

# EFFA Guidance Document for the Production of Natural Flavouring Substances and (Natural) Flavouring Preparations in the EU

## General Introduction

With EFFA Information Letter 13/04 we informed you about the revision of the EFFA Guidance Document on the EC Regulation on Flavourings (Regulation (EC) No 1334/2008).

That Guidance Document of 19/02/2009 as revised/amended (V3.0) on 11/03/2013 mainly focused on the practical application of the Flavouring Regulation and highlighted the major changes compared to the current Flavouring Directive 88/388/EEC. It further aimed at establishing EU wide industry guidance on the interpretation of the new rules, e.g. on labelling of flavourings both for B2B sales and on final foodstuffs.

It was also intended to harmonise the interpretation of the Regulation within the European Flavour Industry and thus to serve fair competition.

However although clear interpretations were given on definitions and labelling aspects (e.g. when "natural" can be used in the labelling of flavourings for B2B) it did not elaborate further on the processes that are considered as permissible to obtain / produce natural flavouring substances and (natural) flavouring preparations (i.e. "natural flavouring ingredients") in the EU.

The current Guidance Document, as amended (V2.0), should be regarded as a further addition / additional platform that provides a consistent interpretation of what constitutes a natural process under the new Flavouring Regulation.

This Guidance Document is built up of six Chapters outlining the general principles, various processes and methods:

- Chapter I** General Principles
- Chapter II** Traditional food preparation processes
- Chapter III** Appropriate physical processes
- Chapter IV** Microbiological and enzymatic processes
- Chapter V** Processes used in the production of natural flavouring ingredients
- Chapter VI** Analytical methods to assess authenticity

With regard to the Analytical methods used to assess authenticity as described in Chapter VI, it should be noted/emphasised that the use of analytical methods in assessing the authenticity of natural flavouring substances has limitations. The user of the substances remains responsible for obtaining compliant documentation from the supplier / manufacturer and, if necessary, to initiate or conduct a full process audit and traceability at the manufacturer's premises.

# DISCLAIMER

## Important Legal Notice:

The present Guidance Document on the Regulation has been produced by EFFA solely with the aim of providing informal guidance. It should be read in conjunction with the relevant legislation, being understood that only European Union legislation published in paper editions of the Official Journal of the European Union is deemed authentic. The guidance given by EFFA should not be used as a substitute for legal advice and should not be considered as an authoritative interpretation of the law, as only the European courts have the power to interpret statutory provisions.

Everyone should be aware of and fulfill all their obligations under applicable national and European laws and regulations. The guidance given by EFFA does not relieve members or any other persons of their obligations under those laws and regulations and members and any other persons should always satisfy themselves in any particular instance that the guidance provided by EFFA can be properly followed.

The Guidance Document was established with great care. However, EFFA can not be held accountable for the correctness of information, suggestions, advice and possible printing errors.

Neither EFFA nor any individual members or officers, can be held liable for any loss or damage suffered by any member or by any other person as a result of following or relying on this Guidance Document or other EFFA documentary materials. By using this Guidance Document, you will be deemed to have accepted the above limitation of liability.

EFFA has the copyright for this Guidance Document. The reproduction of the total text or parts thereof is permissible only if title and author are named.

# Table of Contents

## GUIDANCE DOCUMENT FOR THE PRODUCTION OF NATURAL FLAVOURING SUBSTANCES AND (NATURAL) FLAVOURING PREPARATIONS IN THE EU

---

<b>Chapter I. General Principles</b>	<b>45</b>
Introduction	45
Regulatory framework	45
Guiding principles	46
Source materials in the production of natural flavourings	46
Identified in nature	46
Natural processes	47
Self-policing	47
Schematic representation	48
<hr/>	
<b>Chapter II. Traditional food preparation processes</b>	<b>49</b>
Introduction	49
Regulatory context	49
Flavour formation during food processing	50
Regulatory interpretation	51
Industrial implications	52
Annex	53
<hr/>	
<b>Chapter III. Appropriate physical processes</b>	<b>55</b>
Introduction	55
Regulatory context	55
Practical implications	56
<hr/>	
<b>Chapter IV. Microbiological and enzymatic processes</b>	<b>59</b>
Biosystems and enzymes	59
Biosystems	59
Enzymes	60
General requirements for biosystems and enzymes	60
<hr/>	
<b>Chapter V. Processes used in the production of natural flavouring ingredients [substances and (natural) flavouring preparations]</b>	<b>61</b>
List of all permissible processes	61
Description of all processes – Definitions	62
Annex	69
<hr/>	
<b>Chapter VI. Analytical methods to assess authenticity</b>	<b>71</b>
Preamble	71
Use of discriminating analytical methodology	71
Lack of discriminating analytical methodology	74



# CHAPTER I

## General principles

### 1. Introduction

On December 31, 2008 Regulation (EC) No 1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on food, hereafter referred to as "The Regulation", was published.

EFFA has published extensive guidance on the general interpretation of the Regulation (EFFA guidance document on the EC Regulation on Flavourings: see EFFA Information Letter FL/09/02).

The Regulation amends the former definition of natural for flavours as stipulated in Directive 88/388/EEC. This aspect is not extensively reviewed in the EFFA Guidance document referred to above. It is the purpose of the current document to provide a consistent interpretation of what constitutes a natural process under the new Regulation. This interpretation:

- is ready to serve as a European Flavour Industry proactive proposition on the interpretation of Article 3.2(c, d, k) and Annex II of Regulation (EC) 1334/2008, while taking into account the scope of Recital (25),
- serves as a platform for the European (EFFA) Flavour Industry self-policing (for the distribution or sale or use of natural flavouring substances and flavouring preparations),
- constitutes the Guidance Document that should be taken as a basis for an EFFA Code of Practice on this subject,
- serves as a platform for explaining the same topic with other stakeholders within the EU and with IOFI.

In addition the document will provide guidance on the practical implications of self-policing instruments, e.g. the use of analytical techniques.

### 2. Regulatory framework

The Regulation defines natural in the Articles 3.2(c), (d) and (k). Recitals (16) & (25) reiterate the main guiding principles.

The applicable text is provided below for reference.

Recital (25)

Flavouring substances or flavouring preparations should only be labelled as 'natural' if they comply with certain criteria which ensure that consumers are not misled.

Article 3.2(c)

'natural flavouring substance' shall mean a flavouring substance obtained by appropriate physical, enzymatic or microbiological processes from material of vegetable, animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II. Natural flavouring substances correspond to substances that are naturally present and have been identified in nature;

Article 3.2(d)

'flavouring preparation' shall mean a product, other than a flavouring substance, obtained from:

- food by appropriate physical, enzymatic or microbiological processes either in the raw state of the material or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II;
- and/or
- material of vegetable, animal or microbiological origin, other than food, by appropriate physical, enzymatic or microbiological processes, the material being taken as such or prepared by one or more of the traditional food preparation processes listed in Annex II;

Article 3.2(k)

'appropriate physical process' shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring, without prejudice to the listing of traditional food preparation processes in Annex II, and does not involve, *inter alia*, the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.

Recital (16)

Regulation (EC) No 178/2002 defines food as any substance or product, whether processed, partially processed or unprocessed, intended to be, or reasonably expected to be, ingested by humans. Materials of vegetable, animal or microbiological origin, for which it can be sufficiently demonstrated that they have hitherto been used for the production of flavourings, are considered to be food materials for this purpose, even though some of these source materials, such as rose wood and strawberry leaves, may not have been used for food as such. They do not need to be evaluated.

### 3. Guiding principles

The regulatory context of natural flavouring ingredient production is based on three guiding principles:

- The origin of the source material
- The identification in nature of the manufactured flavouring ingredient
- The natural processes, their sequence and conditions thereof applied during manufacture

All three aspects are further detailed in the paragraphs below.

### 4. Source materials for the production of natural flavourings

The Regulation stipulates that sources of natural flavourings are material of vegetable, animal or microbiological origin in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II.

Minerals are not listed as an accepted source.

It is acknowledged and agreed that these sources may be foods as well as non-foods. Source materials for the production of natural flavouring ingredients may also include less routinely consumed parts of plant material as well as co- and/or by-products of food production such as fibre, hulls, stems, shells etc. (See in this respect Recital (16) of the Regulation.)

### 5. Identified in nature

In this section the term "identified in nature" is clarified and guidance is provided on the application of this definition to molecules that may exist as geometric and optical isomers.

#### 5.1. Definition

The Regulation does not contain a definition of the term "identified in nature". The term identified in nature has been clarified in the EFFA guidance document on the Regulation as follows:

*"Identified in nature" means:*

- *identified in materials of plant, animal, microbiological, or mineral origin,<sup>1</sup> and/or*
- *identified in food in the raw state or processed or partly processed for human consumption and*
- *meeting the criteria for the validity of identifications in nature as further described in the attachment to IOFI Information Letter 1333 (Flavour Fragr. J., 2006, **21**: 185).*

It is acknowledged and agreed that a process used to produce a natural flavouring substance may not in itself be used to qualify the resulting product as natural if the end product cannot be found in nature or in products traditionally used as foods by human beings.

<sup>1</sup> "Identified in nature", should not be confused with "recognised as a source material" for the production of natural flavouring substances or flavouring preparations, e.g. mineral origin (which should not be regarded as a source material for the preparation/production of natural flavouring ingredients).

## 5.2. Geometric and optical isomers

Flavouring substances may exist as distinct geometric isomers<sup>2</sup> (Z/E, more commonly referred to as *cis/trans*). Mixtures of geometric isomers do not have to be produced in the same ratio as they are found in a specific food or in a natural source. If all geometric isomers have been identified in nature, the production of a mixture of geometric isomers in any ratio should be allowed.

Flavouring substances may have one or more chiral centres and hence can exist as different optical isomers (enantiomers and diastereoisomers resp.). Mixtures of optical isomers shall be allowed in any ratio provided that all the isomers are identified in nature.

If one of the geometric or optical isomers has not (yet) been identified in nature, it must be interpreted as an artefact of the natural process. Any flavouring substance thus produced would not qualify as natural unless the artefacts present are in small amounts that neither characterise nor contribute to the flavour of the natural ingredient.

In this context it should be mentioned that the naturalness of the ingredient is not the sole regulatory requirement. The natural flavouring substance thus produced must comply with the appropriate regulatory specifications for that substance<sup>3</sup>.

## 5.3. Salts of natural flavouring substances

Salts of natural flavouring substances with cations such as  $\text{NH}_4^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{++}$  or anions such as  $\text{Cl}^-$ ,  $\text{SO}_4^{--}$  and  $\text{CO}_3^{--}$  are acceptable as natural flavouring substances.

## 6. Natural processes

The Regulation first clearly defines the sources which may be used in the production of natural flavouring ingredients. These are sources of vegetable, animal or microbiological origin. The sources themselves may or may not be processed according to the traditional food preparation processes which are listed in the Annex II of the Regulation. This difference is essential since it recognizes that a number of vegetable, animal or microbiological sources already intrinsically contain flavour while other source materials only generate flavour during the food preparation process. The interpretation of traditional processes allowed under Annex II is covered in Chapter II.

The source material (whether processed or not) is subjected to physical, enzymatic or microbiological processes to obtain the flavouring substance or flavouring preparation.

The legislator has introduced in Article 3.2(k) a definition of appropriate physical processes (which was not included in the former Directive 88/388/EEC) as being those which do **not intentionally** modify the chemical nature of the components of the flavouring. However, the phrasing "*without prejudice to the listing of traditional food preparation processes in Annex II*" in Article 3.2(k) acknowledges that processes that are listed in Annex II may be used irrespective of whether they induce such modification. In Chapter III detailed guidance is provided on the interpretation and practical implications of Article 3.2(k).

Enzymatic and microbiological processes do have the actual intention of chemically modifying the nature of the components and as such the legislator approves their use for this purpose. The regulatory context and practical implications for enzymatic and microbiological processes are covered under Chapter IV.

From the text of the Regulation it is clear that the food preparation processes take place before the physical, enzymatic and microbiological processes. It should be noted that the physical, enzymatic and microbiological processes themselves may be used sequentially and repetitively in any order as is also the case for the food preparation processes in Annex II. The legislator does not provide any limitations to this extent. In this context it must be emphasized that natural processes do not have to mimic the route of formation by which the flavouring substances or preparations are formed in the vegetable, animal or microbiological source and / or in traditional food processing.

A natural flavouring substance or preparation may be produced by consecutive manufacturing steps involving a series of intermediates. It is important to emphasize that each step must be recognised as a natural process. However, the intermediates in themselves do not have to be recognised as flavouring ingredients (substances) nor as food intended for human consumption as such.

<sup>2</sup> Positional isomers are different substances (with different CAS-numbers, identifiers and specifications) and are not the subject of this paragraph.

<sup>3</sup> Relevant (legal) instruments may be the EU-Community List, FCC, JECFA and national specification references (sometimes referred to as standard of identity, e.g. for ethyl acetate or ethyl butyrate) as applicable.

It should be noted that several processes may find useful application in different phases of the production of natural flavouring ingredients. To further facilitate a transparent view of the processes used in natural flavouring production and how they are covered in the Regulation, Chapter V provides an open-ended list of such processes and includes concise descriptions and conditions of use.

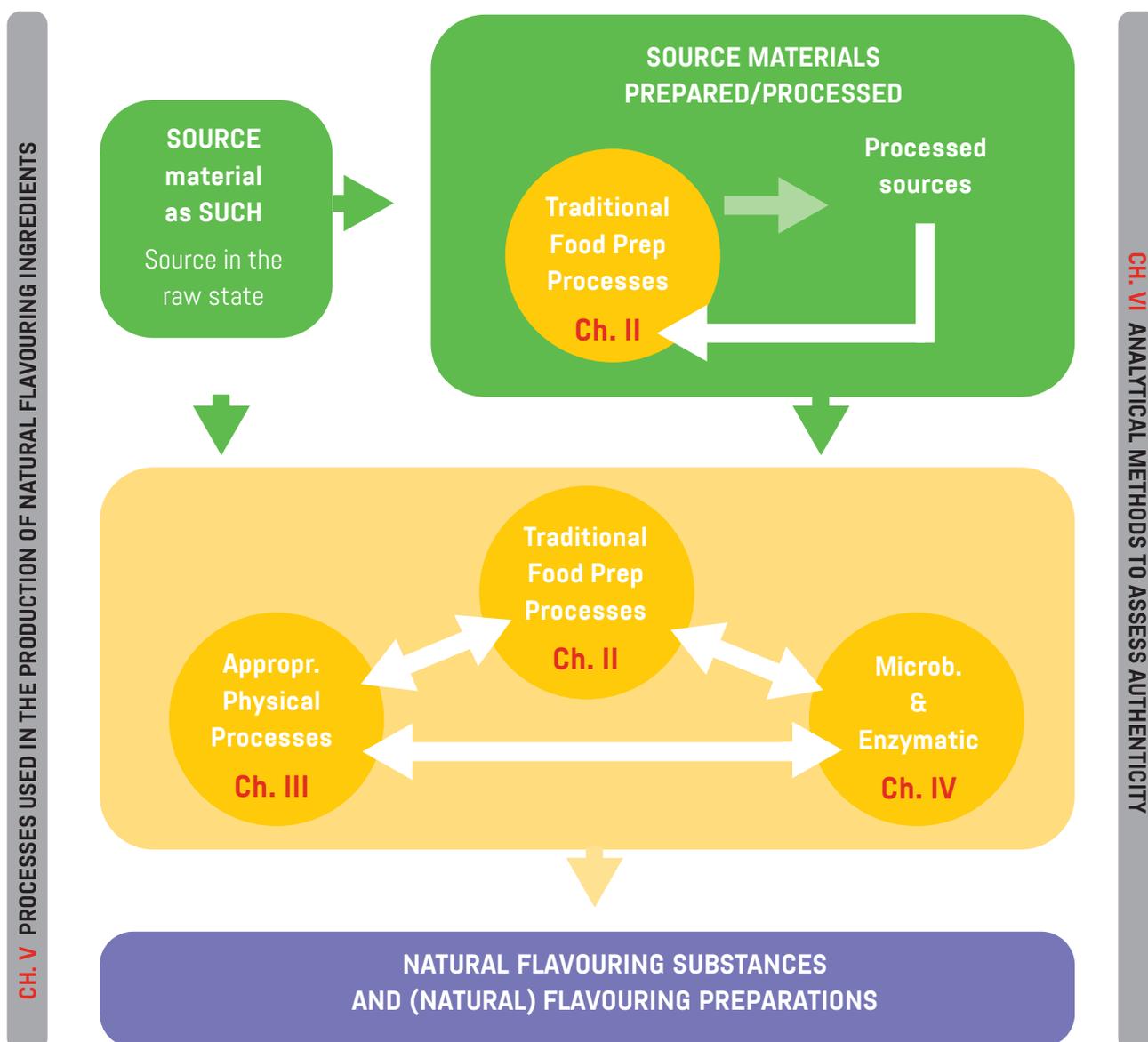
## 7. Self-policing

Every operator placing a natural product in the market is responsible for ensuring compliance with the Regulation. However, not every operator will be freely offered access by its supplier to the detailed process information required to qualify the natural claim. Chapter VI provides guidance on the potential use of analytical techniques to assess natural claims.

## 8. Schematic representation

For easy reference a pictorial representation of the interpretative guidance on the production of natural flavouring substances and flavouring preparations is provided below. The scheme includes (in red) the references to the respective Chapters in this Guidance Document.

FRAMEWORK SCHEME ON NATURAL PROCESSES



## CHAPTER II

# Traditional Food Preparation processes

## 1. Introduction

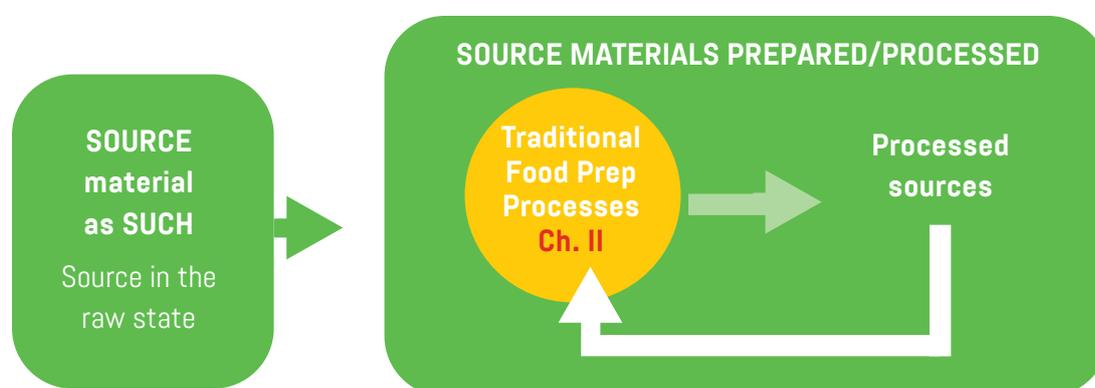
The Regulation defines the source materials from which natural flavouring substances or flavouring preparations may be obtained as follows:

Article 3.2(c) & (d)(ii) "...material of vegetable or animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the traditional food preparation processes listed in Annex II."

In this chapter the context of this definition is discussed, the legal text is interpreted and guidance notes are provided for appropriate industrial practice.

## 2. Regulatory context

The Regulation defines in Articles 3.2(c), (d)(i) and 3.2(d)(ii) the source materials which may be used in the production of natural flavouring ingredients, i.e. material of vegetable, animal or microbiological origin. These sources themselves may be food or non-food, either in the raw state or may be processed according to traditional food preparation processes which are listed in Annex II.



The regulatory context of the Article is important. The legislator herewith acknowledges that two distinct sources of natural flavouring ingredients exist:

- **Source Raw (Intrinsic)**

Natural flavouring ingredients may be intrinsic to the raw source from which they have been isolated. This is predominantly the case for products that originate from fruits, spices, herbs, meat, fungi, and yeast.

- **Source Processed (*in situ*)**

Natural flavouring ingredients may be formed from sources of vegetable, animal or microbiological origin as a result of food processing which may include microbiological and enzymatic processes. The natural flavouring ingredients are subsequently isolated from the processed source material.

The difference between raw and processed sources is essential since it recognizes that a number of vegetable, animal or microbiological sources already intrinsically contain the flavour whilst other source materials only generate the flavour during the food preparation process. This view is consistent with the well-established definition for natural occurrence.

### Summary:

Flavouring substances occur naturally either as an intrinsic component of the source material in the raw state and/or as formed during food processing.

## 3. Flavour formation during food processing<sup>4</sup>

Foods contain proteins, fats, carbohydrates, micro-nutrients, and other constituents. The interactions between all these constituents during food processing are numerous and complex: new materials are formed whereas others disappear. Some of these interactions and transformations have been extensively studied and can be described in pathways such as Amadori and Heynes rearrangements, Strecker degradation, retro-aldol reactions, all of which are transformation steps that are part of non-enzymatic browning reactions, referred to as Maillard reactions<sup>5</sup> and (hetero)cyclisation reactions.

The type of ingredients that are formed during food processing depend, amongst others, on the texture/composition of the source materials, the processes applied, the process conditions (time, temperature) and the water activity. The resulting ingredients are not necessarily present in the unprocessed source material and include nitrogen, sulphur and oxygen containing compounds such as pyrazines, pyrroles, thiazoles, trithiolanes, furans, and oxazolines.

In the table below some typical examples of flavour formation during food processing are concisely reviewed.

Table 1. Some typical examples of flavour formation during food processing

Source material	Process	Compounds
Onion	Cutting, cooking	Tissue disruption during cutting initiates the enzyme mediated production of the intermediate sulfenic acid along with ammonia and pyruvate. Further non-enzymatic reactions yield the characteristic lachrymatory substance thiopropanal S-oxide. Cooking enhances the formation of further reaction products of sulfenic acid by re-arrangements and decomposition. These resulting substances are of the classes mercaptans, disulfides, trisulfides and thiophenes.
Cabbage (Brassica plants)	Cutting, cooking	Tissue disruption initiates the enzyme mediated production of the characterising isothiocyanates. Cooking destroys the isothiocyanates and promotes the formation of nitriles and sulphur-containing degradation and re-arrangement products.
Cocoa	Roasting	During roasting the characterising substance 5-methyl-2-phenyl-2-hexenal is formed by an aldol condensation from phenylacetaldehyde (from phenylalanine) and 3-methylbutanal (from leucine). <sup>6</sup> Other substances formed are pyrazines and heterocycles.
Coffee	Roasting	Extensive literature is available on the transformations (Strecker degradation and Maillard reactions) responsible for the formation of volatiles during coffee roasting. <sup>7</sup>
Dairy	Fermentation	Homofermentative lactic acid bacteria produce only lactic acid, acetaldehyde and ethanol in milk cultures. Blue mold cheeses produced by fermentation with <i>Penicillium roquefortii</i> are characterized by the formation of methyl ketones from fatty acids.
Meat, Fat	Frying	(E,E)-2,4-decadienal is produced from the oxidation/degradation of unsaturated fatty acids.
Caramel	Heating	4-hydroxy-2,5-dimethyl-3[2H]-furanone is produced by the thermal degradation-dehydration of sugars. <sup>8</sup>
Cereal	Baking	Pyrazines and pyrrolines are produced from the thermal degradation of amino acids in the presence of sugars, following fermentation of the dough.

<sup>4</sup> *Food Chemistry* third edition, edited by Owen R. Fennema. Marcel Dekker Inc. 270 Madison Avenue, New York, New York 10016 ISBN: 0-8247-9691-8. Chapter 11 by Robert C. Lindsay (p. 723–765). 1996

<sup>5</sup> *On Food and Cooking, the Science and Lore of the Kitchen*. Harold McGee. Editor: Scribner, 1230 Avenue of the Americas, New York, NY 10200. ISBN 0-684-80001-2 (p. 778).

<sup>6</sup> Phenylacetaldehyde and 3-Methylbutanal are themselves produced via the Strecker degradation of the respective L-amino acids phenylalanine and leucine.

<sup>7</sup> *Coffee Flavor Chemistry*. Flament, Ivon. John Wiley & Sons, Ltd., West Sussex UK, 2002, pp 37-ff

<sup>8</sup> The production of the flavouring substance 4-hydroxy-2,5-dimethyl-3[2H]-furanone by appropriate isolation from an intermediate complex mixture obtained by dehydration of rhamnose in the presence of the natural amino acids L-lysine and/or L-proline is consistent with the use of natural processes on natural raw materials. The mere fact that the intermediate complex mixture, obtained by heating a reducing sugar and a source of nitrogen (amino), when used as such without purification should be classified as a "process flavour" does not conflict with this position/statement.

### Summary:

During food processing natural flavouring substances are formed of which important representatives are not known to occur in the raw source.

These flavouring substances are formed as a result of a cascade of transformations which often include both enzymatic and non-enzymatic pathways.

The thermal processing of food particularly promotes the formation of complex substances such as sulphur and nitrogen containing heterocyclic substances by non-enzymatic pathways.

## 4. Regulatory interpretation

Article 3.2(c) & (d) “...material of vegetable or animal or microbiological origin either in the raw state or after processing for human consumption by one or more of the **traditional food preparation processes** listed in Annex II.”

It should be noted that the text refers to processes used in **traditional food preparation processes**. This statement should not literally be interpreted as domestic cooking practices. The list in Annex II is interpreted as covering food and food ingredient processes “traditionally” used in domestic cooking and industrial processing in the flavour and food related industries. In this context the word “traditional” is interpreted as being used to emphasize that a process listed in the Regulation should have a history of use (either domestic or industrial) and as such would be expected to be known to, and accepted by, the general public (familiarity).

In line with Article 22 and Recital (32) of the Regulation this list can be updated in the future. It has already been acknowledged that the list is incomplete, for example enzymatic processes are missing (cheese preparation). Another example is extrusion which is a process used in the production of, amongst others, breakfast cereals.

### 4.1. Subsequent processing

The allowable processes are not bound to the type of source material and may be applied sequentially and repetitively in any order. In other words processes in the list can be used for further processing of intermediate products/fractions (food and/or materials other than food) that were obtained by previous processing steps.

This is an important element for the flavour industry. In the preparation of natural flavouring ingredients the flavour industry does not normally use the source as a whole but rather processes the specific fractions responsible for the formation of the flavouring ingredients. In this respect the flavour industry, in the spirit of their intent, respects the use of traditional food preparation processes and applies them to particular fractions in order to specifically generate the flavouring substances rather than the nutritional value of the food.

### Summary:

- The traditional food preparation processes may be applied sequentially and repetitively in any order.
- These processes are applied to material of vegetable, animal and/or microbiological origin (being either raw or processed; being either food or non-food).
- It is acceptable to apply these processes so as to specifically optimize the yield of the flavouring substances from the food precursor.
- It is understood and accepted that the use of traditional food preparation processes may intentionally modify the chemical nature of the components of the food or food ingredient to which the process is applied.

## 5. Industrial implications

The use of processes by the flavour industry to obtain natural flavouring substances and flavouring preparations must conform to the regulatory interpretation presented above. In this section a number of guidance notes are formulated to further support a consistent interpretation of the requirements.

### Ingredients

The materials to which the traditional food preparation processes may be applied are from animal or vegetable or microbiological origin, either in the raw state or from earlier processing.

These materials may be food or may be material other than food.

### Process

The processes are those listed in Annex II or those deemed appropriate by EFFA (see also Chapter V).

The traditional food preparation processes may be applied sequentially and repetitively in any order.

The traditional food preparation processes are purposely applied to intentionally modify the chemical nature of the source material. The modifications are those which are demonstrated to occur (or likely to occur) during traditional food preparation processes or "as in the kitchen".

### Catalysts

Article 3.2(k) on "appropriate physical processes" excludes, *inter alia*, the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.

Although not strictly formulated in the Regulation, EFFA considers that the same limitation applies to the traditional food preparation processes referred to in Article 3.2(c) & (d).<sup>9</sup>

### Conditions

With the exception of frying and pressure cooking Annex II does not limit the conditions under which the processes are applied. However, EFFA considers the use of elevated time, temperature and pressure as unacceptable if applied to initiate a process which cannot be demonstrated to occur under the commonly accepted conditions of traditional food preparation processes.

Industrial processes are commonly applied in closed vessels for environmental considerations and for the safety of workers. These requirements dictate that industrial processes cannot necessarily be compared, one to one, with traditional food preparation processes. The industrialized (closed vessel) process may require the modification of physical parameters to yield an acceptable product. EFFA considers these modifications acceptable provided it is demonstrated that they do not alter the intrinsic characteristics of that process.

EFFA considers that temperature should be limited to 400°C (see also Chapter V). Pressure should preferably be maintained below 400 bars (conditions which may be reached in a typical supercritical extraction of herbs and spices).

### Auxiliary agents

The use of auxiliary agents such as natural organic acids and bases is allowed to accelerate the formation of the desired product. In other words the auxiliary agent improves the yield of a conversion but is not essential for the transformations to occur and does not change the overall mechanism of formation.

Materials such as acids and bases used in transformations in which they are essential to initiate the conversion are not auxiliary agents but reactants. In this respect these materials should be regarded as source materials and shall be natural.<sup>10</sup>

<sup>9</sup> For example the use of *p*-toluenesulfonic acid as an "organic soluble" acid catalyst is not permitted.

<sup>10</sup> The use of natural occurring acid and base is for example allowed in ester hydrolysis, esterification, cyclisation (lactones formation) provided its use solely changes the reaction kinetics of an already existing equilibrium.

## Annex II. List of traditional food preparation processes

Chopping	Coating
Heating, cooking, baking, frying (up to 240 °C at atmospheric pressure) and pressure cooking (up to 120 °C)	Cooling
Cutting	Distillation/rectification
Drying	Emulsification
Evaporation	Extraction, incl. solvent extraction in accordance with Directive 2009/32/EC
Fermentation	Filtration
Grinding	
Infusion	Maceration
Microbiological processes	Mixing
Peeling	Percolation
Pressing	Refrigeration/Freezing
Roasting/Grilling	Squeezing
Steeping	





# CHAPTER III

## Appropriate Physical Processes

### 1. Introduction

The Regulation defines appropriate physical processes from which natural flavouring substances or flavouring preparations may be obtained as follows:

Article 3.2(k): '*appropriate physical process*' shall mean a physical process which does not intentionally modify the chemical nature of the components of the flavouring, without prejudice to the listing of traditional food preparation processes in Annex II, and does not involve, inter alia, the use of singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.

In this chapter the context of this definition is discussed and an interpretation of the legal text provided.

### 2. Regulatory context

Article 3.2(k) stipulates three distinctly different requirements which will be separately reviewed.

#### 2.1. Physical processes within the context of “without prejudice to the listing of traditional food preparation processes in Annex II”

Article 3.2(k) determines an appropriate physical process as one which does **not intentionally** modify the chemical nature of the components of the flavouring. Article 3.2(k) contains an important additional element stipulating “*without prejudice to the listing of traditional food preparation processes in Annex II*”.

The legislator herewith acknowledges that traditional food preparation processes are appropriate physical processes which may intentionally change the chemical nature of the components of the flavouring.

Consequently it is only physical processes that are not listed in Annex II which may not be used to intentionally modify the chemical nature of the components of the flavouring.

#### 2.2. Physical processes within the context of intentional modification

The Regulation refers to physical processes. A physical process may lead to a physical and/or a chemical change.

- Physical change means altering properties such as physical state, colour and appearance.
- A chemical change would be, for instance, a decomposition of constituents and/or formation of novel constituents.

Not in all cases is the difference clear. Some instances may be argued as either a physical or a chemical change. The solution of a salt in water is generally positioned as a physical process resulting in a physical change but it does involve the dissociation of the salt in the solution.

In the interpretation of the phrasing of "... does not intentionally modify the chemical nature of the components of the flavouring", the term *intentionally* is the most important. Herewith the legislator has recognized and accepted to a certain extent that physical processes will induce chemical changes.

The term intentional in this context is interpreted as meaning processes where the primary intent is to chemically modify and to yield specific components not initially present (or not present in the desired quantity) in the source material, the abundance of which is critical for the organoleptical performance of the desired product. In all other cases the formation is interpreted as unintentional (unavoidable by-product/artefact).

## 2.3. Physical processes within the context of "and does not ...UV radiation".

Article 3.2(k) specifically mentions that physical processes covered under this article may not "*inter alia*" use singlet oxygen, ozone, inorganic catalysts, metal catalysts, organometallic reagents and/or UV radiation.

The term "*inter alia*" is used to emphasize that the list of compounds included in the definition is not exhaustive but merely provides examples of chemical reagents typically used to intentionally or irreversibly modify the chemical nature of the components of the source material with which they are reacted. In the table below, the reagent groups are listed and typical representatives of each group provided.

Table 2. Typical examples of reactions and reagent groups disallowed under Article 3.2(k)

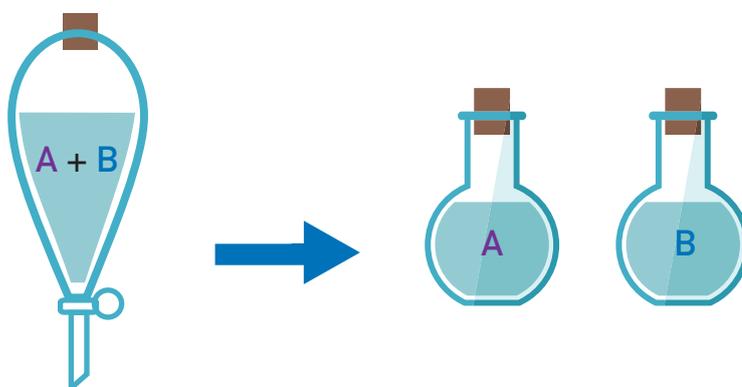
Reagent Group	Definition	Typical Representatives
Singlet oxygen, ozone	O <sub>2</sub> in an excited phase.	Singlet O <sub>2</sub> , O <sub>3</sub>
Inorganic catalysts or reagents	All chemical compounds that act as catalyst except those containing C-H bonds, metal catalysts and organometallic reagents.	HCl, H <sub>2</sub> SO <sub>4</sub> , NaOH, KOH, MgSO <sub>4</sub> , NH <sub>3</sub> , LiAlH <sub>4</sub> , KMnO <sub>4</sub> , DiBAIH, NaBH <sub>4</sub>
Metal catalysts	Elemental metals.	Typical examples are Fe, Cu, Ni and Pd
Organometallic reagents	Chemical compounds containing bonds between carbon and metal that act as reagents.	Sodium ethoxide, sodium methoxide, Grignard reagents, organolithium reagents
UV radiation	Electromagnetic radiation with a wavelength in the range of 10 – 400 nm.	UV Light, Rose Bengal
Inter alia	Other non-natural catalysts, sources of radicals and reagents considered unacceptable by EFTA.	Hydroperoxides (such as tert.-butylhydroperoxide), pTSA, TEMPO

## 3. Practical implications

Physical processing induces changes in the composition of the product in comparison with that of the starting material. As indicated in the previous section this has been acknowledged and accepted by the legislator. In this section these changes are characterized and discussed to the extent relevant for the interpretation of Article 3.2(k).

### 3.1. Compositional changes (redistribution)

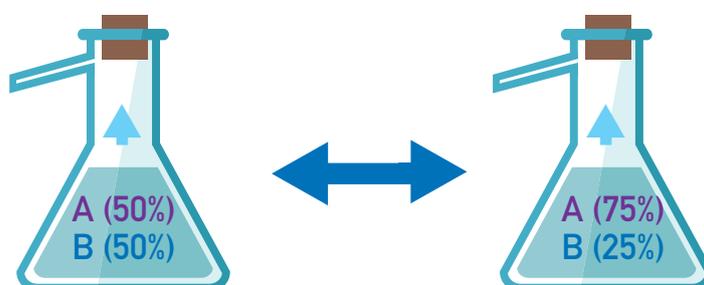
Physical processes used for isolation/purification may segregate or redistribute the starting material into two or more fractions, an example being simple phase separation in ambient conditions (e.g. oil/water phase separation). Transformation of the chemical composition does not occur. A physical process fitting this description complies with Article 3.2(k).



Physical process redistributes materials

### 3.2. Compositional changes (influencing equilibrium)

A physical process applied for isolation/purification may influence the composition by changing the existing equilibrium between the constituents in the mixture, meaning the equilibrium will move to the most favoured composition from a thermodynamic perspective. This phenomenon will not introduce novel constituents in the composition, only the ratio of existing constituents will change.

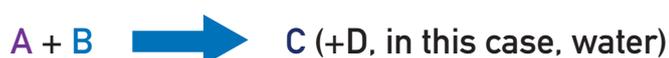


Physical process shifts equilibrium

The extent to which this type of compositional change applies depends heavily on the constituents in the starting material, their stability, the type of physical processes applied and the conditions thereof. A physical process fitting this description complies with Article 3.2(k).

### 3.3. Compositional changes (novel formation)

Last, but not least, a physical process applied for isolation/purification may also lead to formation of constituents not identified in the starting material. These novel constituents are formed as a result of a cascade of transformations initiated by the process applied and the conditions thereof. This may apply, for example, to thermal esterification without catalysis whilst removing water.



Physical process: formation of novel substances

If the physical process introduces novel constituents the question of *intention* becomes relevant. It is acknowledged and agreed that physical processes applied with the intention of isolation/purification, and by which certain novel components may be unintentionally formed as artefacts (unavoidable by-products), are acceptable under Article 3.2(k). For example during (steam) distillation to produce essential oils novel esters may be formed by trans-esterification from the corresponding acids and alcohols. The most well-known example of novel formation is lime oil. The organoleptical difference between cold pressed and distilled lime oil is due, inter alia, to the novel formation of alpha-terpineol from the conversion of citral in the presence of citric acid.

In cases where a process is designed such that it deliberately (intentionally) promotes the formation of novel substances rather than isolation / purification of the intrinsic substances, then the process applied must be a traditional food preparation process listed in Annex II.

### 3.4. Elimination processes

Elimination processes are very important in the flavouring industry. They may be applied to remove both undesired flavouring material as well as to remove undesirable non-flavouring components.

It should be mentioned that chemical conversion may be applied to remove non-flavouring components from the source material, for as long as the target material remains unchanged. There are important reasons to remove undesirable non-flavouring matter. One purpose is detoxification, e.g. in the production of benzaldehyde from amygdalin ex prunus seeds (almond, *prunus dulcis*; apricot, *prunus armeniaca*) where the released HCN is precipitated with iron sulphate.



Undesired material B is eliminated via B-X precipitate, A remains unchanged

In this case, B-X is produced by/via a chemical reaction and cannot be deemed natural as it has undergone intentional chemical change (see also Chapter V under absorption/adsorption).

#### Summary:

- Physical processes applied in order to separate constituents into multiple fractions do comply with Article 3.2 (k).
- Physical processes applied in order to move/shift the equilibrium of constituents do comply with Article 3.2 (k).
- Physical processes applied with the intention of isolation/purification but by which certain novel components may be formed as artefacts (unavoidable by-products eliminated as much as possible) do comply with Article 3.2 (k).
- Processes applied in order to eliminate undesirable components from the flavouring by changing the chemical nature of the components to be removed do comply with Article 3.2 (k).
- Physical processes that are not listed in Annex II and are applied in order to intentionally modify the chemical nature of the components of the flavouring DO NOT comply with Article 3.2 (k).

# CHAPTER IV

# Microbiological and Enzymatic Processes

## 1. Biosystems and Enzymes

Biological entities comprising micro-organisms such as bacteria, yeasts and fungi, or higher organisms such as algae, plants or animals, used as such or in cell or tissue cultures, and enzymes derived thereof are permitted for the production of natural flavouring ingredients. The biological entities other than isolated enzymes will be referred to in the document as biosystems.

Biosystems are grown and/or maintained during a fermentation process. Since the terms 'enzyme' and 'ferment' are synonyms the term fermentation also refers to a process involving enzymes from microbial and non-microbial origin.

## 2. Biosystems

The biosystems used should be either those that are traditionally employed in food preparation or those that are regarded as safe (safe strain lineages) under the conditions used for the production of natural flavourings.

### 2.1. Definitions

**Medium:** In this context 'medium' refers to the mixture that is used to culture micro-organisms such as bacteria, yeasts and fungi or higher organisms such as algae, plants or animals cells and tissue cultures. Liquid media are generally but not exclusively aqueous mixtures of nutrients. Solid media are typically composed of solid organic material and/or aqueous mixtures of nutrients supplemented with a thickener such as agar. The medium has to be particularly suited/adapted to the biosystem.

**Nutrients:** 'The substances from which microorganisms synthesize their cell material and obtain their energy'.<sup>11</sup> Flavouring materials obtained from biosystems may be derived in whole or in part from nutrients provided for this purpose.

Fermentation nutrients are generally classified as sources of carbon, nitrogen, sulfur, minerals and vitamins.<sup>12</sup> Nutrients are not restricted in origin (they do not need to be natural). However, the carbon source for the biosystem must be of natural origin or derived from CO<sub>2</sub> from air.

**Substrates:** Substrates are not usually defined for living entities (biosystems) such as bacteria. ECHA however considers that when the action of a biosystem has similarity with a single enzymatic conversion, the same restrictions should apply to the substrate/precursor as in an enzymatic reaction (see below: Enzymes).

**Co-Factors, Nutrients, Hormones, etc:** Materials used at any stage of the process, such as co-factors, minerals, nutrients, vitamins, hormones, pH adjusting agents, oxygen, nitrogen or carbon dioxide may only be used at levels required for the productive functioning of the biosystems. These materials are not restricted in origin (they do not need to be natural). Residual amounts of co-factors, minerals, nutrients, vitamins, hormones and pH adjusting agents shall be removed from flavouring preparations and natural flavouring substances to the greatest extent technically possible and shall not compromise the safety of consumers nor mislead them in violation of the general conditions of use as defined in Article 4 of the Regulation.

<sup>11</sup> *General Microbiology* by Hans G. Schlegel (1993), Press Syndicate of the Cambridge University, The Pitt Building, Trumpington Street, Cambridge CB2 1RP, 40 West 20<sup>th</sup> street, New York, NY 10011-4211, USA, page 193.

<sup>12</sup> *Fermentation and Biochemical Engineering Handbook, Principles, Process design and Equipment, second edition (PY)*. Edited by Henrt C Vogel and L Todaro. Chapter 3, "Nutritional Requirements in Fermentation Processes" (Willem H. Kampen), pages 122-160.

## 3. Enzymes

Until the application of the Community List of food enzymes as referred to in Article 17 of Regulation (EC) 1332/2008 on food enzymes, EFTA considered there should be no limitation to the origin of the food grade enzymes used.

However, enzymes utilised in the production of natural flavouring ingredients shall already comply with the remainder of Regulation (EC) 1332/2008.

### 3.1. Definitions

**Substrates:** 'The substance on which an enzyme acts, and which is activated by the enzyme, is termed the substrate of the enzyme'.<sup>13</sup> All substrates in this context must be derived from vegetable, animal or microbiological sources either directly or after transformation by approved traditional food processes as described in Chapter II.<sup>14</sup>

**Medium:** The term medium is sometimes also used to characterize the 'reaction medium' of an enzyme (e.g. organic solvents listed in the Solvent Directive: 2009/32/EC as extraction solvents for natural flavouring complexes; water, humidified gas etc.).

### 3.2. EFTA Guidance on Enzymatic processes

Although "Enzymatic processes" are not included as such in Annex II to Regulation (EC) 1334/2008 listing the "Traditional Food Preparation Processes", it is EFTA's understanding that the enzymatic processes are covered by the entry "Fermentation" listed in the Annex II.

## 4. General Requirements for Biosystems And Enzymes

**Biosystems and enzymes cannot be used as 'alibis' for non-acceptable chemical processes.**

Biotechnological processes should be thoroughly investigated in order to ensure that the described reaction is genuinely catalysed by the biosystem or enzyme. If the conditions of the reaction (pH, use of co-factors etc.) induces almost the same yield of product with or without the presence of the biosystem or enzyme then this clearly demonstrates a placebo effect. The reaction must be further scrutinised under a category other than those of the present Chapter.

**Temperature, Pressure, etc:** Temperatures and pressures which are applied are self-limiting since they should be compatible with the stability and efficiency of the biosystems or enzymes. Techniques such as sonication, mechanical disruption and enzymatic lysis may also be used for breaking up cell membranes.

**Carriers:** In a situation where there is a possibility that a carrier, for example, might react with the natural flavouring ingredient generated by the biotechnological process during isolation/purification then this reaction must comply with guidelines of EFTA on physical processes (Chapters II&III of this document).

Carriers may remain in the final product provided they comply with the Regulation (EC) No 1333/2008 on food additives.

**Immobilisation Matrices:** Immobilisation matrices are not limited provided that they are approved for food use.

**Isolation/Purification:** Any physical process used to recover, isolate or purify the flavouring substances or flavouring preparations formed by the biosystem or enzyme will not detract from their natural status provided they do not alter their chemical composition (see Chapter V).

<sup>13</sup> *Enzymes* by M. Dixon and E.C. Webb, Academic Press Inc, Publishers, New York, 1958; page 5.

<sup>14</sup> An example of a substrate that could not be used to produce a natural flavouring ingredient would be the use of a substance of mineral origin such as sodium sulfide where the sulfur atom is incorporated by enzymatic processes into the chemical structure(s) of the resulting flavouring ingredient.

## CHAPTER V

## Processes Used in the Production of Natural Flavouring ingredients

This Chapter aims to provide an overview of all processes used to obtain natural flavouring substances and/or (natural) flavouring preparations.

All substrates for these processes are required to be eligible for qualification as natural according to this EFFA Guidance Document.

As the Flavour Industry in Europe we have always alerted our membership on other legislation, e.g., solvent extraction Directive, and therefore drafted our Guidance document to establish a clear preference for those solvents that are permitted by the Directive 2009/32/EC as amended.

## 1. List of all permissible processes

The processes listed below are further described in this Chapter. The limitations and permissibility of each process, in the opinion of EFFA, is also discussed.

This list is an open, positive list and is neither exclusive nor restrictive: it may be expanded after submission to, and discussion within, EFFA.

ABSORPTION	GRINDING
ADSORPTION	HEATING, COOKING (including Extrusion Cooking), BAKING, FRYING
AGGLOMERATION	INFUSION
CENTRIFUGATION	ION EXCHANGE
CHOPPING	LYOPHILIZATION
CHROMATOGRAPHY	MACERATION
COATING	MICROBIOLOGICAL PROCESSES
COOLING	MIXING
CRYSTALLIZATION	OSMOSIS
CUTTING	PEELING
DIGESTION	PERCOLATION
DISTILLATION/RECTIFICATION	PRECIPITATION
DRYING/VACUUM DRYING	PRESSING
ELECTROPHORESIS	REFRIGERATION/FREEZING
EMULSIFICATION	ROASTING/GRILLING
ENCAPSULATION	SALTING OUT
ENZYMATIC PROCESSES	SQUEEZING
EVAPORATION	STEEPING
EXTRACTION	SUBLIMATION
FERMENTATION	ULTRASONIC TREATMENT
FILTRATION	

## 2. Description of all processes – Definitions

### ABSORPTION

### ADSORPTION

This is restricted to processes in which the starting material is recovered intact and in which the adsorbent is not purposely used as a catalyst for an irreversible chemical conversion of a natural substrate into another substance.

This applies to mixtures as well as individual substances.

**Example of an allowable use of adsorbent:** to remove impurities (even after chemical transformation of this impurity on the adsorbent, provided this by-product does not qualify for the labelling of Natural Flavouring Substance) from a crude natural flavouring substance obtained by a natural process.

E.g.: Mixture (A & B) gives (A & C) where B is converted to C

A = natural substance untouched by the adsorption process (to be purified)

This process leaves A intact while B is converted into C and removed (C **cannot** be regarded as a natural substance).

Another example of an allowed use of an adsorbent is the use of activated charcoal to remove or standardize the colour of essential oils, provided the activated charcoal does not purposely alter the chemical composition of other constituents of the essential oil, e.g. by isomerisation.

**Example of an unacceptable use:** the conversion of citronellal to isopulegol purposely promoted by adsorption on silica gel.

The use of ion exchange resins is a further example of adsorption on a support and is detailed later.

### AGGLOMERATION

This process is usually used to modify the particle size of a product. The simplest way is to use a fluidized bed into which a powder is introduced, moistened and agglomerated. Other equipment can be used (rotogranulators, vortex granulators, compactors...).

If a binding agent is used it must comply with the appropriate legislation.

### CENTRIFUGATION

The purpose of centrifugation is to separate two liquids or solid/liquid mixtures which are not miscible with each other (e.g. centrifugation of cold pressed essential oils of citrus away from the water phase created by squeezing and washing citrus peels).

Centrifugation can also be used to separate the solvent phase from the water phase in an extraction step, prior to removal of the solvent in vacuo.

Centrifugation can also be used to purify extracts from suspended particulate matter which would otherwise only settle after a long period of time.

### CHOPPING

See under CUTTING.

### CHROMATOGRAPHY

This is a separation process which is used for preparative purposes.

Low pressure column chromatography, HPLC and GLC, either preparative and/or chiral, can be used.

For the production of natural flavouring ingredients, all solvents that are acceptable according to the EFTA Guidance Document can be used for chromatography – the solvents do not have to be natural themselves.

Solvents for this use are preferably those solvents permitted by the Directive 2009/32/EC on extraction solvents and its subsequent modifications.

Indeed, EFTA recognizes that other eluents may be used as well. In the absence of regulations, the residue limits as listed in the IOFI IL 1352 and its attachments are considered as appropriate.

Chromatography should not be used to trigger isomerization or act as an alibi for chemical transformation.

Chromatography should only be used on substrates which are produced via an acceptable natural process. Example of unacceptable use: preparative chiral chromatography of racemic gamma-undecalactone prepared by isomerization of undecylenic acid with highly concentrated mineral acid (such as H<sub>2</sub>SO<sub>4</sub>).

## COATING

No particular comments.

## COOLING

This process lowers the temperature of a product or the solution of a product. A synonym is "refrigeration".

In Traditional Food Preparation Processes, cooling (principally of liquids or of the surface of solids) slows down oxidation caused by oxygen in air. It also reduces the modification of the food composition via its own enzymes and spoilage initiated by surface or air borne bacteria and other microorganisms.

Solidification of a liquid by lowering its temperature is called "freezing" (e.g. of water into ice). Other types of "freezing" include "deep freezing" of vegetable and animal substrates to facilitate their preservation for transportation purposes, or to prolong their shelf-life either for direct consumption or for further processing.

See "freeze drying" under DRYING.

In purification processes, cooling can be used to induce a variety of outcomes:

- crystallization of a substrate to be purified (see under "Crystallization");
- precipitation of undesirable material in a solvent solution (e.g. maceration, infusion) in order to clarify that solution ;
- precipitation of undesirable waxes (e.g. dewaxing of citrus essential oils).

## CRYSTALLIZATION

This is a process for purifying solid substances from their impurities.

For the production of natural flavouring ingredients, all solvents that are acceptable according to the EFTA Guidance Document can be used for chromatography – the solvents do not have to be natural themselves.

Solvents for this use are preferably those solvents permitted by the Directive 2009/32/EC on extraction solvents and its subsequent modifications.

Indeed, EFTA recognizes that other eluents may be used as well. In the absence of regulations, the residue limits as listed in the IOFI IL 1352 and its attachments are considered as appropriate.

## CUTTING

There are many definitions of cutting :

- making "cuts" as from a **fractional distillation** process,
- standardization of, for example, an oleoresin with an approved diluent or edible solvent (e.g.: standardization of ICU or Scoville Units with vegetable oil, addition of a solubilizer to an oleoresin etc.),
- dicing, chopping or comminuting fresh vegetables.

**Note:** *in certain cases of food preparation processes, chopping or cutting fresh foods may induce unavoidable chemical modifications. For instance, chopping fresh garlic will provoke rupture of certain cell membranes and contact between a precursor (alliin) with an in-built enzyme (alliinase) transforming the precursor into volatile substances which are not naturally occurring inside the fresh garlic pod.*

## DIGESTION

Digestion is understood as a form of enzymatic conversion. See Chapter IV.

## DISTILLATION/RECTIFICATION

Distillation is a very wide-spread process.

For the production of natural flavouring ingredients, all solvents that are acceptable according to the EFTA Guidance Document can be used for chromatography – the solvents do not have to be natural themselves.

### AZEOTROPIC DISTILLATION

Azeotropic distillation is a generally accepted process. The continuous removal of water is an example.

Solvents for this use are preferably those solvents permitted by Directive 2009/32/EC on extraction solvents and its subsequent modifications.

There is no limitation as regards time or temperature.<sup>15</sup>

### FRACTIONAL DISTILLATION

Fractional distillation can be performed under vacuum or under pressure (e.g.: the purification of ethanol).

It is acceptable provided :

- the packing of the column does not intentionally induce irreversible chemical transformations of the substrates being fractionated,
- the process is not purposely used to induce chemical transformations that generate artefacts, isomers and other substances not identified in nature.

### MOLECULAR DISTILLATION

Is acceptable provided any non food-grade solvents used as fluidisers or diluents remain **exclusively** in the residue.

### RECTIFICATION

“Rectification” is a distillation process by which some portion of a natural substrate is removed by distillation for the purpose of standardization and to ensure compatibility of the substrate with established quality standards or specifications (e.g. the removal of the non-volatile portion from an essential oil).

However, “rectification” may encompass other commonly accepted processes such as the “topping” or “tailing” of essential oils and the continuous feed process utilised in the rectification of ethanol.

### STEAM DISTILLATION

Steam distillation occurs when, for instance, vegetables are boiled or pressure cooked: the generation of water vapour or steam inevitably leading to a loss of the most volatile flavouring substances from the food. Alternatively, steam can react with some of the flavouring substances contained in the substrate and transform them by hydrolysis. This can occur in the presence of a native enzyme such as during the distillation of bitter almond oil. Here the formation of the benzaldehyde rich essential oil is as a consequence of the hydrolysis of a precursor and the co-generation of hydrocyanic acid (which has to be carefully removed so as not to present a hazard to the operator). Similarly, the steam distillation of rose inevitably leads to the formation of racemic linalool (not present in the headspace of rose) generated by the allylic transposition (hydration and dehydration) of geraniol which is naturally occurring.

### DRYING/VACUUM DRYING

Acceptable processes include **spray-drying**, **freeze drying**, **vacuum drying** and **microwave drying** of herbs, spices etc. All these processes are susceptible to the loss of some flavouring components.

### ELECTROPHORESIS

EFTA could not suggest any industrial application of this process and questions its relevance to the Flavour Industry.<sup>16</sup>

### EMULSIFICATION

This is considered a necessary technology in fermentative bioprocessing (e.g. the use of lipases in bioconversion) and for spray-drying. The emulsifiers are required to be approved for use in flavourings and/or food in the case of lipase bio-processing and for flavourings only when spray-drying.

<sup>15</sup> EFTA considers that the nature of the solvents allowed is self limiting to acceptable temperatures.

<sup>16</sup> One of the members of EFTA has seen a patent on the isolation of propionic acid by electrodialysis.

## ENCAPSULATION

Only carriers and encapsulating agents which are approved for use in flavourings are allowable.

## ENZYMATIC PROCESSES

The use of enzymes in the manufacturing processes for flavouring preparations and/or natural flavouring substances is authorized within the applicable legal requirements for the use of such enzymes in the EU.

See Chapter IV.

## EVAPORATION

Transformation of a liquid into a vapour.

## EXTRACTION

Solvents for the production of flavouring preparations are restricted to those permitted by Directive 2009/32/EC on extraction solvents and its subsequent modifications.

The additional use of ultrasound or microwaves in the extraction process is allowed.

The use of carriers as co-solvents (e.g.: propylene glycol, triacetin etc.) which are legally approved for the dilution of flavourings is also allowable. In this case, these carriers remain in the flavouring preparation. However, it is strictly forbidden to purposely introduce these carriers in order to form artefacts (e.g.: dioxolanes, acetals, or hemiacetals, etc.). Conversely, artefacts (especially acetals) are known to form unintentionally during solvent extraction of spices and herbs containing aliphatic aldehydes and during the distillation of spirits.

Extraction is widely used as a purification process to remove metabolites from fermentation broths for instance, or to extract flavouring substances which are highly water soluble. Solvents used for the production or purification of natural flavouring substances are preferably those permitted by Directive 2009/32/EC on extraction solvents and its subsequent modifications or are themselves foodstuffs (e.g. edible oils).

## FERMENTATION

In traditional food preparation processes fermentation is undoubtedly used to transform precursors into other types of molecules and of course flavouring substances (e.g.: beer, cheese or wine making).

The use of micro-organisms in the manufacturing processes for flavouring preparations and/or natural flavouring substances is authorized within the applicable legal requirements for the use of such organisms in the EU.

See Chapter IV.

## FILTRATION

Membrane Filtration (Ultrafiltration, Pervaporation and Reverse Osmosis) and other modes of filtration (cloth, filter press, etc.) are allowed in the same way they are used to produce ingredients for the Food Industry.

## GRINDING

No particular comments. See CUTTING.

## HEATING, COOKING (including EXTRUSION COOKING), BAKING, FRYING

EFFA recommends temperatures similar to those obtained in the other traditional food preparation processes:

- heating,<sup>17</sup>
- cooking (including extrusion cooking),
- baking,
- frying (up to 240°C at atmospheric pressure) and
- pressure cooking (up to 120°C).

Heating in the presence of air such as in frying or stir-frying leads to the production of many degradation and oxidation products from non-saturated lipids, for instance as a result of the decomposition of hydroperoxides.

<sup>17</sup> 240°C limitation as for frying does not apply to heating – temperatures exceeding 240°C should remain exceptional, e.g.: the cleavage of fats with superheated steam is performed industrially at temperatures around 250°C.

Extrusion Cooking is an increasing ubiquitous traditional process to extrusion as a lower energy, more rapid way of cooking food.

Extrusion cooking can only be used within the conditions (pressure, temperature) which are appropriate for the processing of conventional foods (such as biscuits, cereals, expanded snacks, textured vegetable proteins etc.).

## INFUSION

This is the solubilisation of extractibles from a plant or animal substrate into an appropriate edible solvent, typically water or ethanol, or mixtures thereof.

Removal of the spent material is achieved with pressing, filtration, centrifugation, etc.

The unintentional formation of acetals cannot be avoided, especially if the substrate and the solvent offer the appropriate pH conditions for this to occur.

A synonym is maceration, mostly used to qualify an infusion in ethyl alcohol or fats and oils.

## ION EXCHANGE

Ion exchange should be limited to the sole purpose of pH adjustment during the isolation of acids and bases.

## LYOPHILIZATION

See under DRYING (freeze drying).

## MACERATION

See under INFUSION.

## MICROBIOLOGICAL PROCESSES

The use of micro-organisms in the manufacturing processes for flavouring preparations and/or natural flavouring substances is authorized within the applicable legal requirements for the use of such organisms in the EU.

See Chapter IV.

## MIXING

No particular comments.

## OSMOSIS

EFFA only considers reverse osmosis as relevant to the Flavour Industry. See under FILTRATION.

## PEELING

This is the physical removal of the skin, shell or outer envelope of a natural substrate to be further processed (e.g. peeling of fruit, shelling of pistachios, filberts etc.).

## PERCOLATION

This is a particular form of extraction by which a solvent is forced through stacks of comminuted natural substrate contained within an appropriate extraction vessel, either by pressure (e.g. percolation of coffee in pressurized extractors<sup>18</sup>) or by circulation (e.g. percolation of chopped vanilla beans by a hydrated alcohol).

## PRECIPITATION

Precipitation is used mainly to eliminate undesirable impurities during a purification step (e.g. the precipitation of undesirable minerals from the crystallization of glycosidic compounds).

## PRESSING

Pressing of oil seeds to obtain 'first pressing oil' for instance.

<sup>18</sup> Coffee percolation machines using super heated steam usually work at pressures around 16 bar.

## ROASTING/GRILLING

There are different kinds of roasting processes, or processes which are analogous to roasting, which must be considered under this heading.

### CONVENTIONAL ROASTING (such as the roasting of coffee, tea, cocoa, seeds, nuts, vegetables etc.)

Involves self-limiting temperatures and air flow that ensure that the resultant flavour is palatable and acceptable for human consumption.

### GRILLING

This is a traditional cooking process in which dry heat is applied to the surface of a foodstuff.

This normally takes place at atmospheric pressure in the presence of air.

The temperature at which grilling occurs is recommended not to exceed 400°C.

The use of a pure oxygen atmosphere is forbidden. "Grilling" must occur as it does in the kitchen, either in an open or closed environment.

### THERMAL DECOMPOSITION

Thermal decomposition of a suitable source material (See Chapter I) can also be used to generate natural flavouring substances. As above it is recommended not to exceed a temperature of 400°C. An example is the formation of *n*-heptanal from castor oil.<sup>19</sup>

### SALTING OUT

Separation of a water soluble material may be effected by the saturation of a salt (e.g. NaCl) in the water phase in order to reduce the solubility of the material and effect its "oiling out". The product is separated by decanting, centrifugation, etc. (e.g. salting out of terpeneless and sesquiterpeneless citrus oils from "weak" ethanolic solutions).

### STEEPING

No particular comments.

### SQUEEZING

An example is the production of apple juice or lemon juice by squeezing of the respective fruit.

Synonym: PRESSING.

### SUBLIMATION

This is a natural process used to purify natural flavouring substances or separate them from undesirable tar or polymeric materials (e.g. separation of maltol from fir balsam).

### ULTRASONIC TREATMENT

See EXTRACTION

<sup>19</sup> According to Kirk-Othmer's Encyclopedia of Chemical Technology, the pyrolysis of castor oil at 340-400°C splits the ricinoleate molecule at the hydroxyl group to form heptanal and undecylenic acid.

- Jean Garnero reports in Rivista EPPOS (October 1973) that the pyrolytic distillation of castor oil starts becoming effective at 280°C. Then temperature raises and stabilizes between 300 and 340°C.





## Introductory and Descriptive Note

- This Annex consolidates within its first column the various lists of processes used in the production of Natural Flavouring Substances and (Natural) Flavouring Preparations.
- The second column lists those traditional food preparation processes as per Annex II of the Regulation (Chapter II of this Guidance Document).
- The third column lists those physical processes which are commonly used for the purification of Natural Flavouring Substances in the EU by the Flavour Industry.
- The fourth column lists those processes which are most commonly used in microbiological and enzymatic processes (Chapter IV of this Guidance Document).
- The fifth column lists other processes currently in use in the EU by the Flavour Industry which are not appropriately or exhaustively described in the Chapters II to IV of this Guidance Document.
- The sixth column lists those processes which are currently considered to be useable for the production of Natural Flavouring Ingredients in the latest version of the IOFI Code of Practice.

<b>PROCESS (APPROPRIATE PHYSICAL AND/OR TRADITIONAL FOOD PREPARATION)</b>	<i>Food preparation process (generation) [Annex II]</i>	<i>Purification process</i>	<i>Microbiological and enzymatic processes</i>	<i>Other process</i>	<i>Process as per IOFI CoP</i>
Absorption		•			•
Adsorption		•			•
Agglomeration		•			
Centrifugation		•			
Chopping	•				
Chromatography		•			
Coating	•				
Cooling	•	•			
Crystallization		•			•
Cutting	•				•
Digestion			•		
Distillation/rectification	•	•			•
Drying/Vacuum Drying	•	•	•		•
Electrophoresis		•			
Emulsification	•		•		•
Encapsulation				•	•
Enzymatic Processes	•		•		
Evaporation	•				
Extraction	•	•			•
Extrusion				•	•
Fermentation	•		•		
Filtration	•	•			•

Continued on next page

PROCESS (APPROPRIATE PHYSICAL AND/OR TRADITIONAL FOOD PREPARATION)	Food preparation process (generation) [Annex II]	Purification process	Microbiological and enzymatic processes	Other process	Process as per IOFI CoP
Grinding	●				●
Heating, Cooking (including Extrusion Cooking), Baking, Frying	●				●
Infusion	●				
Ion exchange		●			
Lyophilization		●		●	
Maceration	●				
Microbiological processes	●		●		
Mixing	●	●			●
Osmosis		●			●
Peeling	●				
Percolation	●				
Precipitation		●			●
Pressing	●	●			
Refrigeration/Freezing	●	●			
Roasting/Grilling	●				●
Salting out		●			
Squeezing	●				●
Steeping	●				
Sublimation		●			●
Ultrasonic treatment				●	●

Examples for production pathways of natural flavouring ingredients are given in Attachment IIc:



**Attachment IIc:**

- Example 1: Natural Flavouring: Fish Extract
- Example 2: Natural Flavouring: Cooked Onion Extract
- Example 3: Natural Flavouring: Methyl Butyric Acid
- Example 4: Natural Flavouring: Gamma Decalactone

## CHAPTER VI

# Analytic Methods to Assess Authenticity

## 1. Preamble

The analytical methods outlined in this document find useful application in the review of the authenticity of natural flavouring substances. For each type of analysis EFFA has identified which kind of transformation pathway may be characterized by the method. If, and where, applicable interpretative limitations to the method have been included.

The methods of analysis as laid down in this document are for the sole purpose of identifying compliance with the natural processes as laid down in Chapter I. These analytical techniques are not necessarily suitable for the identification of the natural source from which they are produced (source authentication).

The analytical techniques which are recommended herein to establish the naturalness of a given process should have been published in a peer-reviewed publication and preferably validated by a round-robin analysis. If a standard round-robin test has not been performed, a method where the repeatability and/or the reproducibility (or other statistical tests) has been evaluated should be preferred.

A method without the previous characteristics can be suggested, but cannot be considered as recommended until validated by a group of Flavour Industry analytical experts (such as the IOFI WGMA) or another official authority.

EFFA recognizes that analytical methods are not static and the opinions contained herein should be periodically reviewed pending the publication of new methods or refinements.

All necessary precautions should be exercised when isolating the substance to be analysed from a complex mixture or substrate since the process itself may affect how representative it is of the original substance (e.g. enantiomeric excess affected by pH; discrimination of certain isotopes by distillation/extraction processes).

When a single method fails to bring a clear answer about the authenticity of a natural process, the association of several methods may help to draw a more conclusive and robust diagnosis.

EFFA recognizes there are differences between the analytical methods which assess source authenticity and those which can be used to assess the naturalness of a process. The available analytical techniques are generally more likely to be useful in the discrimination of the source of the flavouring substances. However, to repeat, the methods of analysis laid down in this document focus solely on those used to identify compliance with the natural processes as elucidated in Chapter I.

EFFA emphasizes that the use of analytical methods in assessing the authenticity of natural flavouring substances has limitations. The user of the substances remains responsible for obtaining compliant documentation from the supplier / manufacturer and, if necessary, to initiate or conduct a full process audit and traceability at the manufacturer's premises.

## 2. Use of Discriminating Analytical Methodology

### 2.1. Fingerprint Analysis

**Field of application:** Examination of impurities characteristic of a natural process or a non-natural process. The presence of trace impurities could indicate the synthetic nature of the process.

**Examples:** the use of a strong mineral oxidant for the conversion of 2-methylbutanol to 2-methylbutyric acid may lead to the presence of 2-hydroxy-2-methylbutyric acid.

A careful inspection by GLC is also recommended in the case of substances that can be produced by aldolization (e.g. cinnamaldehyde).

## 2.2. Chiral Analysis

**Field of application:** Chiral analysis has shown to be a reasonably effective analytical method when reviewing the source authenticity of juices and nectars.

Chiral analysis is even more effective when coupled with an isotopic method such as chiral GC/IRMS (isotopic ratio mass spectrometry).

Publications<sup>20</sup> on citrus oils report that the enantiomeric excess of terpenic alcohols ( $\alpha$ -terpineol, terpinen-4-ol and linalool) can vary as a function of fruit maturity (bergamot, lemon) or production process (distillation in the presence of low pH juice induces high levels of racemization). Terpenes may also show considerable variations in a single species owing to the geographic area of cultivation or the hybrids cultivated.

The high enantiomeric purity of a given flavouring substance is no guarantee that the material has been produced by a natural process. Examples of processes producing almost pure enantiomeric substances and which are not eligible to qualify as natural are:

- the resolution by enzymes or micro-organisms of a racemate obtained by a non-natural process, possibly after derivatization of the enantiomers of the racemic mixture;
- the resolution of lactones or other racemates obtained by a non-natural process by physical means such as HPLC or GLC with a chiral column or any other chromatographic process;
- the production of optically active flavouring substances by chemical modification of an optically active natural substrate (e.g. the conversion of *d*-limonene from orange oil into *l*-carvone or the hydrogenation of menthone to menthol by a chemical reaction).

## 2.3. Site Specific Deuterium NMR

**Field of application:** This method can be useful in the discrimination of natural processes involving hydrogen at "characteristic" positions on the molecule.

The analysis may be applied to e.g. the oxidative degradation of eugenol to vanillin. Oxidation through a non-natural process would normally give a deuterium enriched carbonyl hydrogen whereas biosynthesis would give a lower deuterium value on the carbonyl site.

The method also finds useful application in the discrimination between a natural process involving enzymes (*vide supra*) and chemical hydrogenation e.g.:

- on the carbon bearing the alcohol functionality in the case of the reduction of a carboxylic group (acid, ketone or aldehyde);
- on the two adjacent carbons receiving the hydrogen atoms in the case of the reduction of a carbon-carbon double bond (e.g.: the enzymatic reduction of massoia lactone to D-decalactone).

<sup>20</sup> *Enantiomeric distribution of volatile components of citrus oils by MDGC* – G. Dugo, L. Mandella, A. Cotroneo, I. Bonaccorsi and G. Lamonica – *Perfumer & Flavorist*, Vol. 26, January/February 2001.

+ "Multidimensional capillary GC-GC for the analysis of real complex samples. Part II. Enantiomeric distribution of monoterpene hydrocarbons and monoterpene alcohols of cold-pressed and distilled lime oils" – L. Mondello & al – *J. Microcolumn Separations*, 10(2), 203-212 (1998).

## 2.4. IRMS (Isotope Ratio Mass Spectrometry) / SIRA (Stable Isotope Ratio Analysis) Coupled with High Resolution Gas Chromatography (HRGC), Chiral Multidimensional Gas Chromatography (MDGC) or Elemental Analyzer with Combustion or Pyrolysis Mode (EA-C or EA-P)

### 2.4.1. Carbon SIRA

**Field of application:** EFTA is unaware that this method can be used in the authentication of a natural process. Typically it is used in the assessment of the natural origin of the raw materials used. For instance it offers the possibility of differentiation between  $C_3$ -,  $C_4$ - and CAM plant material versus synthetic source material.

### 2.4.2. Deuterium SIRA

**Field of application:** All processes likely to exchange hydrogen atoms in the target molecule.

The literature<sup>21</sup> shows that the global deuterium abundance of (*E*)-2-hexenal seems to be higher than that of (*E*)-2-hexenol when both are produced by the same plant through a cascade of enzymatic reactions. This suggests that a kinetic isotope effect occurs in the course of the enzymatic reduction.

Confirmation of the naturalness of a process can be based on the comparison of the deuterium abundance in the aldehyde and the corresponding alcohol provided:

- the water used for the steam distillation and isolation of these flavouring substances has no effect on the labile hydrogen of the hydroxyl group;
- the pH of the steam distillation is maintained neutral;
- chemical reduction of the aldehyde with hydrogen from common hydrides or catalytic hydrogenation shows significant discrimination from variations seen naturally.

### 2.4.3. Oxygen SIRA

**Field of application:** Verification that the extraneously introduced oxygen comes either from a natural source (air in the case of fermentation) or from a disallowed oxidation agent (mineral origin).

This method of analysis is applicable to oxidation of natural alcohols to carboxylic acids (e.g. natural butyric acid from natural *n*-butanol) since the extra oxygen either originates from air (fermentation) or from the oxidation chemical (when a strong mineral oxidant is used).

In the case of the enzymatic oxidation of a natural secondary alcohol to a natural ketone, the oxygen 18 to oxygen 16 ratio should remain unchanged. The same may apply to the conversion of a primary alcohol to an aldehyde but EFTA considers that the possibility of oxygen scrambling from the fermentation medium cannot be excluded and warrants further investigation.

## 2.5. $^{14}C$ Radiocarbon Determination

**Field of application:** EFTA is unaware that this method can be used in the authentication of a natural process. Typically it is used in the assessment of the natural origin of the raw materials used.

If a flavouring substance analysed by radiocarbon analysis is found devoid of any radioactivity, this is a **robust proof** that the material has been generated from fossil fuel substrates.

Flavouring substances can be considered to have a "modern" activity when they show typical radiocarbon determinations between 100-115% of the standard modern activity of 14.5 dpmg ( $\pm 5\%$ ). Radiocarbon activity below 13.8 dpmg may be indicative of mixtures of natural flavouring substances with their non-natural counterparts.

<sup>21</sup> Flavor authenticity studies by 2H/1H ratio determination using on-line gas chromatography pyrolysis isotope ratio mass spectrometry – K. Hörr, C. Ruff, B. Weckerle, T. König and P. Schreier – J. Agric. Food Chem., 2001, 49, 21-25.

## 3. Lack of Discriminating Analytical Methodology

A number of chemical reactions involved in the natural production of flavouring ingredients lack a discriminating analytical methodology. Typical representatives of this group are:

### 3.1. Ester Hydrolysis

The oxygen in the alcohol moiety originates from a natural plant material (e.g. (*Z*)-3-hexenol from the naturally abundant esters in *Mentha arvensis* oil) and therefore it is **for the time being** quasi-impossible to discriminate between a natural hydrolysis process and a non-natural one.

As far as the acid moiety is concerned, EFFA feels that the scrambling of both the oxygen and the hydrogen is highly dependent on the isotopic composition and amount of water used in the reaction and it is not currently possible to make a clear differentiation between a natural process (enzymatic hydrolysis or physical process) and a chemical hydrolysis.

### 3.2. Esterification

EFFA felt that it is even more difficult to discriminate between natural esters obtained by enzymatic esterification and autocatalytic processes and the same esters **from exactly the same raw materials** obtained using non-approved acid catalysis.

Original version, 03/09/10

Revised version (V2.0), 11/03/13