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REVIEW

The European Flavouring regulation and how to deal with "Restricted Substances"

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Abstract

The European Flavouring Regulation contains a list of so-called "restricted substances" (RS), i.e. substances that occur naturally in source materials for flavourings and food ingredients with flavouring properties, but whose presence in certain foods is restricted and/or for which maximum levels are set. This list is the Annex III to the Flavouring Regulation, the most important part of which is Part B, listing 11 substances which are naturally present in flavourings and food ingredients with flavouring properties and to which "maximum levels" apply in specific food categories. The current paper provides some legal aspects with regard to those "restricted substances" and reports on a new method which has been developed by the Working Group on Methods of Analysis of the International Organization of the Flavor Industry (IOFI) for the rapid routine determination of β-asarone, coumarin, menthofuran, methylchavicol, methyleugenol, pulegone, safrole, and α- and β-thujones in flavourings and their raw materials by gas chromatography-mass spectrometry (GC-MS), using selected-ion monitoring and internal standards. The paper will further focus on Business-to-Business requirements when flavourings are sold to food producers (customers) and provide some elements from EFFA's Guidance Document.

Keywords: Volatile Restricted Substances, European Flavouring Regulation, Annex III, Methods of Analysis, routine determination, GC-MS

Introduction

The European Flavouring Regulation (EC) No 1334/2008 (Official Journal, 2008) entered into force on 20 January 2009 and applies since 20 January 2011. According to this Regulation certain substances, most of which are common constituents of natural (food) ingredients, are restricted. These "restricted substances" (RS) as they are called, are substances that occur naturally in source materials for flavourings and food ingredients with flavour properties, but whose presence in certain foods is restricted and/or for which maximum levels are set (Demyttenaere, 2012).

Thus, two types of restrictions are foreseen: Annex III Part A of the Flavouring Regulation lists 15 substances "*which shall not be added as such to food*", whereas Part B of Annex III lists 11 substances which are naturally present in flavourings and food ingredients with flavouring properties and to which "*maximum levels*" apply in specific food categories. Also elsewhere in the world flavour regulations contain such lists of so-called "Restricted substances". For example a list of substances to be controlled can be found in the Mercosur Technical Regulation Concerning Flavourings (Mercusor, 2006), the new Russian Federation Customs Union Technical Regulation on Food Additives (Russian Federation Customs Union, 2012), and many flavour regulations of South-East Asian countries, such as Malaysia, Indonesia, Singapore, etc.

Only a few publications refer to the determination of RS in compound flavourings or their raw materials, and the latter only concern the analysis of one or two individual RS in single essential oils (Royal Society of Chemistry, 2002; Archer, 1988; De Jager, Perfetti, & Diachenko, 2008; Otto, Wohlschlager, Grüner, Weinreich, & Parlar, 2001). This paper reports on a method for the determination of some of these RS in

flavourings and their raw materials by gas chromatography-mass spectrometry using selected-ion monitoring (GC-MS-SIM) and internal standards. The method is intended for flavour-industry laboratories in order to enable them to inform their customers (food industry) of the amounts of these substances in commercial flavourings, but is not intended for their analysis in finished foods.

Legal background

Current Flavouring Regulation in EU

On 31 December 2008 the new Flavouring Regulation was published in the Official Journal of the EU, which entered into force on 20 January 2009 and which officially applies since 20 January 2011.

The full title of this Regulation is: *Regulation (EC) No 1334/2008 of the European Parliament and of the Council on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC*.

Since the application of this new Regulation, the former Council Directive 88/388/EEC of 22 June 1988 (Official Journal, 1988a) as well as its amendment Directive 91/71/EEC (Official Journal, 1991) and the Commission Decision 88/389/EEC (Official Journal, 1988b) have been repealed. As many essential oils and extracts either contain flavouring substances, or are regarded as "*food ingredients with flavouring properties*" this new Flavouring regulation will have an impact on essential oils and their use as flavouring ingredients for food products. Extracts and essential oils contain certain constituents (substances) that according to this regulation "*should not be added as such to food*" or to which maximum levels apply. In particular the application of maximum levels of these substances will have an impact on how and when extracts, essential oils but also herbs and spices may or can be applied to food.

Maximum levels of Restricted Substances

Apart from the fact that the former Flavouring Directive 88/388/EC now has been replaced by a Regulation, there are many changes that will have an impact on how essential oils and extracts will be used as source of flavours. The most important issue is how the "Restricted Substances" are addressed. This is addressed by Art. 6 of the Flavouring Regulation: "*Presence of certain substances*" which refers to Annex III with the same title. This article clearly states in the first paragraph that "*Substances listed in Part A of Annex III shall not be added as such to food*."

However, when it concerns the levels of these substances coming from the use of flavourings and food ingredients with flavouring properties (such as extracts, essential oils, herbs and spices) the Regulation further specifies (Art. 6.2):

2. *Without prejudice to Regulation No 110/2008 maximum levels of certain substances, naturally present in flavourings and/or food ingredients with flavouring properties, in the compound foods listed in Part B of Annex III shall not be exceeded as a result of the use of flavourings and/or food ingredients with flavouring properties in and on those foods. The maximum levels of the substances set out in Annex III apply to foods as marketed, unless otherwise stated. By way of derogation from this principle, for dried and/or concentrated foods which need to be reconstituted the maximum levels apply to the food as reconstituted according to the instructions on the label, taking into account the minimum dilution factor.*

This means that maximum levels of these substances also apply when the substances come from any type of food ingredients with flavouring properties; the only exception is given to dried and/or concentrated foods which can have higher levels before they are diluted, and/or reconstituted. Upon dilution, and/or reconstitution, the normal maximum levels apply again.

The main difference between the former Flavouring Directive 88/388 and the new Flavouring Regulation is that in the Directive 88/388 there was only one list (Annex II) of substances to which the maximum levels apply – all those substances may not be added as such to food. In contrast, in the new Flavouring Regulation, the Annex III is split in two parts: Part A with "*Substances which shall not be added as such to food*" and Part B establishing: "*Maximum levels of certain substances, naturally present in flavourings and food ingredients with flavouring properties, in certain compound food as consumed to which flavourings and/or food ingredients with flavouring properties have been added*."

Part A of Annex III contains 15 substances, whereas Part B contains 11 substances.

Table 1 lists the 15 Substances of Part A of Annex III "*which shall not be added as such to food*" and Table 2 lists the 11 substances of Part B with their respective maximum levels in the various compound foods according to the current Flavouring Regulation.

Table 1. Annex III, Part A of Regulation (EC) No 1334/2008: Substances which shall not be added as such to food

Agaric acid	Aloin	**Capsaicin**
1,2-Benzopyrone, coumarin	Hypericine	Beta-asarone
1-Allyl-4-methoxybenzene, estragole[a]	Hydrocyanic acid	**Menthofuran**
4-Allyl-1,2-dimethoxybenzene, methyleugenol	Pulegone	Quassin
1-Allyl-3,4-methylene dioxy benzene, safrole	**Teucrin A**	Thujone (alpha and beta)

[a]Substances in bold are "new" (i.e. not in the former Directive 88/388/EC Annex II)

Table 2. Maximum levels of certain substances, naturally present in flavourings and food ingredients with flavouring properties, in certain compound food as consumed to which flavourings and/or food ingredients with flavouring properties have been added (Annex III, Part B to Flavouring Regulation (EC) No 1334/2008).

Name of the substance	**Compound food in which the presence of the substance is restricted**	**Maximum level mg/kg**
Beta-asarone	Alcoholic beverages	1.0
1-Allyl-4-methoxybenzene, Estragol(*)	Dairy products	50
	Processed fruits, vegetables (incl. mushrooms, fungi, roots, tubers, pulses and legumes), nuts and seeds	50
	Fish products	50
	Non-alcoholic beverages	10
Hydrocyanic acid	Nougat, marzipan or its substitutes or similar products	50
	Canned stone fruits	5
	Alcoholic beverages	35
Menthofuran	Mint/peppermint containing confectionery, except micro breath freshening confectionery	500
	Micro breath freshening confectionery	3000
	Chewing gum	1000
	Mint/peppermint containing alcoholic beverages	200

4-Allyl-1,2-dimethoxy-benzene, Methyleugenol (*)	Dairy products	20
	Meat preparations and meat products, including poultry and game	15
	Fish preparations and fish products	10
	Soups and sauces	60
	Ready-to-eat savouries	20
	Non-alcoholic beverages	1
Pulegone	Mint/peppermint containing confectionery, except micro breath freshening confectionery	250
	Micro breath freshening confectionery	2000
	Chewing gum	350
	Mint/peppermint containing non-alcoholic beverages	20
	Mint/peppermint containing alcoholic beverages	100
Quassin	Non-alcoholic beverages	0.5
	Bakery wares	1
	Alcoholic beverages	1.5
1-Allyl-3,4-methylene dioxy benzene, safrole (*)	Meat preparations and meat products, including poultry and game	15
	Fish preparations and fish products	15
	Soups and sauces	25
	Non-alcoholic beverages	1
Teucrin A	Bitter-tasting spirit drinks or bitter[1]	5
	Liqueurs[2] with a bitter taste	5
	Other alcoholic beverages	2
Thujone (alpha and beta)	Alcoholic beverages, except those produced from *Artemisia* species	10
	Alcoholic beverages produced from *Artemisia* species	35
	Non-alcoholic beverages produced from *Artemisia* species	0.5
Coumarin	Traditional and/or seasonal bakery ware containing cinnamon in the labelling	50
	Breakfast cereals including muesli	20
	Fine bakery ware with exception of traditional and/or seasonal bakery ware containing cinnamon in the labelling	15
	Desserts	5

(*) The maximum levels shall not apply where a compound food contains no added flavourings and the only food ingredients with flavouring properties which have been added are fresh, dried or frozen herbs and spices. After consultation with the Member States and the Authority, based on data made available by the Member States and on the newest scientific information, and taking into account the use of herbs and spices and natural flavouring preparations, the Commission, if appropriate, proposes amendments to this derogation.
(1) As defined in Annex II, paragraph 30 of Regulation (EC) No 110/2008.
(2) As defined in Annex II, paragraph 32 of Regulation (EC) No 110/2008.

Another significant change is that under the former Directive 88/388 the limitations applied to all food or beverage categories mentioned under Annex II to this Directive, i.e. all categories covered by the term "Foodstuffs" were limited to a certain (same) maximum level and the same applied to all "Beverages"; apart from the general limitations/restrictions some exceptions (for certain more particular food categories) applied (i.e. "special restrictions").

Under the current Flavouring Regulation, the maximum levels only apply to the specific food categories (referred to as "*Compound food in which the presence of the substance is restricted*") mentioned in the second column of Annex III, with a specific maximum level, mentioned in the third column. Thus, only those categories contributing most to the consumers' exposure are restricted. This is also referred to as the "major contributor approach" and is outlined in Recital (10): "*Maximum levels for certain naturally occurring undesirable substances should focus on the food or food categories which contribute most to dietary intake.*"

It is a pragmatic solution which was adopted to make the controls by the Member States more efficient as they will focus on foodstuffs who contribute the most to the intake of substances of toxicological concern and no longer to foodstuffs and beverages in general.

This means that "compound foods" (i.e. certain foodstuffs/food categories or beverages) that are not mentioned in Annex III are not restricted. For example thujone used to be restricted to all foodstuffs (0.5 mg/kg) and all beverages (0.5 mg/kg) with the exception of certain alcoholic beverages and foodstuffs to which higher levels applied (old Annex II); today thujone is only limited to alcoholic beverages (with two different levels depending on whether or not they have been produced from *Artemisia* species: respectively 35 and 10 mg/kg) and to non-alcoholic beverages produced from *Artemisia* species (maximum level 0.5 mg/kg),but not to any other non-alcoholic beverages, nor to foodstuffs.

"New" restricted substances

Further compared to the former Flavouring Directive 88/388, some "restricted substances" are new, e.g. methyleugenol, estragole, menthofuran, etc. This is because since the publication (and amendment) of the former Directive some new scientific evidence has become available that suggested that there would be some toxicological concern for these substances. As explained in Recital (8): "*Since 1999, the Scientific Committee on Food and subsequently the European Food Safety Authority* [EFSA] […] *have expressed opinions on a number of substances occurring naturally in source materials for flavourings and food ingredients with flavouring properties which, according to the Committee of Experts on Flavouring Substances of the Council of Europe, raise toxicological concern. Substances for which the toxicological concern was confirmed by the Scientific Committee on Food should be regarded as undesirable substances which should not be added as such to food.*"

It should be noted that for the same reason, as stipulated in Art. 22 of the Flavouring Regulation, the Annex III can be amended "*to reflect scientific and technical progress*" […] "*following the opinion of the Authority*" (i.e. European Food Safety Authority, EFSA).

An important example is methyleugenol (4-allyl-1,2-dimethoxybenzene). In 1999 methyleugenol was evaluated by the Committee of Experts on Flavouring Substances (CEFS) of the Council of Europe. The conclusions of this Committee were:

"*Available data show that methyleugenol is a naturally-occurring genotoxic carcinogen compound with a DNA-binding potency similar to that of safrole. Human exposure to methyleugenol may occur through the consumption of foodstuffs flavoured with aromatic plants and/or their essential oil fractions which contain methyleugenol. In view of the carcinogenic potential of methyleugenol, it is recommended that absence of methyleugenol in food products be ensured and checked with the most effective available analytical method*" (Council of Europe, 1999).

Methyleugenol was subsequently evaluated by the Scientific Committee on Food (SCF) and an opinion on its safety was published in 2001 (Scientific Committee on Food, 2001).

The conclusion of the SCF was:

"*Methyleugenol has been demonstrated to be genotoxic and carcinogenic. Therefore the existence of a threshold cannot be assumed and the Committee could not establish a safe exposure limit. Consequently, reductions in exposure and restrictions in use levels are indicated*."

An equally important but similar example is estragole (1-allyl-4-methoxybenzene) also known as methylchavicol. In 2000 the Committee of Experts on Flavouring Substances (CEFS) of the Council of Europe evaluated estragole and based on their findings (it was found to be a naturally occurring genotoxic carcinogen in experimental animals), a limit of 0.05 mg/kg (detection limit) was recommended (Council of Europe, 2000).

Estragole was subsequently evaluated by the Scientific Committee on Food (SCF) and an opinion on its safety was published in 2001 (Scientific Committee on Food, 2001).

The conclusion of the SCF was:

"*Estragole has been demonstrated to be genotoxic and carcinogenic. Therefore the existence of a threshold cannot be assumed and the Committee could not establish a safe exposure limit. Consequently, reductions in exposure and restrictions in use levels are indicated*."

As a consequence, both methyleugenol and estragole have been added to Annex III of the new Flavouring Regulation as "restricted substances".

Analysis of volatile restricted substances

Many published methods exist for the determination of RS in finished foods and beverages, however only a few refer to their determination in compound flavourings or their raw materials – the latter mainly concern the analysis in single essential oils of one or two individual RS, and are often based on a direct GC or LC analysis (Royal Society of Chemistry, 2002; Archer, 1988; De Jager et al., 2008; Otto et al., 2001).

Sample preparation for compound flavourings is generally simpler than for finished foodstuffs, but their complexity necessitates the use of GC-MS with selected-ion monitoring in order to deal with the interferences arising from the wide variety of constituents present in a typical compound flavouring.

Reporting obligations by flavour industry

Flavour companies need a rapid routine method for the simultaneous determination of multiple RS in compound flavourings, since it is in their interest to inform their customers of the levels of any of these in their flavourings, and the guidelines of EFFA commit the industry to provide such information. According to the first principle of HACCP (Hazard Analysis and Critical Control Point) as laid down in Regulation 852/2004/EC (Official Journal, 2004), the flavour manufacturers shall put in place suitable procedures in order to identify any hazard that must be prevented, eliminated or reduced to acceptable levels.

The following recommendations are stated in the EFFA Guidance Document (EFFA, 2013) in relation to the reporting of the presence of Restricted Substances in flavourings to customers (food business operators):

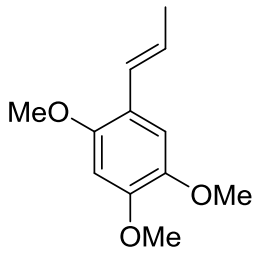
> Recommendation 1)
>
> *Flavour producers commit themselves to control the potential presence of ANNEX III A substances in flavourings in case these substances are also listed in Annex III part B and hence subject to maximum limits in specified applications. In the Quality Assurance System of flavour producers, these substances should be identified.*
>
> Recommendation 2)
>
> *Flavour producers commit themselves to communicate to the customer any relevant "RS" levels in flavourings irrespective of the intended use of the flavourings, even if the flavoured food is not covered by any food category mentioned in Annex III B.*

Development of a method of analysis of Restricted Substances

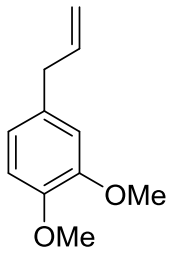
The Working Group on Methods of Analysis (WGMA) of the International Organization of the Flavour Industry (IOFI) has previously already published guidelines for the quantitative analysis of volatile flavouring substances by GC (IOFI, 2011) and by GC/MS using SIM (IOFI, 2012). The IOFI WGMA has now developed a method suitable for this purpose (IOFI, 2015). It should be noted that the method is not intended for the analysis of RS in finished foods and beverages.

The method is based on the analysis of flavourings and their volatile raw materials by gas chromatography-mass spectrometry (GC-MS), using selected-ion monitoring (SIM) and internal standards. It has been evaluated by 9 flavour-industry laboratories using a complex surrogate flavouring. This surrogate flavouring contained the following added volatile restricted substances (as standards): β-asarone, coumarin, menthofuran, methylchavicol, methyleugenol, pulegone, safrole, and the thujones (α- and β-isomers) (See Figure 1).

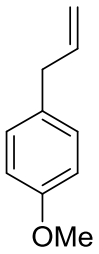
Figure 1. Volatile restricted substances covered by the method of analysis



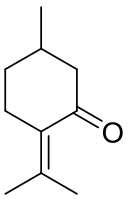
beta-asarone



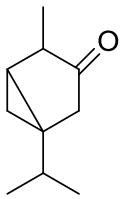
Methyleugenol



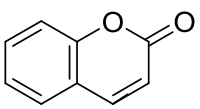
Estragole = Methylchavicol



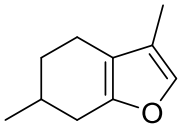
Pulegone



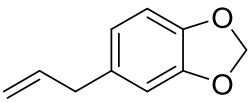
Thujone



Coumarin



Menthofuran



Safrole

For more details about the developed method of analysis we refer to the paper of the IOFI WGMA (IOFI, 2015).

The main principles of the method are summarised below:

- dilution of flavourings or their volatile raw materials in a suitable solvent of low volatility, e.g. iso-octane, ethanol;
- addition of 1 or more internal standards;
- direct split injection in a GC/MS system with a quadrupole or a magnetic-sector analyser;
- suggested internal standards are 1,4-dibromobenzene and 4,4'-dibromodiphenyl which provide very distinctive isotopic patterns, always in a ratio close to 1:2:1, making it possible to use the central molecular ion as quantifier and the two others as qualifiers;
- non- or semi-polar columns are recommended; columns with a polar phase can be used, but long-term stability should be monitored.

Some general considerations with regard to the instrumentation of choice are summarised below:

- Gas Chromatograph coupled with Mass Spectrometer: quadrupole or magnetic-sector analyser;
- ion-trap detectors for quantification are unsuitable – inter-laboratory testing has shown that results from these are unreliable for the quantitation of volatile flavouring substances;
- Capillary columns with non-polar, polar or semi-polar column (all types were used during the method development & validation) can be used;
- Carrier gas: Helium;
- Injection volume: varying from 0,2 to 1 µL;
- Split ratio: varying from 1/10 to 1/100;
- Injector Temperature: 250°to 300°C;
- Typical temperature program: from 60°C to MAOT @ 5°C/min.

Details about the MS-parameters (recommended selected ions for SIM mode) can be found in the paper of the IOFI WGMA.

Conclusions on method performance

The method was evaluated with a surrogate flavouring purposely designed to provide a "worst-case" product in terms of complexity and which contained all of the analytes at concentrations that would be likely to produce levels in finished foods at around those fixed as maximum under EU legislation (flavourings are used at a dilution of at least 200-fold, usually more). In this validation test, 9 laboratories took part, using four different column types and two different flavour carriers (ethanol and 1,2-propanediol).

Overall, the reproducibility was very high, resulting in relative standard deviations of less than about 20%, and recoveries of 80-120 %.

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DISCLAIMER

The views expressed are purely those of the author and may not in any circumstances be regarded as stating an official position of the European Flavour Association

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