GHIC
Enforcement Statement

Proteomics
as Direct Evidence of Non-Compliant Honey

Honey is not a formulated product. Honey is a natural product produced by bees. Under the EU Honey Directive, a product marketed as honey must not contain added food ingredients, food additives, or any other addition other than honey. This is the legal foundation of the issue. Once a foreign substance is added, the product is no longer compliant honey.

GHIC considers the detection of artificial foreign enzymes in honey by proteomics to be a decisive analytical development in honey enforcement.

The reason is simple: traditional diastase testing measures activity. Proteomics detects the foreign protein itself.

The Weakness of Traditional Diastase Testing

Diastase activity measures the starch-degrading activity of amylase-type enzymes in honey. These enzymes are naturally present in honey, mainly because bees add them during honey production. Diastase activity is therefore used as a quality parameter and forms part of the compositional criteria applied to honey. Codex and EU-related standards generally require diastase activity of not less than 8 Schade units, with specific exceptions for honeys with naturally low enzyme content.

However, diastase activity is not a source-identification method. It does not determine whether the enzyme activity comes from the honeybee or from an industrial enzyme added afterwards.

This creates a major loophole. If honey has been diluted with syrup, overheated, overprocessed, or otherwise manipulated, its natural enzyme activity may be too low. Fraudsters can then add industrial amylase or other enzymes to restore the measured activity. The product may pass the official activity threshold while still being adulterated.

This is exactly the enforcement problem: the test result may look compliant, while the product is not compliant.

Eurofins describes this weakness clearly: conventional enzyme activity tests cannot reliably distinguish bee-derived amylase or invertase from enzymes originating from other organisms. This matches the GHIC working position: standard tests look at activity, not at the specific enzyme protein.

What Proteomics Changes

Proteomics closes this loophole. Proteomics does not ask only:
“Is there enzyme activity?”

Proteomics asks: “Which protein is present, and from which organism does it originate?”

Even if two enzymes perform the same function, their amino acid sequences are different. An amylase from *Apis mellifera* and an amylase from *Aspergillus*, *Bacillus*, *Rhizopus*, or another industrial source may all degrade starch. But they are not the same protein.

Modern LC-MS/MS and LC-HRMS proteomics can detect the characteristic peptides of these proteins. These peptide sequences act as molecular fingerprints. If the fingerprints match a foreign enzyme, the conclusion is direct: the product contains a foreign protein.

Eurofins describes a targeted bottom-up proteomics workflow for honey in which proteins are purified, digested into peptides, and measured by LC-MS/MS or LC-HRMS. The method is specifically designed to detect foreign enzymes that common activity tests such as Phadebas or Schade cannot detect.

Why a Positive Foreign-Enzyme Finding Matters

A positive proteomics finding is not merely a suspicious profile. It is not only an indirect statistical deviation. It is direct analytical evidence that a foreign protein is present in the product.

That distinction is crucial for enforcement.

Where an artificial foreign enzyme is detected in a product sold as honey, the relevant question is no longer whether the honey “looks unusual”. The relevant question is whether a foreign substance has been found in a product whose legal definition prohibits such additions.

GHIC’s position is therefore clear:

If an artificial foreign enzyme protein is detected in a product marketed as honey, that product should be treated as suspected non-compliant honey unless the operator can provide a scientifically credible and legally acceptable explanation for the presence of that foreign protein.

In practical terms, such an explanation will rarely be plausible. Industrial amylases are not part of normal beekeeping practice. They are not a natural component of honey. Their accidental presence in a finished retail honey is not a credible default assumption.

Foreign Enzymes Are Not a Minor Technical Detail

The addition of artificial enzymes is not a harmless processing aid in honey. It is a method that can be used to disguise adulteration.

Foreign enzymes can be used to raise diastase or amylase activity after syrup dilution or improper processing. That allows a manipulated product to satisfy an official quality parameter that was originally intended to protect honey authenticity.

In other words, artificial enzymes can be used to make fake or manipulated honey appear compliant.

This makes foreign-enzyme detection an enforcement trigger. It should not be treated as a laboratory curiosity. It should be treated as evidence of a possible deliberate attempt to bypass existing honey controls.

Practical Use of the Method

Eurofins has publicly described the use of targeted bottom-up proteomics for the detection of foreign enzymes in honey. The Eurofins workflow currently includes, among others, α-amylase from *Aspergillus oryzae*, α-amylases from *Bacillus licheniformis* and *Bacillus amyloliquefaciens*, amyloglucosidase from *Aspergillus niger*, invertase from *Saccharomyces cerevisiae*, glucoamylase from *Rhizopus* species, and natural honey proteins such as MRJP1, α-glucosidase from *Apis mellifera*, and α-amylase from *Apis mellifera*.

Eurofins also states that a foreign enzyme is reported as positive only when the required peptides and qualifiers are detected, and that the method is qualitative, with detection limits in the low µg/g range depending on equipment and sample amount.

Eurofins' honey and bee products testing portfolio states that its laboratory is accredited according to ISO 17025. For formal enforcement use, the exact accreditation status of the specific proteomics parameter should always be checked in the individual laboratory report and the current accreditation annex. This wording is important: GHIC should rely on the actual report scope, not on general marketing language.

Scientific Support

The scientific literature supports the principle behind this approach.

Erban et al. showed in 2021 that LC-MS/MS proteomics can identify adulteration by foreign amylases in honey. In their dataset of 45 honey samples, four honeys contained foreign amylases from *Aspergillus niger*, *Bacillus amyloliquefaciens*, and/or *Bacillus licheniformis*.

Takahashi et al. investigated 67 commercial honeys in Japan and detected α-amylases from *Aspergillus* and *Geobacillus*in several samples, supporting the practical relevance of foreign-amylase findings in commercial honey.

Zheng et al. used SWATH-MS proteomics to generate honey proteomic signatures for identifying syrup-adulterated honey and for classifying honey by country of production and nectar source. Zhang et al. later demonstrated that targeted and untargeted proteomics can identify peptide markers for rape honey and apply them to adulteration analysis, including the differentiation of acacia honey, rape honey, and adulterated samples.

The wider trend is clear: protein and peptide markers are becoming increasingly useful in honey authenticity work. Proteomics is not merely a research concept. It is moving into practical authenticity testing.

Enforcement Consequence

A positive foreign-enzyme result should lead to immediate action.

GHIC recommends that retailers, importers, packers, authorities, and producer organisations treat a confirmed foreign-enzyme finding as a serious non-compliance signal. The minimum response should be:

1. Immediate quarantine of the affected lot.
2. Verification of chain of custody and sampling documentation.
3. Confirmation by an independent counter-sample where required.
4. Full review of supplier documentation, batch records, blending records, and import documents.
5. Additional authenticity testing by LC-HRMS, NMR, IRMS, metagenomics, and other suitable methods where necessary.
6. Notification of competent authorities where legal thresholds for suspicion are met.
7. Withdrawal from sale if the finding is confirmed and no credible legal explanation exists.

The European Commission's coordinated action "From the Hives" showed that 46% of 320 imported honey samples were suspicious of non-compliance and that fraudsters may use sophisticated methods to adapt honey/sugar blends to evade detection by clients and authorities. This confirms the need for stronger, more direct tools.

Proteomics provides such a tool.

GHIC Position

GHIC's position is therefore:

A product marketed as honey that contains an artificial foreign enzyme protein should not be treated as compliant honey.

The presence of a foreign enzyme is direct evidence of a foreign substance in the product. Since honey law prohibits additions other than honey, the finding is sufficient to raise a serious suspicion of adulteration and non-compliance.

Traditional diastase activity testing can be manipulated. Proteomics exposes the manipulation.

For GHIC, this method should become part of modern honey enforcement, especially where products show suspicious pricing, unusual import patterns, reduced natural honey protein profiles, questionable supplier histories, or previous analytical inconsistencies.

Proteomics does not replace the full investigative process. But where it detects a foreign enzyme, it gives authorities, retailers, and the honey sector a clear analytical fact:

This product contains a protein that should not be there.

That is enough to act.

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