GERMAN STANDARD METHODS FOR THE ANALYSISOF FATS, FATTY PRODUCTS, SURFACTANTS AND RELATED SUBSTANCES

JOINT COMMITTEE FOR THE ANALYSIS OF FATS, OILS, FATTY PRODUCTS, RELATED SUBSTANCES, AND RAW MATERIALS (GA FETT)

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Supplementary notes on the DGF standard methods C- VI 17 (10) and C- VI 18 (10) on the determination of 3-MCPD fatty acid esters and glycidyl fatty acid esters

Analytics

The German Society for Fat Science (Deutsche Gesellschaft für Fettwissenschaft e.V.) has published two standard methods for the determination of levels of fatty acid-bound 3-chloropropane-1,2-diol (synonymous: 3-monochloropropane-1,2-diol / **3-MCPD**) and fatty-acid-bound **glycidol** in edible oils and fats:

The evaluated **DGF standard method C-VI 17 (10)** describes a procedure for the total determination of ester-bound 3-MCPD and ester-bound glycidol in edible fats and oils by means of gas chromatography/mass spectrometry (GC/MS) following ester cleavage with methanolic sodium methylate and derivatisation of the non-esterified analytes with phenylboronic acid. Under the given process conditions, the released glycidol is converted virtually quantitatively into 3-MCPD (so-called induced 3-MCPD). This induced 3-MCPD mixes inseparably with the 3-MCPD originally contained in the sample. The 3-MCPD results of this method thereby correspond to the sum of bound 3-MCPD and bound glycidol. This is synonymous with the sum of 3-MCPD esters and glycidyl esters expressed as 3-MCPD. Using this method, it is not possible to establish the proportion of 3-MCPD generated from glycidol that makes up the final result.

The new method C-VI 17 (10) corresponds to Part A of original Method C-III 18 (09) and contains the relevant data from an international ring trial that the GA Fett organised in 2010. The precision data of this method meet the applicable standards for repeatability and comparability with regard to determining the sum of 3-MCPD esters and glycidyl esters, expressed as 3-MCPD [**Fiebig 2010**].

The DGF standard method C-III 18 (09) has been withdrawn and should no longer be used or cited because studies as part of an international ring trial that the GA Fett organised in 2010 showed that for Part B of the method to determine the original fatty-acid-bound 3-MCPD without quantities of glycidol could result in false-high findings. Consequently, this could lead to false-low findings when calculating the quantities of bound glycidol from the difference of the two method parts.

The evaluated **DGF standard method C-VI 18 (10)** describes a process to determine the sum of ester-bound 3-MCPD and ester-bound glycidol (Part A) or exclusively of ester-bound 3-MCPD (Part B) in edible fats and oils by means of gas chromatography/mass spectrometry (GC/MS) following alkali- catalyzed ester cleavage and derivatization of the core analytes with phenylboronic acid. In Part A, the released glycidol is predominantly converted using chloride into induced 3-MCPD, which is inseparably mixed with the 3-MCPD originally contained in the sample. In Part B, the released glycidol reacts in absence of chloride to give other derivatives that are not detected. The 3-MCPD results of this method in Part A thereby correspond to the sum of bound 3-MCPD and bound glycidol determined as 3-MCPD. This is synonymous with the sum of 3-MCPD esters and glycidyl esters expressed as 3-MCPD. In Part B of this method, the 3-MCPD results correspond to the quantity of bound 3-MCPD. This is synonymous with the quantity of 3-MCPD esters expressed as 3-MCPD. Using this method, any free 3-MCPD present in samples would contribute to the results. At the same time, the method of indirectly determining ester-bound glycidol is used as a differential procedure, which assumes that besides glycidol, there are

no other substances present that can also react at room temperature with inorganic chloride to produce 3-MCPD. This differential procedure requires a transformation factor to be experimentally determined, which quantitatively describes the conversion of the glycidol in 3-MCPD. The results of the calculated quantities of bound glycidol correspond to the quantities of glycidyl esters expressed as glycidol.

Method C-VI 18 (10) contains the relevant ring trial data from an international ring trial that the GA Fett organised in 2010. The precision data of this method meet the applicable standards for repeatability and comparability with regard to determining the sum of 3-MCPD esters and glycidyl esters, expressed as 3-MCPD, and with regard to determining 3-MCPD esters, expressed as 3-MCPD [**Fiebig 2010**].

To explain further, it should be noted that the DGF method

- DGF C-VI 17 (10) refers to the "**Weißhaar method**" and
- DGF C-VI 18 (10) refers to the "**Kuhlmann method**".

The expanded measurement uncertainty for the 3-MCPD results is expected to be in the 10–30 % range. For the differential method C-VI 18 (10) it should be noted that the measurement uncertainties of both methods (Part A and Part B) increase for the glycidol results, whereby the measurement uncertainty for these analytes also increases with the ratio 3-MCPD : glycidol. Studies and numerous comparative laboratory studies have shown that 2-MCPD fatty acid esters expressed as 2-MCPD can also be determined using Part B of the DGF method C-VI 18 (10) [Sato 2013]. However, this analyte was not included in the validation study that forms the basis of this method.

It should be particularly noted that both methods, C-VI 17 (10) and C-VI 18 (10) were only developed and validated for analysing edible oils and fats and not for investigating other oil and fat products such as emulsifiers, glycerol, phytosterols, sphingolipids etc. The suitability of the DGF methods for these matrices has not been studied. For this reason, results from using the DGF methods C-VI 17 (10) and C-VI 18 (10) for matrices other than edible oils and fats should not be considered valid until their suitability has been proven in corresponding ring trials.

Alternative analysis strategy A fundamental problem in any direct determination of 3-MCPD esters and glycidyl esters is the quantification of many individual isomers and congeners, not all of which are currently commercially available as a standard substance. Consequently, in the mass spectrometry determination, only those compounds are detected that have been methodically established using the reference substances.

Due to the large number of possible 3-MCPD esters and the coelution of isomeric 2-MCPD and 3-MCPD fatty acid esters that occur under standard HPLC conditions, this methodological approach is primarily used for scientific work but is hardly used in routine work. In addition, these methods have not yet been officially validated. In view of the pursued strategy to minimise these process contaminants (reduction of the overall content) it should be evaluated whether monitoring the individual compounds when using direct determination provides relevant and significant information to justify the corresponding additional expense for using this method.

3-MCPD esters and glycidyl esters in fats and oils

In the past, all of the investigated refined vegetable oils contained MCPD esters and glycidyl esters in very different, sometimes high quantities, reaching the double-digit mg/kg range [**Crews 2013, BLE**

2017]. According to current findings, higher quantities of 3-MCPD esters should be expected on average for refined fruit oils compared with refined seed oils. Generally, no 3-MCPD esters or glycidyl esters were evidenced in any of the unprocessed plant oils analysed to date. Free 3-MCPD and free glycidol usually do not occur as process contaminants in fats and oils. However, free 3-MCPD, which frequently occurs as a technical impurity, could also migrate into oils and fats to a limited extent from food contact materials or through roasting and frying processes.

In principle, the formation of 3-MCPD fatty acid esters in the deodorisation of oils and fats is limited by the quantity of chloride available for the reaction, whereby both inorganic chloride and organic bound chlorine can play a role [**Nagy 2011**, **Destaillats 2012a**]. The thermally induced formation of 3- MCPD esters can therefore take place in certain circumstances at relatively low temperatures, significantly below the usual maximum values for deodorisation. Accordingly, it is not only the management of the refining process but also the origin of the oils and fats as well as the quality of the raw materials that influence the quantities of 3-MCPD esters in the refined products. As a rough guideline, it can be assumed that crude oils, which contain more impurities naturally or as a result of unfavourable harvesting and storage conditions and therefore usually need to be deodorised more intensively, have proportionately higher 3-MCPD quantities than those oils and fats that contain fewer undesirable accompanying substances. There are indications that minimising chloride quantities in crude oils by washing as well as preventing chloride entering via all of the upstream refining steps of deodorisation can reduce the formation of 3-MCPD esters.

The following lists various fats and oils, classified according to their potential to contain thermally induced 3-MCPD esters:

3-MCPD

Low contamination potential	Refined rapeseed oil, soybean oil, sunflower oil, groundnut oil, coconut oil, palm kernel oil
Moderate contamination potential	Refined safflower oil, sesame oil, corn oil, olive oil cottonseed oil
High contamination potential	Refined walnut oil, grape seed oil, hazelnut oil, olive pomace oil, palm oil and palm oil fractions, fish oil

The formation of glycidyl esters during deodorisation predominantly occurs as a result of mono- and diacylglycerides that are naturally occurring in oils and fats and is therefore not limited in the same way by a minor component as is the case for the 3-MCPD fatty acid esters. Having said that, glycidyl esters are produced at high temperatures and substantial amounts only start being formed at approx. 200 °C [**Destaillats 2012b**]. At the same time, glycidyl esters - that are present only as mono-fatty acid esters - have a volatility that makes it possible to partially separate them by applying a vacuum in a suitably designed deodorisation system. Depending on the process conditions, an equilibrium can be set between production and removal. Furthermore, the chemically reactive epoxy groups make glycidyl derivatives less stable than MCPD esters, which suggests further technical processes can be used to minimise them. The previous rule of thumb therefore applies, in that oils which usually contain in their crude form a relatively high proportion of mono- and diacylglycerides (such as palm oil), will also have high quantities of glycidyl esters after refinement, only to a limited extent. Above-average quantities of glycidyl esters in refined oils and fats are now presumably related to the use of outdated refining methods.

Toxicology/exposure assessment

The International Agency for Research on Cancer (IARC) has classified 3-MCPD as a possible human carcinogen of class 2B [**IARC 2012**]. In the past, different institutions such as the European Food Safety Authority (EFSA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA), approaches to determine a (provisional) tolerable daily intake ((P)TDI), which led to maximum values of 0.8–4 µg 3-MCPD per kg body weight per day [**EFSA 2016**, **JECFA 2016**]. A re-evaluation by the competent expert body of the EFSA, the EFSA Panel on Contaminants in the Food Chain (CONTAM-Panel) in 2017 resulted in a maximum daily intake being defined as 2 µg 3-MCPD per kg body weight [**EFSA 2018**]. As animal studies have shown that 3-MCPD fatty acid esters are largely hydrolysed during digestion with the release of 3-MCPD [**Abraham 2013**], the TDI is viewed as a group value for the sum of free 3-MCPD and the 3-MCPD proportion of 3-MCPD fatty acid esters (bound 3-MCPD). There are not enough toxicological data on 2-MCPD fatty acid esters in order to derive a classification or a TDI value.

IARC classifies glycidol as class 2A: probably carcinogenic to humans [**IARC, 2000**]. As it has genotoxic properties, no TDI can be specified but rather the risk is assessed via the margin of exposure (MoE). In general, the ALARA principle applies to genotoxic compounds – intake should be as low as reasonably achievable. For glycidyl esters, glycidol was found to be virtually completely released from the ester-bound form in animal studies during digestion [**Appel 2013**]. For this reason, the risk for glycidyl esters is assessed in a similar way to that of the glycidol. On the basis of the available toxicological data, the German Federal Institute for Risk Assessment (BfR) has derived a maximum daily intake for glycidol of 0.406 µg per kg body weight.

Law / Official regulations in the EU

Regulation **EU 2020/1322**, amending Regulation EC No. 1881/2006, applies as of January 1st 2021 with regard to foods in the EU. This specifies maximum quantities for free 3-MCPD in hydrolysed plant protein and soya sauce as well as maximum quantities for *glycidyl fatty acid esters, expressed as glycidol*, in vegetable oils and fats, fish oils and other marine oils, that are placed on the market for end consumers or for use as an ingredient in foods (maximum quantity 1000 µg/kg). Furthermore, this regulation covers the quantities of glycidyl fatty acid esters in vegetable oils and fats, fish oils and other marine oils that are destined for the production of baby food and processed cereal-based food for infants and young children (maximum quantity 500 µg/kg) and for infant formula, follow-on formula and foods for special medicinal purposes for infants and young children as powders (maximum 50 µg/kg from 1.07.2019) and as a liquid (maximum quantity 6.0 µg/kg from 1.07.2019). With the same regulation, maximum quantities for the sum of free and fatty acid bound 3-MCPD (analyte group) are set for the same foods for which maximum levels for GE are established. Due to the low 3-MCPD formation potentials, a stricter maximum level (1250 µg/kg) for the *sum of 3-MCPD and 3-MCPD fatty acid esters, expressed as 3-MCPD* is set for oils and fats from coconut, maize, rapeseed, sunflower, soybean, palm kernel and olive oils and mixtures of oils and fats with oils and fats only from this category. A higher maximum level of 2500 µg/kg applies to other vegetable oils (including olive pomace oils), fish oils and oils from other marine organism and mixtures of oils and fats with oils and fats only from this category. For oil mixes from both categories with quantitatively known ingredients, the category-related maximum levels apply to the single ingredients. In case of oil and fat mixes of unknown composition, the higher maximum level of 2500 µg/kg applies. For vegetable oils and fats, fish oils and other marine oils that are destined for the production of baby food and processed cereal-based food for infants and young children the maximum level for the *sum of 3-MCPD and 3-MCPD fatty*

acid esters, expressed as 3-MCPD is set to 750 µg/kg. Finally, for infant formula, follow-on formula and foods for special medicinal purposes for infants and young children as powders the maximum level is addressed as 125 µg/kg for powders and as 15 µg/kg for liquids.

Implementing Regulation **EU 2019/2093** amending Regulation EC No. 333/2007, which lays down the methods of sampling and analysis, is used for the official control of the level of certain contaminants in foods. Regulation EU 2019/2093 establishes analytical performance criteria for methods of analysis of free 3-MCPD, 3-MCPD fatty acid esters, expressed as 3-MCPD and glycidyl fatty acid esters, expressed as glycidol, in foods.

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