Partenariat Analyse Authenticité Arômes

P3A

Focus
The role of Carbon-14 analysis in checking the regulatory compliance of natural flavourings

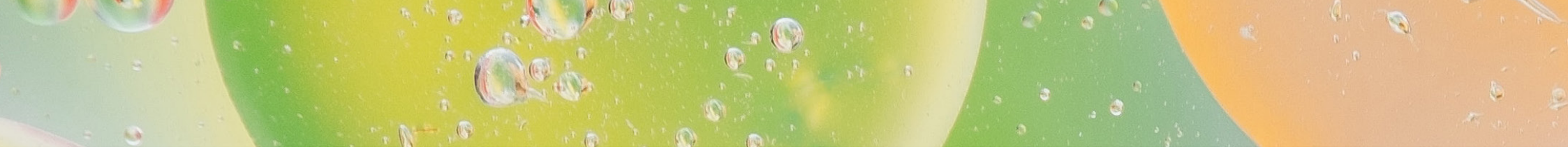
December 2023

Aromalyse
EXPERT CHEMICAL ANALYSIS & MUCH MORE
www.aromalyse.com

BETA
www.betalabservices.com

eurofins
www.eurofins.fr

SNIAA
Syndicat National des Ingrédients Aromatiques Alimentaires
www.sniaa.org



This document has been prepared by the Scientific Committee of the Partenariat Analyse Authenticité Arômes (Flavouring Authenticity Analysis Partnership or P3A). P3A was set up by SNIAA (French Flavour Association) in conjunction with several analytical laboratories with the aim of boosting confidence both in the results produced by analytical laboratories and in the products offered by flavouring manufacturers.

Members of the P3A are indeed frequently called upon to conduct analyses to confirm the compliance of natural flavourings.

The P3A Scientific Committee has decided to draft a series of documents to provide practical and explanatory information regarding the role of each of these analytical methods available for checking the regulatory compliance of natural flavourings.

1.What is Carbon-14?

Carbon-14 (^{14}C) is best known for its use by archaeologists to date archaeological objects, such as mummies or organic residues. However, its natural radioactivity can also be used to determine whether carbon-containing products are derived from biomass or petrochemicals.

^{14}C is produced in the atmosphere by the interaction of cosmic rays with nitrogen atoms (Figure 1). The ^{14}C then combines with oxygen atoms to form $^{14}CO_2$, which is inhaled by plants and becomes part of their composition. All animals will also assimilate ^{14}C in their bodies. So all molecules with a carbon skeleton derived from living organisms contain this natural radioactivity.

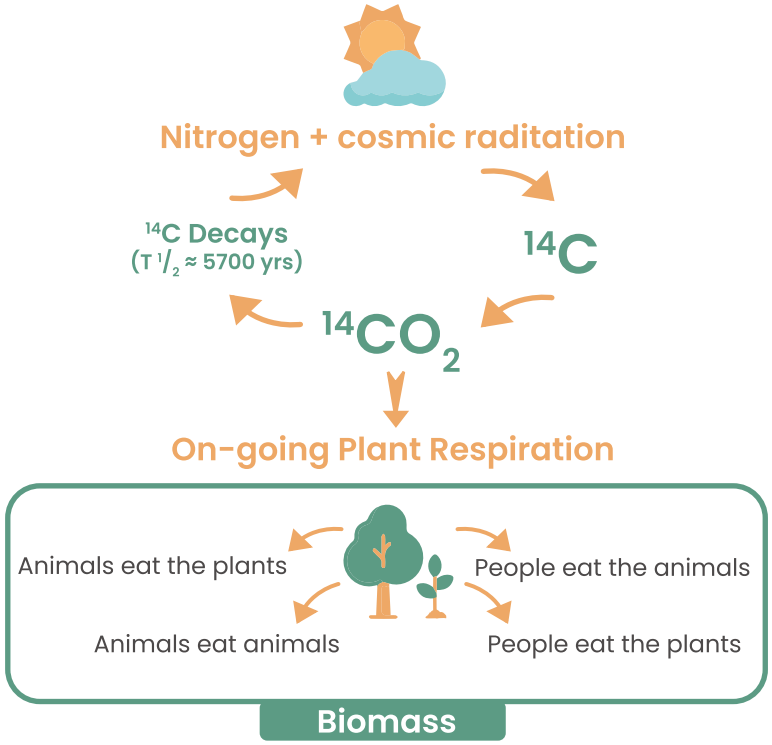


Figure 1. Origin of atmospheric ^{14}C and its incorporation into the biomass (Source: Beta Analytic)

Biomass (including plants, animals, fungi, algae, etc.) therefore contains levels of ^{14}C that reliably reflect those present in the atmosphere during its lifetime.

When an organism dies, the ^{14}C, being radioactive, begins to decay (Figure 2). The half-life[1] of ^{14}C is 5,730 years.

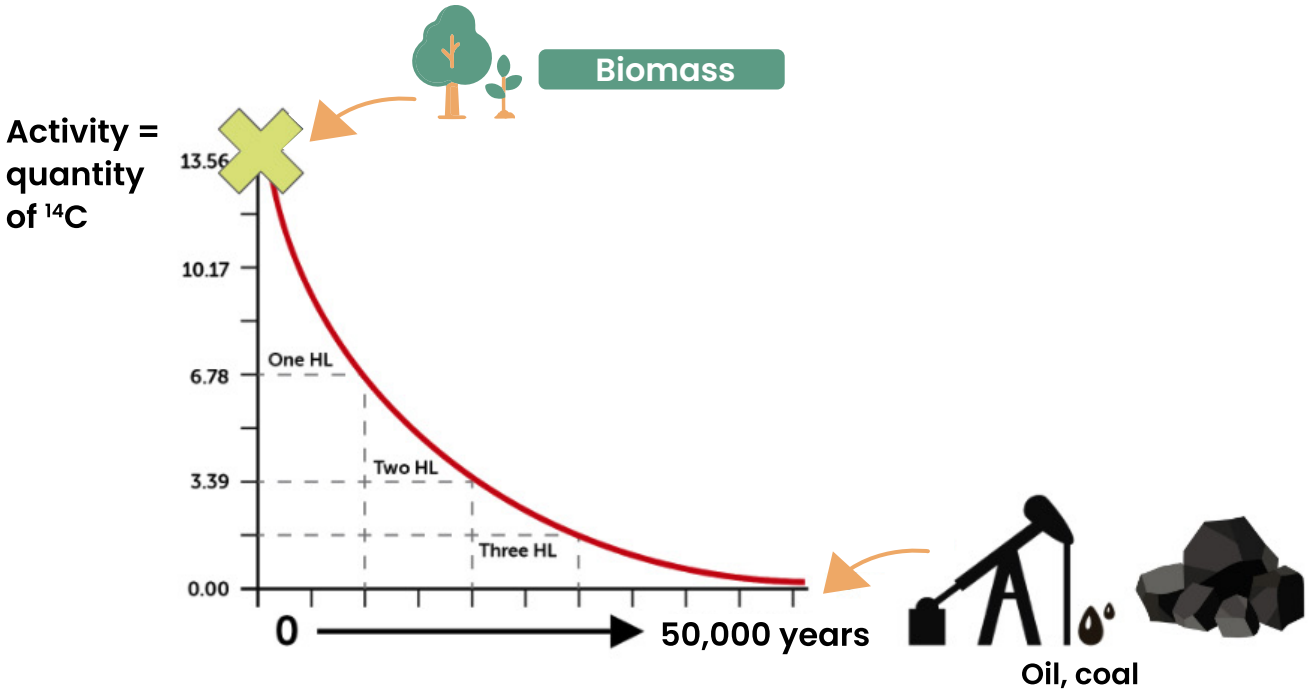


Figure 2 : Radioactive decay of ^{14}C over time following the death of an organism; 50,000 years after the death of an organism, ^{14}C levels are extremely low (Source: Beta Analytic)

[1] The half-life of a radioactive element is the time taken for half its atoms to decay naturally. As shown in Figure 2, radioactive decay is an exponential process. This process is not dependent on environmental factors (temperature, pressure, etc.), but is a property specific to the radioactive element - radionuclide - concerned.

This means that 50,000 years after the death of an organism, the quantities of ^{14}C it contains will be so small as to be undetectable. This is why fossil fuel sources (like oil and coal), which are derived from organisms that died tens or hundreds of millions of years ago, emit no ^{14}C signal.

In this context, the percentage of modern or biobased carbon is defined as the quantity of carbon derived from biomass present in a product relative to its total carbon content. This can be represented schematically as shown below (Figure 3):

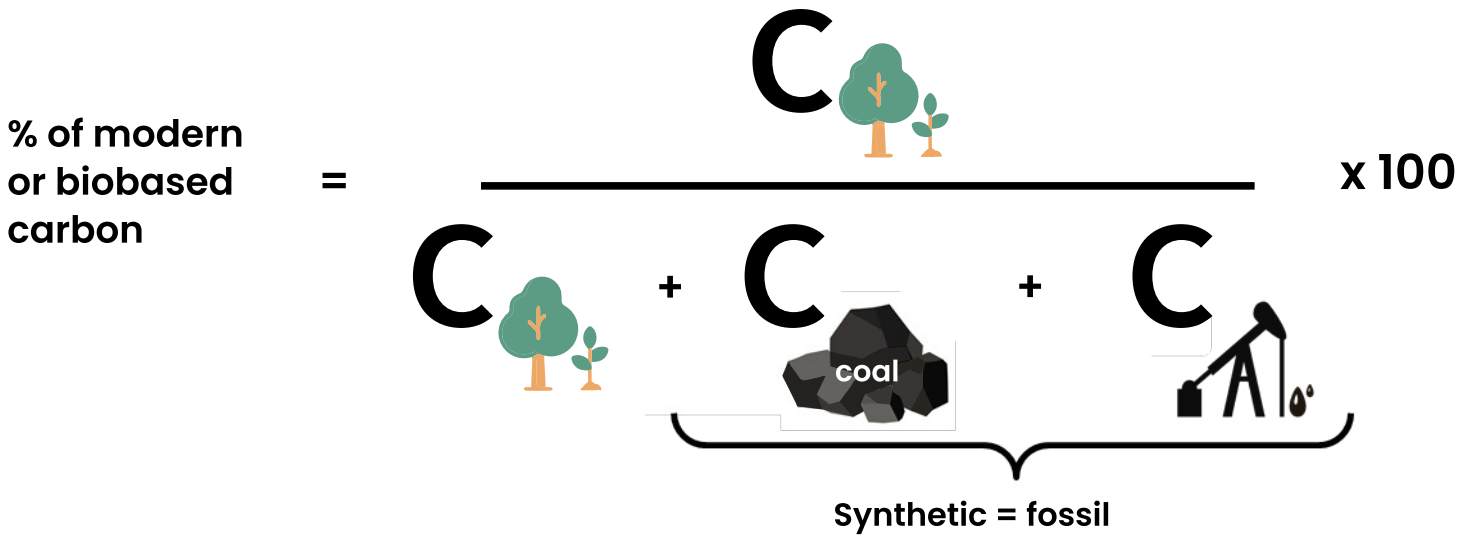
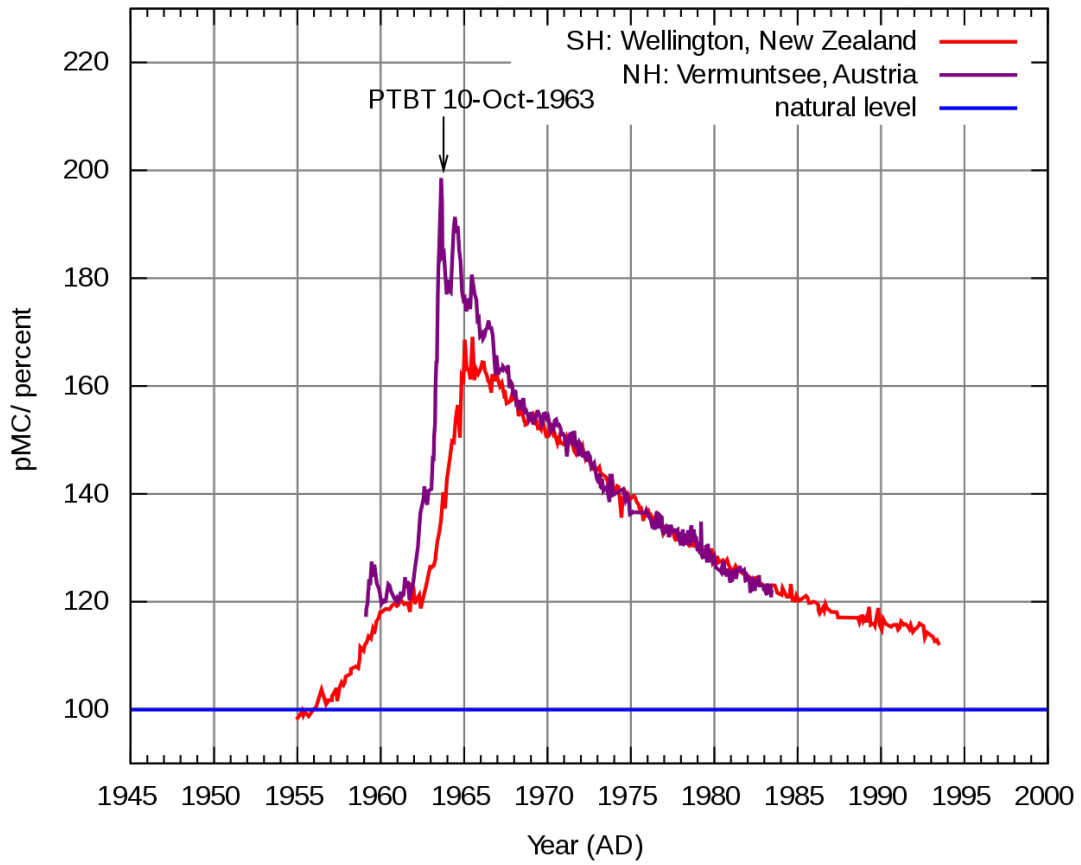


Figure 3: Calculation of the percentage of modern carbon in ^{14}C analysis (Source: Beta Analytic)

To find out more

Some anthropogenic effects have modified atmospheric ^{14}C levels:

- Since the advent of the industrial era, a great amount of fossil carbon has been burnt and therefore released into the atmosphere, resulting in a fall in the overall level of atmospheric ^{14}C.
- Nuclear testing has also disrupted atmospheric ^{14}C levels over the last 7 decades as a result of releasing large quantities of ^{14}C into the atmosphere (Figure 4).



PTBT = Partial Test Ban Treaty
pMC = Pourcentage de Carbone Moderne (cf. page 10)
SH = South Hemisphre
NH = North Hemisphere

Figure 4: The influence of nuclear testing on atmospheric $^{14}CO_2$ levels in the second half of the 20th century. The graph shows the ratio of ^{14}C / ^{12}C relative to the natural level in atmospheric CO_2 over time.

Source : https://en.wikipedia.org/wiki/Bomb_pulse#/media/File:Radiocarbon_bomb_spike.svg

2.Carbon-14 analysis methods - description

2.1. The relevant ASTM and ISO Standards

A number of standards give a frame to the use of ^{14}C analysis to determine the percentage of carbon from natural sources (biomass) relative to carbon from fossil sources (chemical synthesis) found in food flavourings, food additives, fragrances, cosmetics, food supplements and other chemical compounds in food, medicines and beverages.

The results indicate the percentage of biomass-derived carbon atoms, but not the percentage by mass of the product that is biobased (Figure 5). These standards do not differentiate between different types of natural source (plants, animals or microbiological material) or botanical origin, but determine only whether the material is derived from biomass or petrochemical sources, or both. These methods therefore do not set out to determine environmental impact, geographical origin or product performance and functionality.

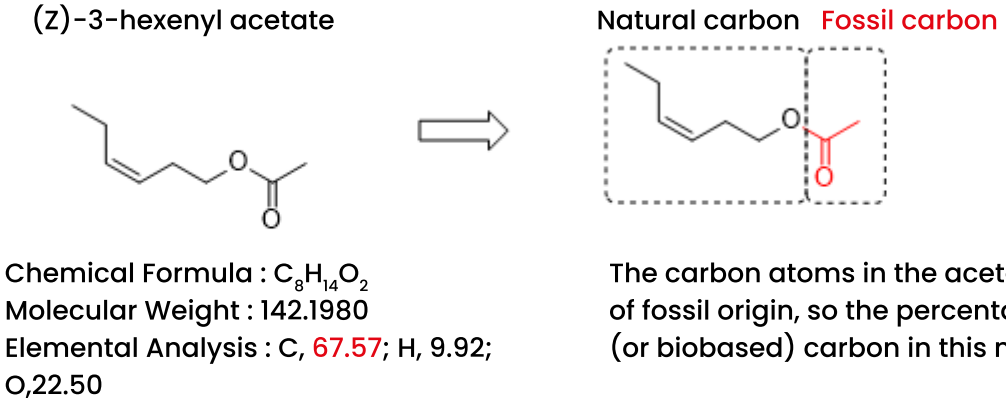
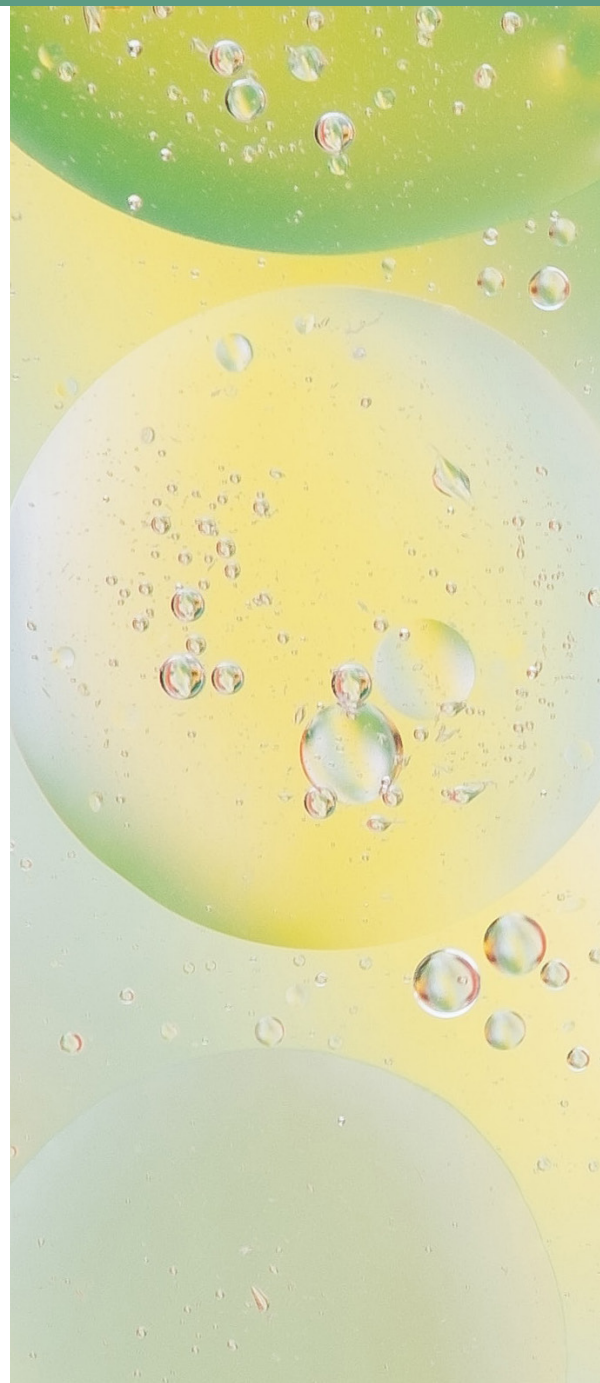


Figure 5: Calculating the percentage of modern carbon in a molecule using ^{14}C analysis.



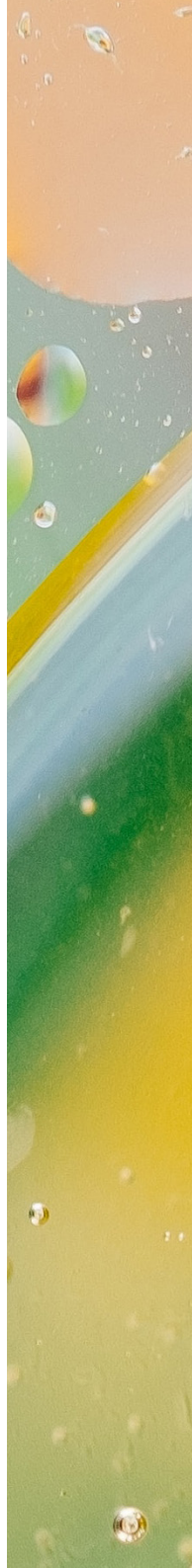
• The oldest standard is ASTM D6866, which was developed in the USA. ASTM D6866 is one of the standards used to determine the biobased content of solids, liquids or gases using ^{14}C. These test methods apply to any product containing carbon-based compounds that can be burnt in the presence of oxygen to produce gaseous CO_2 and apply equally to samples in gaseous form.
The results are reported as a fraction of the Total Organic Carbon (TOC) content of a material, which requires any inorganic carbon (carbonates) present to be removed by acid bath prior to the analysis.

• ISO 16620-2 is an international standard developed by the International Organization for Standardization, and describes a method for determining the biobased content of solid, liquid and gaseous samples using ^{14}C analysis. An ISO 16620-2 analysis report for a flavouring or fragrance sample indicates the percentage of biobased carbon as a fraction of Total Carbon (TC) or Total Organic Carbon (TOC) in a material submitted for analysis. The TC result refers to all carbon present in the material, organic and inorganic.

• The European EN 16640 and EN 16785-1 standards are a priori not used today for flavouring analysis, and are used primarily to analyse bioplastics.

These standards are broadly equivalent, but differ in their approach to sample preparation. They set an absolute error of +/- 2-3%, which reflects the history of their introduction. The main criterion for choosing between these standards is whether or not carbonates are to be included. In the context of flavourings, few differences are expected regarding this point, which would not be the case for wood-based products or other carbonate-containing products. The choice may also be guided by the need to comply with certain labelling schemes that favour a particular standard.

Referred to as 'Method C' in the ASTM standard, Accelerator Mass Spectrometry (AMS) is the most advanced technology for obtaining high-accuracy results. Liquid scintillation offers a second method for analysing ^{14}C content; it is less accurate, but much easier to implement (for direct counting) and less expensive to use on a routine basis.



2.2. Accelerator Mass Spectrometry - AMS

The sample is first examined so that it can be aliquoted (i.e. sub-sampled) as optimally as possible. It can then be tested for the presence of carbonates using hydrochloric acid, since many standards require carbonates - inorganic carbon - to be removed before analysis. Any carbonates detected are removed using phosphoric acid.
The material is placed in an environment of 100% oxygen and burnt at 800°C to convert all the C to CO_2. The CO_2 is then reduced to graphite using a metal catalyst.

The sample is placed in an Accelerator Mass Spectrometer (Figure 6), where the ^{14}C content is measured and then corrected for isotopic fractionation. The isotope ratio measured is then compared with that of a modern standard of known value (Oxalic Acid II or NIST-4990c). The result is multiplied by the current correction factor (these factors are regularly updated) to determine the percentage of biobased carbon.
Multiple quality assurance standards are measured alongside the sample and reported separately in a quality assurance report.

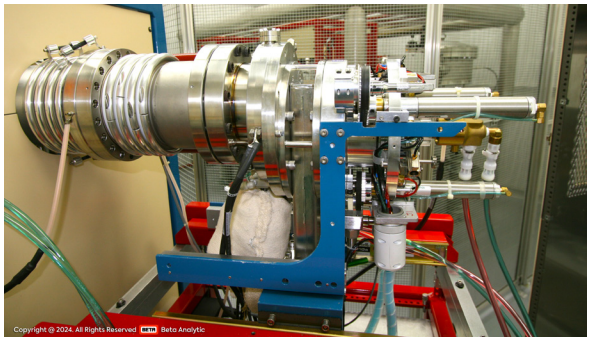


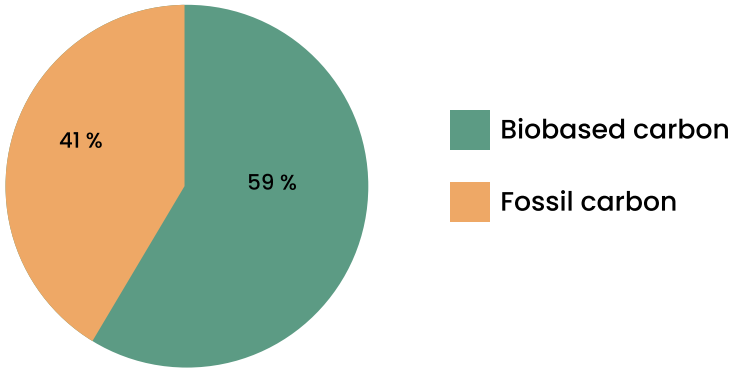
Figure 6: Accelerator mass spectrometer.

The detection limit is expressed as a percentage of modern carbon[2] (pMC) and varies from laboratory to laboratory.

The results are easy to interpret: they express the percentage of carbon (total or organic only) that is biobased (Figure 7). The percentage of modern carbon ranges from 0 to 100%.

RESULT: X_B^{TC} 59% biobased carbon (trace of synthetic origin in the material analysed)

Percentage of modern carbon (pMC)	58.59 +/- 0.16 pMC
Atmospheric adjustment factor (REF)	100.0; = pMC/1.000



RESULT: X_B^{TC} 99% biobased carbon (statistically consistent with entirely natural origin, and no trace of synthetic origin)

Percentage of modern carbon (pMC)	98.86 +/- 0.23 pMC
Atmospheric adjustment factor (REF)	100.0; = pMC/1.000

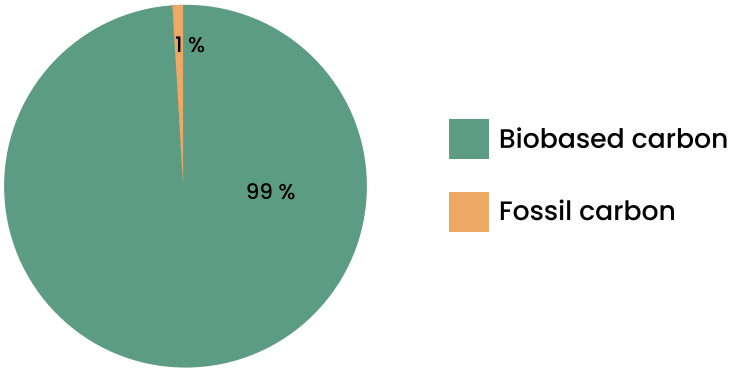


Figure 7: Examples of results obtained using the Accelerator Mass Spectrometry method in accordance with ISO 16620-2:2019

[2] pMC is the abbreviation for percentage of modern carbon. It refers to the percentage of ^{14}C measured in the sample relative to a modern reference standard (NIST 4900C).

2.3. Liquid scintillation

Liquid scintillation is based on detecting the ionising radiation released by radioactive atoms as they decay. For Carbon-14, it is used to detect a beta particle, which in this case is an electron (Figure 8).

$$^{14}_{6}C \rightarrow {}^{14}_{7}N + e^{-} + \bar{\nu}e$$

Figure 8: ^{14}C decay reaction.

The radiation released when a nucleus decays will excite the solvent, which in turn will emit photons (scintillation) that are then picked up by a photomultiplier in the analyser (Figure 9). The radioactivity present in the sample will therefore be measured indirectly and expressed relative to the current standard of 13.6 disintegrations per minute per gram of carbon (for 100% biobased carbon).

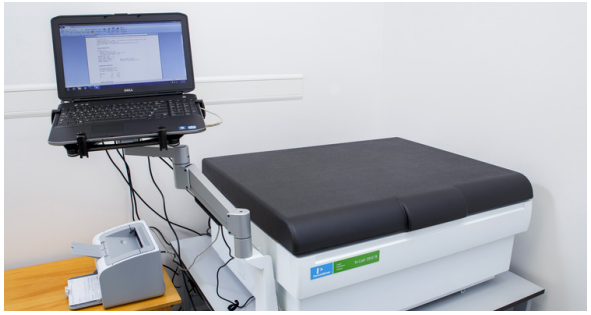


Figure 9: Typical liquid scintillation analyser.

Two methods are commonly used in laboratories:

• **The (ASTM) standardised method:**

This is the method developed initially by geologists and archaeologists, and now accepted by the international community. Its aim is to recover the carbon from the studied molecules and transform it into a molecule that can be observed using liquid scintillation (without artefacts or measurement disturbances). The principle involves burning the studied molecule, recovering the resulting CO_2 and then reducing it first to lithium carbide and then to acetylene. In the final stage, the acetylene is converted to benzene by trimerisation (the Kekulé reaction), and it is this molecule whose radioactivity will be measured using liquid scintillation (Figure 10).

• **The pragmatic approach: direct counting**

This approach involves detecting ^{14}C radioactivity within the molecules themselves without the need for combustion, as was the case in the preceding paragraph. The measurement is made using the same type of equipment as the standardised method. This method has the advantage of requiring much less preparation and handling time but may not be appropriate for certain molecules. For example, coloured and/or highly conjugated products and certain chemical functions are simply incompatible with the direct counting method. In fact, it is reported that in the presence of these particular structural/chromatic properties (conjugation/colouring) or for aldehydes (for example), there is a 'quenching' of the signal. In other words, the sample itself inhibits the beta particles released by its own ^{14}C atoms. In general terms, a direct count (in pulses per minute) must be accompanied by a signal transmittance value (transformed Spectral Index of External Standard or tSIE), which is used to gauge measurement validity. If the involved molecules result in signal quenching, it is necessary to use the standard method (combustion/conversion to benzene) or AMS.

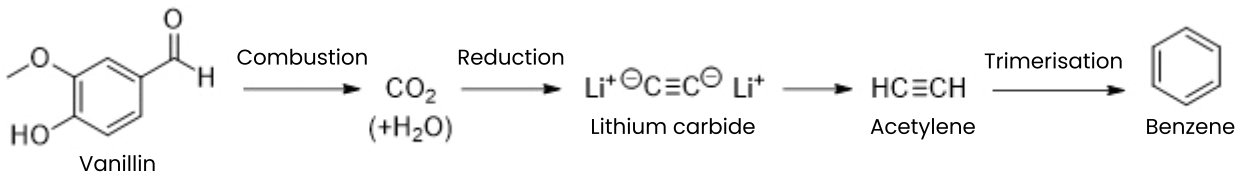


Figure 10: Sample preparation (of vanillin in this instance) for ^{14}C detection by liquid scintillation.

2.4. GC-AMS coupling

In its standardised (ASTM) version, liquid scintillation provides the same type of result as AMS by making it possible to trace back to a ^{14}C content per gram of total carbon (or total organic carbon). Despite its benefits in everyday practice, direct counting should be used only to give an indication of whether the molecule tested is biobased or of fossil origin, since this method is not able to correctly estimate the percentage of natural carbon in relation to fossil carbon. In the jargon that surrounds this technique, a sample is said to be 'hot' (radioactive and therefore natural), or 'cold' (completely dead, and therefore of fossil origin).

AMS measures ^{14}C isotopes directly, whereas liquid scintillation measures them indirectly; AMS is therefore more accurate and is often favoured by regulations. Liquid scintillation also requires larger sample sizes than AMS.

For complex samples (flavouring preparations, formulated flavourings, etc.), the methods described above can be combined with sample preparation and separation.
The samples are first extracted and/or hydrolysed to make the organic compounds soluble. Various separation techniques can then be used: gas chromatography (GC), high-performance liquid chromatography (HPLC), etc.
HPLC is used mainly for non-volatile polar compounds such as sugars, amino acids, polar lipids and polyamines. Gas chromatography (GC) is the preferred method to use with flavourings.

The separative power of gas chromatography makes it possible to isolate a target molecule and transform it into an elemental gas using a combustion or pyrolysis furnace. This gas is then injected directly into an AMS system. This combination of techniques makes it possible to determine the biobased carbon content of each peak obtained by gas chromatography (GC) separation, and therefore of each element in complex samples. It is accepted practice that clear separation of elements within a complex sample must be verified for correct interpretation of AMS results.

3.Carbon-14 analysis and regulatory compliance

At European level, Regulation (EC) 1334/2008 sets out the regulatory requirements for flavourings and certain food ingredients with flavouring properties.

Regulatory compliance of natural status flavourings is governed by multiple factors, as detailed in the **Analytical controls and regulatory compliance of natural flavourings** document prepared by P3A.

Measuring the percentage of modern carbon by ^{14}C analysis allows to determine the percentage of biobased carbon atoms in a molecule. This method therefore provides an important indication of the regulatory compliance of certain ingredients, such as flavouring substances. When assessing the naturalness of a flavouring, regulatory compliance is based solely on the flavouring part. Extraction solvents and carriers may be of fossil origin without compromising the naturalness of the flavouring itself.

Furthermore, a result indicating 100% biobased carbon does not guarantee regulatory compliance of a natural flavouring. Certain processes not authorised for the manufacture of natural flavourings (Regulation (EC) 1334/2008), such as oxidation or hydrogenation reactions, and certain chemical reactions involving natural precursors or non-biological catalysts (not authorised by Regulation (EC) 1334/2008), do not involve or modify carbon atoms.

Accordingly, to confirm or invalidate the results obtained, this analysis should be combined with:

› Other molecule identification techniques (isotope analysis, chiral analysis[3], detection of the presence of adulterants undetectable by gas chromatography, synthetic impurities, etc.)
› An assessment of the guarantees of process, formulation and other compliance offered by the flavouring producer.

Lastly, the regulatory definition of natural flavourings is not the same in all regions of the world. In the USA, for example, manufacturing processes are not taken into account. So on the basis of a ^{14}C analysis, a flavouring substance may be considered natural in the USA, but not in Europe.

[3] For more information, please refer to P3A Focus Documents 'The role of chiral analysis in checking the regulatory compliance of natural flavourings' and 'The role of isotopic analysis in checking the regulatory compliance of natural flavourings' (forthcoming)

Partenariat Analyse Authenticité Arômes

P3A

Aromalyse
EXPERT CHEMICAL ANALYSIS & MUCH MORE
www.aromalyse.com

BETA
www.betalabservices.com

eurofins
www.eurofins.fr

SNIAA
Syndicat National des Ingrédients Aromatiques Alimentaires
www.sniaa.org