Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

Guideline on sampling and analysis for the detection of pollen from genetically modified plants in honey

This guideline was established in co-operation with the § 64 LFGB (*German Food and Feed Act*) working group “Development of methods to identify foodstuffs produced by means of genetic engineering techniques“ of the Federal Office of Consumer Protection and Food Safety (BVL), the German federal state authorities responsible for surveillance as well as with the assistance of the working group of food chemistry experts of the German federal states and of the BVL (ALS). It is a recommendation with the aim of harmonising the sampling and analysis of honey for traces of pollen of genetically modified plants in the framework of the surveillance carried out by the responsible authorities of the German federal states as well as of in-house controls by the industry.

Sampling

Sampling is performed according to DIN 10742:2011-06 [1]. The sampling quantity is at least 1 kg. At the laboratory two test portions of 50 g are taken from the sample.

Sample preparation and DNA extraction

The honey may be warmed up for homogenisation; mix thoroughly by stirring. Each test portion is submitted to a CTAB-based DNA-extraction method for honey, with optional subsequent clean-up [2, 3]. The procedure is as follows:
Distribute each test portion into four parts of 12.5 g of homogenised honey to sterile 50 ml centrifugation tubes, add approx. 40 ml of distilled water to each tube, dissolve for approx. 10 min at 30-40℃ by shaking, and finally centrifuge for 10 m in at approx. 4.000 x g. Suspend the pollen removed by centrifugation with 5 ml of distilled water and reunite the four parts of the test portion. Fill up the pollen suspension with distilled water to a volume of approx. 20-30 ml and centrifuge again for 5 min at approx. 4.000 x g. Decant the supernatant, re-suspend the pollen in approx. 0.5 ml of sterile water and transfer the suspension into a 2 ml reaction vessel containing approx. 100 mg of glass beads (particle size approx. 500 µm).
Allow the suspension to stand over night. Subsequently, shake it in an oscillating mill at a frequency of 20 s^{-1} or in a mechanical high-speed shaker (e.g. Vortex) for 1 min [2, 3].
For extraction add 1 ml of CTAB buffer and incubate for 30 min at 65℃ and at maximum shaking frequency in a thermo-shaker. As an option, an RNase digest may be carried out at this point. Add Proteinase K-solution and incubate again for 60 min at approx. 65℃ while shaking. Let the samples cool down at room temperature and centrifuge for 10 min at 13.000 x g or more. Transfer 1 ml of the clear supernatant into a new 2 ml reaction vessel. Add 500 µl of chloroform and mix for approx. 30 s. Centrifuge for 10 min at 13.000 x g, then transfer the upper aqueous layer into a new 1.5 ml reaction vessel. Precipitate the DNA with 0.8 volumes of isopropanol for 30 min at room temperature and pelletize by centrifugation for 10 min at 15.000 x g. Wash the pellet with ethanol (70%), dry and dissolve in 50 µl of TE [3].
Other methods can be used if they produce results of the same or better quality.
As an option, the obtained DNA can be used to carry out a clean-up procedure for the elimination of possible inhibitors [2].

The undiluted DNA-solutions should be tested for the presence of inhibitors by applying a suitable procedure (e.g. [4]). To control the amplificability of the extracted DNA, a plant-specific PCR procedure can be applied (e.g. [5]).

PCR detection

For the PCR the DNA should be undiluted if possible. DNA which is used for the measurement of the calibration curve should be isolated from reference material by applying a suitable extraction method [6].

To test for the presence of pollen from genetically modified plants in honey, an analytical strategy is employed which is based on an increase in specificity in the order of the real-time PCR methods applied.

Screening method

At first validated screening methods [7, 8, 9, 10] are used to analyse for traces of genetically modified plants (e.g. P35S, T-nos, bar, CTP2-CP4EPSPS). Positive findings can be specified by combining different screening methods [11, 12]. In order to limit the screening methods to be employed, preliminary tests for plant species-specific reference genes (e.g. rape, maize, soya and possibly further species) can be carried out [7, 8, 9, 13].

Event-specific detection

Following the orientational screening, specification and quantification are realised, ideally using validated event-specific methods [7, 9, 13]. If no event-specific methods are available, a specification can be reached by applying further screening and construct-specific methods [7, 8, 9, 10, 14]. The reference material used for quantification must be described in detail in the analytical report.

Evaluation of PCR results

The results for the test portions and the control samples must be unambiguous and the controls must show the expected results (according to DIN EN ISO 24276 [15]).

For quantification with an event-specific method, the practical limit of detection and limit of quantification must be determined via the ratio of the theoretically achievable limit of detection and limit of quantification of the transgene detection to the sample-related quantity of amplified species-DNA [6].

The use of quality control DNA, e.g. [16], can be helpful to decide when a real-time PCR result is to be assessed as positive.

References

[1] DIN 10742:2011-06, Untersuchung von Honig - Leitfaden zur Probenahme. Beuth, Berlin.

[2] Waiblinger HU, Ohmenhäuser M, Pietsch K, Ritter W, Steegmüller J, Krech A, Horn P, Schroeder A: Die Untersuchung von transgenem Rapspollen in Honigen mittels Real-time-PCR. Dtsch. Lebensm. Rdsch. (2005) 101, Heft 12: 543–549.

[3] Waiblinger HU, Wurz A, Freyer R, Pietsch K: Spezifischer Nachweis von gentechnisch verändertem Raps in Honig. Dtsch. Lebensm. Rdsch. (1999) 95, Heft 5: 192–195.

[4] European Network of GMO Laboratories: Verification of analytical methods for GMO testing when implementing interlaboratory validated methods. Version 22.07.2011. http://gmocrl.jrc.ec.europa.eu/doc/ENGL%20MV%20WG%20Report%20July%202011.pdf.

[5] Laube I, Hird H, Brodmann P, Ullmann S, Schöne-Michling M, Chrisholm J, Broll H: Development of primer and probe sets for the detection of plant species in honey. Food Chemistry (2010) 118: 979–986.

[6] Untersuchung auf gentechnisch veränderte Lebensmittel (2007/43) J. Verbr. Lebensm. 2 (2007): 440−444. http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/nachweis_kontrollen/probenahmeschema.pdf?__blob=publicationFile&v=1.

[7] Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (Hrsg.): Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB, § 35 Vorläufiges Tabakgesetz, § 28 a GenTG, Beuth-Verlag.

[8] DIN EN ISO 21569:2005, Lebensmittel - Verfahren zum Nachweis von gentechnisch modifizierten Organismen und ihren Produkten - Qualitative auf Nukleinsäuren basierende Verfahren. Beuth, Berlin.

[9] DIN EN ISO 21570:2006, Lebensmittel - Verfahren zum Nachweis von gentechnisch modifizierten Organismen und ihren Produkten - Quantitative auf Nukleinsäuren basierende Verfahren. Beuth, Berlin.

[10] Methodensammlung der Bund/Länderarbeitsgemeinschaft Gentechnik (LAG). http://www.laggentechnik.de/.

[11] Bundesamt für Verbraucherschutz und Lebensmittelsicherheit. http://www.bvl.bund.de/SharedDocs/Downloads/09_Untersuchungen/screening_tabelle_gvoNachweis.xls?__blob=publicationFile&v=4.

[12] Waiblinger HU, Grohmann L, Mankertz J, Engelbert D, Pietsch K: A practical approach to screen for authorised and unauthorised genetically modified plants. Anal Bioanal Chem (2010) 396: 2065–2072.

[13] European Union Reference Laboratory for GM Food and Feed. http://gmocrl.jrc.ec.europa.eu/statusofdoss.htm.

[14] European Union Reference Laboratory for GM Food and Feed. http://gmocrl.jrc.ec.europa.eu/default.htm.

[15] DIN EN ISO 24276:2006, Lebensmittel – Verfahren zum Nachweis von gentechnisch modifizierten Organismen und ihren Produkten – Allgemeine Anforderungen und Definitionen. Beuth, Berlin.

[16] Waiblinger HU, Graf N, Broll H, Grohmann L, Pietsch K: Evaluation of Real-time PCR results at the limit of detection. J. Verbr. Lebensm. (2011) DOI 10.1007/s00003-011-0669-4.

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