

DIN/TS 10068



ICS 67.050

**Lebensmittel –
Bestimmung von Mikroplastik –
Analytische Verfahren; Text Englisch**

Food –
Determination of microplastics –
Analytical methods; Text in English

Aliments –
Détermination des microplastiques –
Méthodes d'analyse; Texte en anglais

Gesamtumfang 38 Seiten

DIN-Normenausschuss Lebensmittel und landwirtschaftliche Produkte (NAL)
DIN-Normenausschuss Materialprüfung (NMP)
DIN-Normenausschuss Wasserwesen (NAW)

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Foreword

This document has been prepared by Working Committee NA 057-08-05 AA „Determination of microplastics in foodstuffs” of *DIN-Normenausschuss Lebensmittel und landwirtschaftliche Produkte (NAL)* (DIN Standards Committee Food and Agricultural Products).

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Introduction

WARNING 1 — Suitable precaution and protection measures need to be taken when carrying out working steps with harmful chemicals. The latest version of the hazardous substances ordinance, Regulation (EC) No 1907/2006 [1], should be taken into account as well as appropriate national statements.

WARNING 2 — The use of this document can involve hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

According to Council Regulation (EEC) No 315/93 [2], a possible occurrence of microplastics (MP) in food can be assessed as a contamination. “Contaminant” means any substance not intentionally added to food, which is present in such food, as a result of the production (including operation carried out in crop husbandry, animal husbandry and veterinary medicine), manufacture, processing, preparation, treatment, packaging, transport or holding of such food, or as a result of environmental contamination. Contamination is to be limited to values as low as can reasonably be achieved through good practice at all levels mentioned in Article 1 of the regulation.

Regulation (EC) No 178/2002 [3] determines the requirements for food safety. Article 14 refers to the fact that food might become unacceptable for human consumption as a result of contamination.

For a uniform understanding of the term “microplastics” a solid definition is needed. Furthermore, standardized analytical methods are necessary to obtain comparable results of microplastic contents in different products/matrices. In order to assess the possible impacts of MP consumption on human health, a reliable toxicological assessment as well as an exposure estimate for the general population and vulnerable groups (infants, breastfeeding women, elderly) must also be available. This technical specification could represent a first starting point for future regulatory initiatives.

1 Scope

This specification specifies methods for the sampling, sample preparation and characterization (identification, mass content, particle number, particle size) of microplastics (MP) in food. This specification does not apply to tap water.

These methods have been established for microplastics of the sizes 1 µm to 1 000 µm and large microplastics of sizes from 1 mm to 5 mm in food (sizes according to DIN CEN ISO/TR 21960). This document does not provide any information on the quality of the methods, as no method performance data (inter- and intra-laboratory comparison exercises) have been collected so far.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

DIN EN ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

DIN CEN ISO/TR 21960, *Plastics — Environmental aspects — State of knowledge and methodologies*

3 Terms, definitions, symbols and abbreviated terms

3.1 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

DIN and DKE maintain terminological databases for use in standardization at the following addresses:

- DIN-TERMinologieportal: available at <https://www.din.de/go/din-term/>
- DKE-IEV: available at <http://www.dke.de/DKE-IEV>

3.1.1

food

substances or products which are intended to be, or may reasonably be expected to be ingested by humans in a processed, partially processed or unprocessed state

[SOURCE: DIN 10528:2017-08]

3.1.2

contamination

impurity of food (3.1.1) not intentionally added, but present in the food (3.1.1) as a result of the manufacture, processing, preparation, treatment, presentation, packaging, transport or storage or as a result of environmental contamination

3.1.3

large microplastic

any solid plastic particle insoluble in water with any dimension between 1 mm and 5 mm

Note 1 to entry: Microplastics (3.1.4) may show various shapes.

Note 2 to entry: Typically, a large microplastic object represents an article consisting of plastic or a part of an end-user product or a fragment of the respective article.

Note 3 to entry: Microplastics in this size range are, for example, plastic pellets as intermediates for further downstream processing such as moulding, extrusion, etc. resulting in semi-finished products which are not final end-user products.

[SOURCE: DIN CEN ISO/TR 21960:2021-02, 3.10, modified – Term number in reference to microplastics adapted, in Note 3 to entry “resulting to” changed to read “resulting in”.]

3.1.4

microplastic

any solid plastic particle insoluble in water with any dimension between 1 µm and 1 000 µm (= 1 mm)

Note 1 to entry: This term relates to plastic materials within the scope of ISO/TC 61. Rubber, fibres, cosmetic means, etc. are not within the scope.

Note 2 to entry: Typically, a microplastic object represents a particle intentionally added to end-user products, such as cosmetic means, coatings, paints, etc. A microplastic object can also result as a fragment of the respective article.

Note 3 to entry: Microplastics may show various shapes.

Note 4 to entry: The defined dimension is related to the longest distance of the particle. Specified: e.g. Feret diameter (max).

[SOURCE: DIN CEN ISO/TR 21960:2021-02, 3.9, modified – In Note 4 to entry, the last sentence was added.]

3.1.5

nanoplastic

plastic particles smaller than 1 µm

Note 1 to entry: According to OECD nanoparticles are up to 100 nm.

[SOURCE: DIN CEN ISO/TR 21960:2021-02, 3.13]

3.1.6

measurement uncertainty uncertainty of measurement uncertainty

non-negative parameter characterizing the dispersion of the quantity values being attributed to a measurand, based on the information used

Note 1 to entry: Measurement uncertainty includes components arising from systematic effects, such as components associated with corrections and the assigned quantity values of measurement standards, as well as the definitional uncertainty. Sometimes estimated systematic effects are not corrected for but, instead, associated measurement uncertainty components are incorporated.

Note 2 to entry: The parameter may be, for example, a standard deviation called standard measurement uncertainty (or a specified multiple of it), or the half-width of an interval, having a stated coverage probability.

Note 3 to entry: Measurement uncertainty comprises, in general, many components. Some of these may be evaluated by Type A evaluation of measurement uncertainty from the statistical distribution of the quantity values from series of measurements and can be characterized by standard deviations. The other components, which may be evaluated by Type B evaluation of measurement uncertainty, can also be characterized by standard deviations, evaluated from probability density functions based on experience or other information.

Note 4 to entry: In general, for a given set of information, it is understood that the measurement uncertainty is associated with a stated quantity value attributed to the measurand. A modification of this value results in a modification of the associated uncertainty.

[SOURCE: JCGM 200:2012]

3.2 Symbols and abbreviated terms

AF4-MALS	Asymmetric Flow Field-Flow Fractionation Multi-Angle Light Scattering
ATR	Attenuated Total Reflection
CCD	Charge-Coupled Device
(C)FM	(Confocal) Fluorescence Microscopy
DSC	Differential Scanning Calorimetry
EA-OEM	Elemental Analysis Overdetermined Equation Method
EI	Electron Ionisation
FTIR	Fourier Transform Infrared Spectroscopy
GC/MS	Gas Chromatography Mass Spectrometry
HSI	Hyperspectral Imaging Spectrometry
ILC	Inter Laboratory Comparisons
IR	Infrared Spectroscopy
LM	Light Microscopy
LOD	Limit of Detection
LOQ	Limit of Quantification
HNT	Hot-Needle-Test
HQI	Hit Quality Index
MALDI-TOF	Matrix assisted Time-of-flight Mass Spectrometry
MCT	Mercury-Cadmium-Telluride
MP	Microplastics
NA	Numerical Aperture
pyr-GC/MS	Pyrolysis Gas Chromatography Mass Spectrometry
PA-6	Polyamide 6
PA-12	Polyamide 12
PC	Polycarbonate
PE	Polyethylene
PET	Polyethylene terephthalate
PMMA	Poly(methyl methacrylate)
PP	Polypropylene
PS	Polystyrene
PSU	Polysulfone
PT	Proficiency Tests
PVC	Polyvinyl chloride
QCL	Quantum Cascade Laser
qNMR	quantitative Nuclear Magnetic Resonance Spectroscopy
SEM	Scanning Electron Microscopy
SERS	Surface-enhanced Raman Spectroscopy
SIM	Selected Ion Monitoring
SOP	Standard Operation Procedure
SWIR	Short Wave Infrared
SRS	Stimulated Raman Scattering
TED-GC/MS	Thermoextraction and Desorption coupled Gas Chromatography Mass Spectrometry

TERS	Tip-enhanced Raman spectroscopy
TGA-GC/MS	Thermogravimetry coupled Gas Chromatography Mass Spectrometry
TMAH	Tetramethylammonium hydroxide
TOF-SIMS	Time-of-Flight Secondary Ion Mass Spectrometry
Vis-NIR	Visible/Near-Infrared
μ -FTIR	FTIR Microscopy
μ -Raman	Raman Microscopy

4 Principle

This Technical Specification describes an analytical concept to determine MP in food. The concept includes a) sampling, b) sample preparation and c) detection methods including data analysis. The determination of a suitable detection method depends on the original question and demand parameters. For characterization of MP different analytical methods can be applied. In order to obtain comprehensive data, the combination of methods is recommended. For different detection methods, sampling and sample preparation are specified depending on the food matrix. In Figure 1 the measurement concept of MP analysis is described.

Most commonly applied analytical methods for the determination of MP are spectroscopic methods like Raman or infrared (IR) spectroscopy and thermoanalytical methods like pyr-GC/MS or TED-GC/MS. Furthermore procedures to prevent contamination and for consistent presentation of results are described.

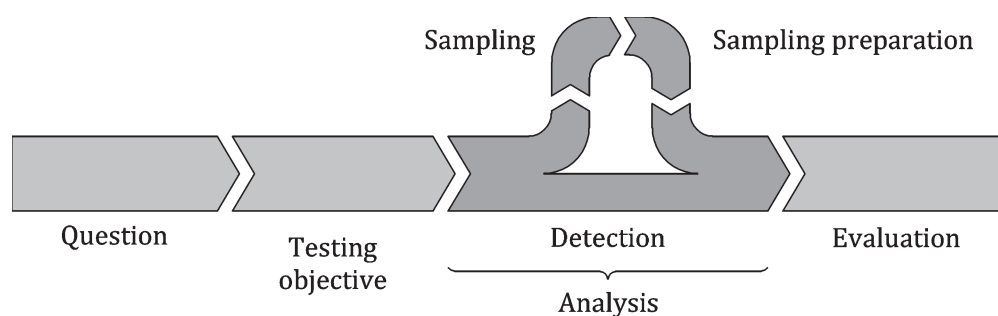


Figure 1 — Measurement concept of microplastics analysis

5 Contamination prevention

5.1 General

The general requirements regarding quality control are described in DIN EN ISO/IEC 17025. During all steps of MP analysis (sampling, sample preparation and detection) exposure to plastic materials and particles shall be minimized to avoid sample contamination:

Laboratory equipment: apparatus and consumable materials should be free of plastic. Alternative materials, e.g. glass or metal, should be used. Specific plastic could be used, if this differs from the analytes.

Facilities and environmental conditions: The facilities and environmental conditions shall be suitable for the laboratory activities and shall not adversely affect the validity of results. Contamination of samples via the ambient air should be avoided. Therefore, containers should be covered all the time. The opening of the containers should be done under verified laminar-flow boxes. By using cleanrooms as an alternative, a classification of air cleanliness by particle concentration regarding DIN EN ISO 14644-1 is recommended. ISO classification number (N) should be ≤ 5 .

The laboratories should be cleaned regularly to remove dirt and dust, so that contamination of the samples is reduced.

5.2 Blank value

All relevant and potential background loads of MP, for example from ambient air, reagents and processes, should be recorded. The blank value should not be deducted from the measured value, but should be listed in the test report (see Clause 9).

The particulate matter in used reagents and at apparatus/consumable materials should be as low as possible.

5.3 Reagents

Reagents include all liquids (e.g. ultrapure water, solutions, solvents) and chemicals used during analysis.

All reagents ideally have to be free from MP. Since this is usually not the case, blank samples have to be continuously carried out and analysed during validation and during the application of the method (see 5.2). Reagents can be filtered prior to their use to reduce microparticle concentrations. As some filters can also lead to contamination of filtered solutions, this step has to be critically checked. Inorganic filters (e.g. metal based or metal coated, glass fibre) are recommended.

5.4 Apparatus and equipment

Apparatus and equipment are all items used during the analysis, which come into direct contact with the sample and therefore can lead to sample contamination.

Plastic materials should be avoided in this context whenever possible. Ideally, glassware or metal objects should be used. If the usage of plastics is unavoidable, sample contamination has to be intensively checked (blank samples, see 5.2). In case of doubt, plastic types used have to be excluded from the results.

All apparatus and equipment have to be thoroughly cleaned to remove adhering microplastics/microparticles. The surface of all items should not show scratches or irregularities, as microparticles can easily adhere and hide from the cleaning procedure. Cleaning can be done e.g. mechanically, supported by an ultrasonic bath or by rinsing with different reagents (e.g. ultrapure water, detergent solutions, solvents) or by thermal treatment. Effectiveness of the cleaning procedure has to be validated and continuously checked before the analysis.

6 Procedure

6.1 Classification of the sample

The status of the sample should be categorized as described below. The sampling and preparation procedures should be chosen accordingly.

Concerning the initial products to be analysed, the following characteristics should be taken into account:

Packaging/processing:

- prepacked (A) or unpackaged (B);
- processed (C) or unprocessed (D).

Physical state:

- liquid (I), chunky (II), paste-like (III), solid (IV).

Sample composition in terms of low (1), mid (2) and high (3) content of:

- suspended matter (a);
- proteins (b);
- fat (c);
- carbohydrates (d);
- inorganic matter (e).

Other characteristics:

- water solubility (solid) (f);
- oil solubility (solid) (g);
- homogeneity (h);
- inhomogeneity (i).

Based on this sample classification different methods for sample preparation can be selected. Depending on the complexity of the sample matrix, a modular sample preparation is possible and similar products can be treated according to a comparable processing scheme. Table 1 shows examples for different matrices. Sample amounts are not given, as they highly depend, e.g. on the sample properties, the analysed size class and mass content of MP as well as the detection method.

Table 1 — Examples for the classification of samples

	Bottled water	Sugar	Salt	Milk (bottled)	Vegetable oil (refined)	Butter	Fish fillet/bivalve molluscs
Prepacked (A)/unpacked (B)	A	A	A	A	A	A	B
Processed (C)/unprocessed (D)	D	C	C	C	C	C	D
Liquid (I)/chunky (II)/paste-like (III)/solid (IV)	I	IV	IV	I	I	III	IV
Content of suspended matter (a)	a1	a1	a1	a2	a1	a2	a3
Content of proteins (b)	b1	b1	b1	b2	b1	b1	b3
Content of fat (c)	c1	c1	c1	c2	c3	c3	c1-c3
Content of hydrocarbons (d)	d1	d3	d1	d2	d1	d1	d1
Content of inorganic matter (e)	e1	e1	e3	e1	e1	e1	e1
Water solubility (f)/oil solubility (g)	f	f	f	f	g	g	—
Homogeneity (h)/inhomogeneity condition (i)	h	h	h	i	h	i	i

6.2 Sampling

6.2.1 General

The particulate character of MP with diverse shapes, sizes, physical properties and the way these particles can get onto the surface or into different kinds of food, lead to an inhomogeneous distribution. The goals and the strategies of sampling need to be flexible, also considering the available analysis methods (e.g. spectroscopic/thermoanalytical). To ensure the plausibility of the results, repeated measurements of samples from the same batch are recommended.

6.2.2 Sampling analytical tasks and strategies

Sampling methods should provide representative samples to guarantee a statistically ensured total amount of MP is reached either as mass content or number and size in the test sample. The kind of sampling will also depend on the nature of the food matrix (categories are shown in 6.1; Table 1).

6.2.3 Storage

Test samples shall be stored in such a way that their composition and sample mass do not change due to e.g. dehydration, evaporation losses, spoilage or decomposition. The test sample should be processed as soon as possible after receipt.

The test samples should be stored as recommended by the producer or as usually done for the relevant product category. For unpacked samples, suitable storage containers are vessels made of glass or metal.

Parameters such as temperature, pH value, light and storage time can have a certain influence on the sample.

6.3 Preparation of the test sample

6.3.1 General

Food samples are generally treated in the same way as prior to consumption (washed, peeled, inedible parts removed). Apart from this preparation, the samples should not be subjected to any further processing, e.g. not boiled, steamed, fried nor heated.

Special treatments (e.g. drying), may be carried out to prevent spoilage of the sample. Particularly for the analysis of MP, a possible loss or change of particles and possible contamination of the test sample have to be carefully considered.

Special measures shall be taken when preparing samples of pre-packed products: Raw food materials, semi-processed products, ready-for-consumption and ready-to-eat products can be offered in pre-packaged form. In the case of such products, sources of contamination on the outside of the packaging, e.g. plastic labels or banderoles, should be removed and the outside of the packaging should be cleaned. The test material should be removed from the packaging as completely as possible. Packages should not be opened more often than necessary to avoid the generation of additional MP. In the case of small packaging quantities, samples should be taken from several containers, if necessary. Alternatively, in the case of large packaging sizes, representative aliquots that can be handled during sample preparation should be taken. If only defined sample components are to be analysed for MP contamination, these should be processed separately prior to analysis.

The goal of sample preparation is to isolate MP from other organic or inorganic suspended matter. Applied techniques are chemical, physical and/or enzyme-based methods, which could be combined. In most cases, high purity of the prepared sample is necessary to prevent matrix effects at detection. The necessary purity grade of the prepared sample depends on the chosen detection method.