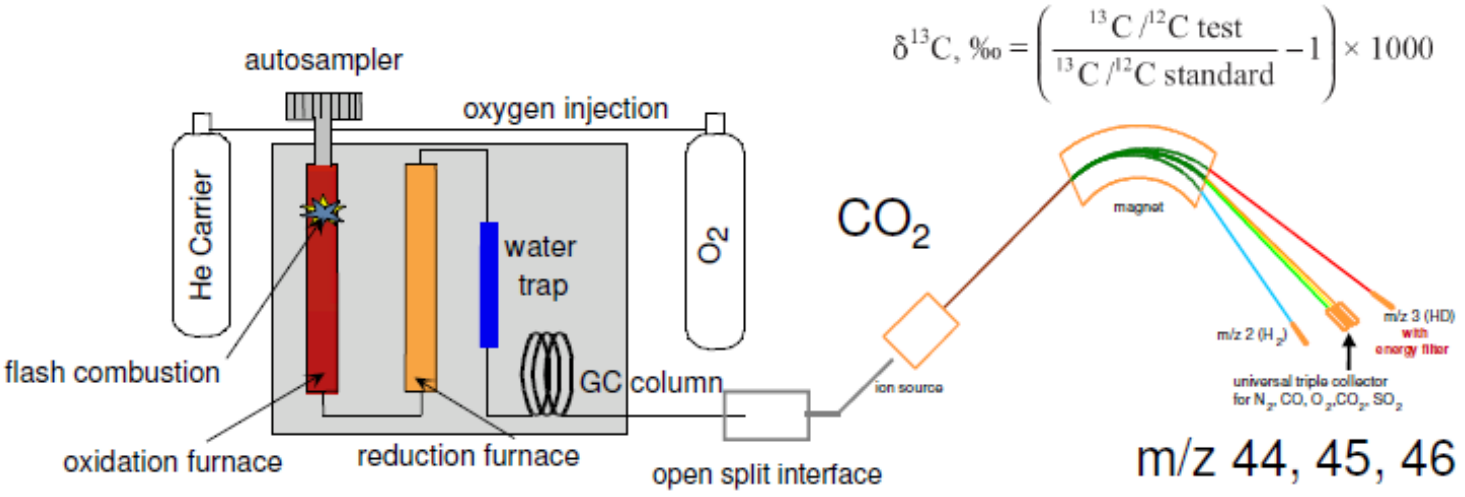
↓

Gases are analysed for ^{12}C and ^{13}C

C-4 Sugar Determination

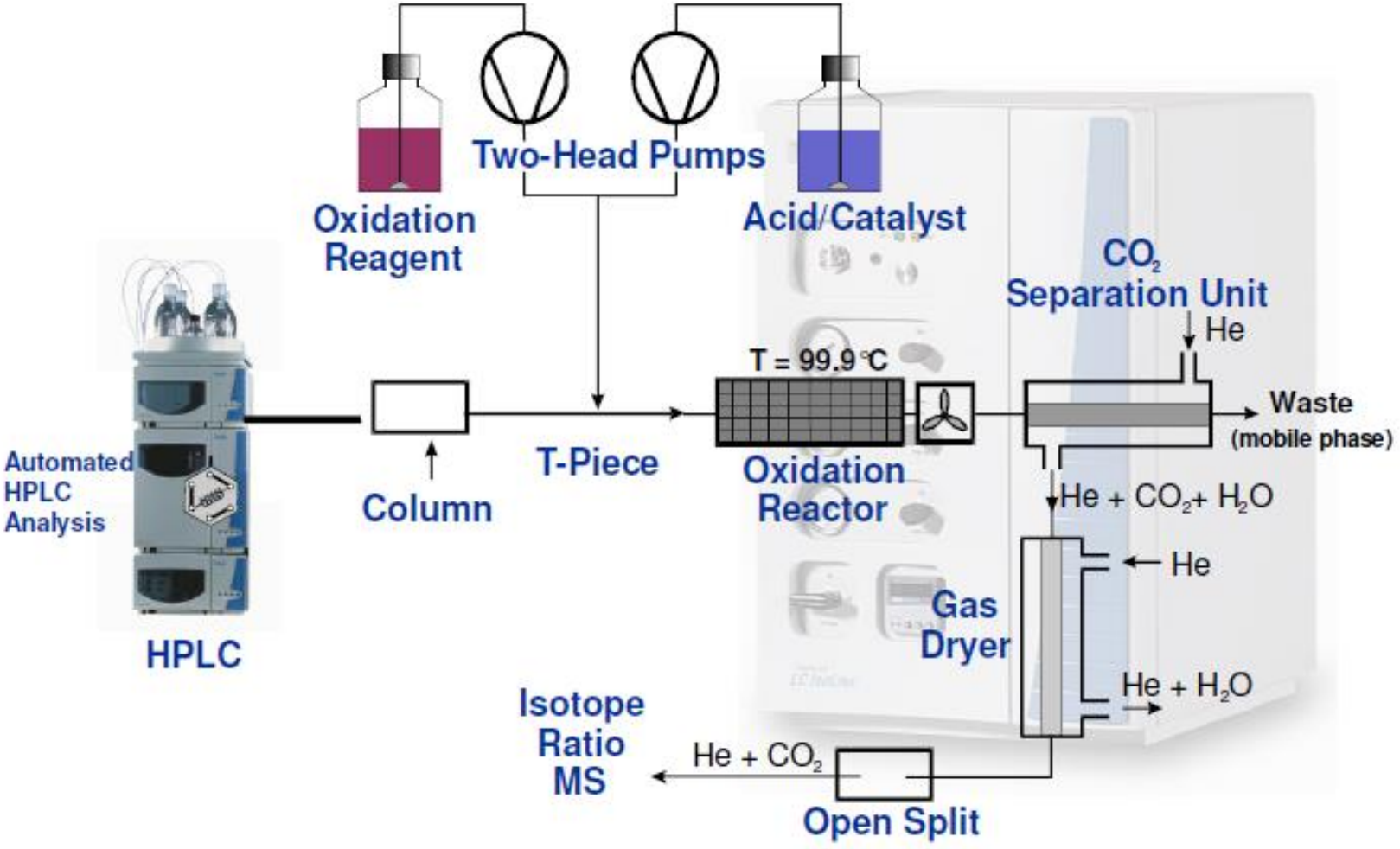
Authenticity Control of Honey — EA-IRMS

- Determination of C4 sugar addition – Cont. Flow Method
  - Analysis 1: Whole Honey ~ $\delta^{13}C_{Sugar}$
  - Analysis 2: Protein Precipitate = $\delta^{13}C_{Protein}$



Cited from Thermo Fischer

C-3 Sugar Determination on LC-IRMS



Cited from Thermo Fischer

Authenticity - Botanical Origin

Pollen analysis is useful to determine botanical origin of honey.

- Honey always include numerous pollen grains (mainly from the plants foraged by honey bees) and honey dew elements that altogether provide a good **fingerprint** where the honey comes from.
- Honey is considered of given botanical origin (unifloral honey), if relative frequency of the pollen of that *taxon / species* exceeds 45 %.

Authenticity - Geographical Origin

- Geographical origin of honey is checked with **traceability documents** and **microscopic pollen** analysis.
- It is based on the entire pollen spectrum being consistent with the flora of a particular region and with any reference spectra or descriptions in the literature.
- The test may be conclusive when a particular floral species is only growing in specific areas **or** when certain pollen combinations are typical from the region in which honey was produced.

Status of Current Testing Facility in India & way forward

- Indian laboratories – Appear to have competence and capabilities for the analysis of Physico- chemical parameters and Contaminants (Heavy metal, Pesticides, Antibiotics & Veterinary drugs).
- Three laboratories have been approved by EIC for Honey testing as per Residue Monitoring Plan (2016-17) for export to EU.
- However, facility need to be upgraded in India for **authenticity** with new equipment.
- Country need to generate spectrum of pollen in planned way for different geographical regions.
- Authenticity check is **getting outsourced** by major exporters due to lack of availability of facilities in India.

Collaboration with National Bee Board for testing of Honey

- NDDB may develop facility at CALF for testing **Authenticity** using EI-IRMS, LC-IRMS and **Contaminants** using LC-MS/MS, GC-MS/MS and ICP-MS with support of National Bee Board.
- The samples from farmers and dairy cooperatives for these parameters can be tested.


"If the bee disappeared off the face of the earth, man would only have four years left to live." (Albert Einstein)

Thank You For Your Kind Attention