Open-Bio
Opening bio-based markets via standards, labelling and procurement

Work package 3

Bio-based content

Deliverable N° 3.2:

Evaluation of applicable techniques for the determination of the bio-based content

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1 Summary

This report presents the comparison between different methods for the bio-based content determination. Radiocarbon method supplemented by elemental analysis (EN 16785-1) is the standardized method used for the determination of bio-based carbon content and total bio-based content. Application of EN 16785-1 to a certain product results in a fixed number for the bio-based content of the product, describing which fraction of the product originates from biomass. This report discusses a possibility to use stable isotope analysis for the determination of total bio-based content.

Sun lotions as a final product and all its constituents were chosen as test product to evaluate the possibility of using stable isotope techniques for the bio-based content determination. The results of the investigations indicate that neither stable isotope analysis of the carbon isotope ($\delta^{13}C$) alone, nor stable isotope analysis of carbon, hydrogen and oxygen isotopes ($\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$) combined cannot be conclusive with respect for the determination of the bio-based content in the selected product and therefore cannot be compared with the bio-based carbon content determined by the radiocarbon method.

In the case of the selected product, $\delta^{13}C$ results of all components and also of the final product fell into the range of $\delta^{13}C$ values that are known for C3 plants (from -33‰ to -20‰) that could indicate that the material can possibly be produced from C3 plants. However, since the $\delta^{13}C$ values derived from fossil feedstock materials are in the same range as for C3 plants, then based only on the $\delta^{13}C$ analysis it cannot be even qualitatively assured whether the material has a bio-based or fossil origin. It shall be noted here, that such qualitative conclusion would be possible with a higher confidence if $\delta^{13}C$ range of the measured product and its components corresponded to C4 plants, since there is a clear distinction between the $\delta^{13}C$ values in C4 plants (from -14‰ to -9‰) and fossil feedstock derived materials. But also for materials when it is known that they are produced from C4 plants, to quantify the result to make it comparable with the bio-based content obtained by radiocarbon analysis methods, remains unclear at the current stage of the research. However, probably stable isotopes can be used for certain product categories where one can observe the correlation between $\delta^{13}C$ and carbon-14, in order to make reliable bio-based content estimations.

Nevertheless, despite that stable isotope analysis cannot be used to obtain a certain number for the bio-based content of a product, multiple stable isotopes analysis is widely used to obtain some indications on the geographical origin of the materials and or feed stock. In the food industry authenticity analysis is used to allow distinction between synthetic and natural products. However, these aspects are beyond the scope of the current report.

To conclude, in order to determine the bio-based content of a product, the radiocarbon method supplemented by the elemental analysis remains to be the most studied and the most accurate. The limitation of this method is that it cannot provide information on the bio-based content for random products and for products that do not contain carbon. For such products, there is a suggestion to use 3H isotope of hydrogen that is also radioactive. Currently there is no standardized methodology developed for the use of ^{3}H analysis for the bio-based content determination. This subject still demands more research.

2 Introduction

Currently, the standardized methods for the bio-based content determination include radiocarbon analysis and radiocarbon analysis in combination with elemental analysis. These methods are described in CEN/TS 16640 (Bio-based products – Determination of the bio-based carbon content using the radiocarbon method) and EN 16785-1 (Bio-based products – Bio-based content – Part1: Determination of the bio-based content using the radiocarbon analysis and elemental analysis). In addition, material/mass balance calculations can be performed for the determination of the bio-mass fraction in a production stream. The distinction between material balance and mass balance, as stated by CEN/TC 411, is based on the fact that for the material balance there is always some biomass present in the finished product, while for the mass balance this is not. For that reason products coming from the mass balance are not considered bio-based products and will be left out of this report (as they are also left out of CEN/TC 411). For the material balance, these calculations take into account material input, material output and material losses during a production process. Material balance methods can provide information about the proportion of bio-mass that is used in the production of a bio-based product. Total bio-based content then is proportional to the amount of biomass that is found at output stage. Material/mass balance methods are described in detail in Deliverable 4.5 of KBBPPS (University of York). However, since material balance is not based on actual measurements but rather on the information about the bio-mass input into the production process, this method (that has not been submitted to CEN/TC 411 as yet) will not be discussed in the current report.

At the current stage there is no method developed for the determination of the bio-based content by using stable isotope analysis of carbon, oxygen, nitrogen and hydrogen. Stable isotope analysis is now most used for detection of the geographical origin, food authentication and in forensic analysis.

It would have certain benefits if the stable isotope analysis could also be used for the evaluation of the bio-based content of the product as the analysis itself is cheaper than the radiocarbon analysis (price comparison). However, the potential of using stable isotopes analysis for establishing a method for bio-based content determination still remains not completely investigated.

Therefore, as a starting point, this report aims to investigate whether it is possible to compare already known methods for bio-based content determination and results obtained from stable isotope analyses for a selected product (sun lotion). In case the stable isotope analyses will enable to establish the bio-based content of a product, then the method can be used in conjunction with the radiocarbon method. Additionally to the analysis of the finished product, the

same analysis (^{14}C, CHN-O and the stable isotopes) will be performed for all constituents of the product in order to check whether there is a correlation between the isotopic footprint of the final product and the isotopic footprint of its constituents.

In case the outcome of the analysis for the chosen product indicates there can be a link between the isotopic footprint and the bio-based content of the final product, further research will be needed to investigate whether it is possible to generalize the method and investigate the limitations of the method (to mention in which cases or for which groups of products the stable isotope analysis gives reliable results on the bio-based content).

The product that will be discussed in this report is a sun lotion and its constituents.
Additionally, at this moment there are also thoughts about using tritium, the radioactive isotope of hydrogen, for bio-based content measurement. The process would be comparable to ^{14}C measurements, but the contents of tritium are much lower and the half time is much faster. Because the first measurements are still in an inquiry phase, this method is not included in this report.

Next paragraphs will describe the use of the ^{14}C and the ^{14}C and the elemental analysis methods for the determination of the bio-based content. Comparison between the methods and their advantages and disadvantages will be also discussed. The results of the investigations on the selected product will be presented followed by the conclusions based on the outcome of the analysis.

3 Stable isotopes: background and principles

All elements are built up from equal amounts of positive protons and negative electrons. In most elements there are also a number of neutrons without charge. Neutrons have the same mass as protons and the mass of an element is the sum of the protons and neutrons. Either an element is stable (stable isotope) or decays in time (radioactive isotope). Most elements have more than one stable isotope, but one stable isotope is mostly much more numerous in nature.
Currently, stable isotopes of carbon, oxygen, hydrogen and nitrogen are most used for food authentication and for tracing the geographical origin. Natural isotopic abundances are the following:

Hydrogen
- ^{1}H - 99.984%
- ^{2}H (or also referred to as D – deuterium) - 0.0156%
- ^{3}H (or also referred to as T – tritium) – trace amounts (radioactive)

Carbon
- ^{12}C - 98.89%
- ^{13}C - 1.11%
- ^{14}C – trace amounts (radioactive)

Nitrogen
- ^{14}N - 99.64%
- ^{15}N - 0.36%

Oxygen
- ^{16}O - 99.763%
- ^{17}O - 0.037%
- ^{18}O - 0.1995%

Heavier isotopes undergo the same chemical reactions as lighter isotopes, but with different (slower) reaction rates. Knowing the precise isotopic ratios in plant and animal tissues allows us to know about the processes by which the materials were formed. This can tell if a plant's roots are tapping recent rain or groundwater, what an animal has consumed throughout its life and where it stands in the food chain, and the global sources for carbon dioxide in the atmosphere.

However, due to mass differences of isotopes of the same element, specific sample type can be affected by isotopic fractionation. Fractionation is caused by the differences in the chemical and physical properties of a certain atomic mass and concerns the concepts of isotope exchange and kinetic processes in reaction rates. As example, changes in temperature are

affecting the isotope exchange process that can cause fractionation in an isotopic ratio. Therefore there must be temperature stability kept in the equipment that is used for the isotopic measurements. Another example of a kinetic isotope effect would be evaporation and condensation (lighter isotopes evaporate faster, heavier isotopes condensate faster).

Stable isotopes ratios are typically represented using δ symbol:

For stable isotope of carbon (^{13}C), $\delta^{13}C$ is determined as

$$\left[\left\{(^{13}C/^{12}C)_{sample} / (^{13}C/^{12}C)_{standard}\right\} - 1\right] \cdot 1000‰$$

For stable isotope of hydrogen (^{2}H), $\delta^{2}H$ is determined as

$$\left[\left\{(^{2}H/^{1}H)_{sample} / (^{2}H/^{1}H)_{standard}\right\} - 1\right] \cdot 1000‰$$

For stable isotope of oxygen (^{18}O), $\delta^{18}O$ is determined as

$$\left[\left\{(^{18}O/^{16}O)_{sample} / (^{18}O/^{16}O)_{standard}\right\} - 1\right] \cdot 1000‰$$

For stable isotope of nitrogen (^{15}N), $\delta^{15}N$ is determined as

$$\left[\left\{(^{15}N/^{14}N)_{sample} / (^{15}N/^{14}N)_{standard}\right\} - 1\right] \cdot 1000‰$$

Stable isotope ratios are usually measured with reference to some predefined materials (called standards) with known stable isotope ratios. Thus for hydrogen and oxygen, for carbon and for nitrogen, the next standards are used:

- SMOW (Standard Mean Ocean Water) - used for $\delta^{2}H$ and $\delta^{18}O$ isotope measurement. The standard is an average of different ocean samples from around the world.
- PDB (Pee Dee Belemnite) - used for $\delta^{13}C$ measurement. The standard is a $CaCO_3$ from a belemnite from the Pee Dee formation in South Carolina.
- Atmospheric Nitrogen - used for $\delta^{15}N$ measurement. The air has a very homogeneous isotopic composition making this an ideal reference.

International standard reference values are given in the table below.

Table 1. Standard reference values for isotopic measurements.

Reference standard	Atom	Isotopic ratio	Value
SMOW	H	$^{2}H/^{1}H$	0.0001557
PDB	C	$^{13}C/^{12}C$	0.011056
SMOW	O	$^{18}O/^{16}O$	0.002004
Atmospheric nitrogen	N	$^{15}N/^{14}N$	0.003663

4 Radioactive isotopes

4.1 Carbon radioactive isotopes and bio-based (carbon) content determination

There are three naturally occurring isotopes of carbon: 99% of the carbon is carbon-12, 1% is carbon-13, and carbon-14 occurs in trace amounts, i.e. making approximately 1 atom per 1012 atoms of the carbon in the atmosphere. From these isotopes ^{12}C and ^{13}C are stable isotopes and ^{14}C is a radioactive isotope.

The ^{14}C is produced in the upper layers of the atmosphere where thermal neutrons are absorbed by nitrogen atoms. When cosmic rays enter the atmosphere, they undergo various transformations, including the production of neutrons that participate in the reaction:
$1n + {}^{14}N \rightarrow {}^{14}C + 1p$, resulting in the ^{14}C formation. The half-life of carbon-14 is 5730±40 years. Carbon-14 decays into nitrogen-14 through beta decay.

During their growth, plants absorb CO_2 from the atmosphere, thus the ^{14}C that is present in biomass originates from recent atmospheric CO_2. Due to radioactive decay, it is almost absent in fossil materials older than 30 000 years. The ^{14}C content may be thus considered as a tracer of products recently synthesized from atmospheric CO_2 and particularly of recently produced bio-products. Till now, the only reliable determination of the biomass content in products is therefore based on the ^{14}C measurements that allow calculation of the bio-based carbon fraction. Schematically this process is shown in **Figure 1**.

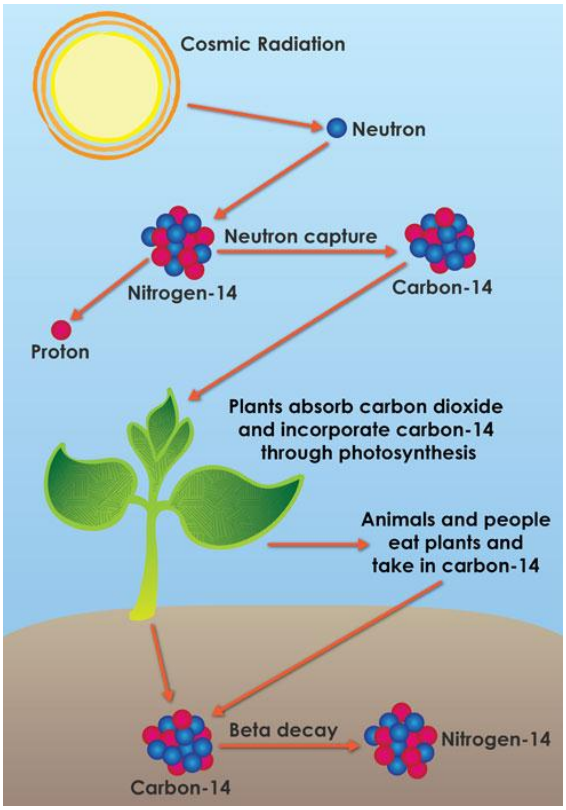

Figure 1. Schematic illustration of formation and transformations of carbon-14 (picture from www.learner.org/chemistry)

Within the ^{14}C determination method, three techniques are distinguished and described further in this report.

1) AMS - accelerator mass spectrometry
2) LSC - liquid scintillation counting
3) BI - *beta-ionization technique*

AMS and LSC techniques are considered to be equivalent.

Since the BI measurements are not included in the normative part of CEN/TS 16640, but only in its informative part and besides its use is limited by only few laboratories in the world, it will also not be described in this report. When necessary, Annex D of CEN/TS 16640 can be referred to for the information on beta-ionization technique for the 14C determination.

AMS and LSC require different amounts of the CO_2: for AMS measurements the minimum amount of CO_2 is 4 ml, and for LSC measurements the required amount of CO_2 depends on the way the sample is prepared for measurement, but at least a few grams will be required.

After the ^{14}C content is determined via AMS or LSC, the bio-based carbon content can be expressed as:

- Percentage of the total carbon of the product
- Percentage of the total mass of the product

The first way of representation is more common. The limitation of this method is that it can be used only in carbon-containing products that ideally should be completely combustible.

4.2 Hydrogen radioactive isotopes and bio-based content determination

Hydrogen (H) has three naturally occurring isotopes, denoted ^{1}H, ^{2}H, and ^{3}H. Other, highly unstable nuclei (^{4}H to ^{7}H) have been synthesized but not observed in nature. The stable isotope ^{2}H (or hydrogen-2) is called deuterium, while the radioactive isotope ^{3}H (or hydrogen-3) is called tritium. The symbols D and T (instead of ^{2}H and ^{3}H) are sometimes used for deuterium and tritium.

The most stable **radioactive isotope of hydrogen** is tritium (^{3}H), with a half-life of 12.32 years. All heavier isotopes are synthetic and have a half-life less 10^{-21} second (less than a zepto-second). Of those, ^{5}H is the most stable, and the least stable isotope is ^{7}H [10].

^{3}H (tritium) contains one proton and two neutrons in its nucleus. It is radioactive, decaying into ^{3}He through β−decay. It is used in thermonuclear fusion weapons, as a tracer in isotope geochemistry. But also, small amounts of tritium occur naturally because of the interaction of cosmic rays with atmospheric gases [9]

The fact that small amounts of tritium occur naturally in the atmosphere can be used to trace the amount of hydrogen that is present in recent biomass. Since the half-life time of tritium is 12.32 years, the ^{3}H can be used to date materials (partially) made from recent biomass. In materials older than a few decades ^{3}H is almost absent. This knowledge can be used to investigate whether ^{3}H can be used for the determination of bio-based fraction of materials or products that do not contain carbon (like many fertilizers) and where therefore the ^{14}C technique cannot provide any insight on the bio-based content. Also it is interesting to see if the use of tritium measurement can be a verification tool for the ^{14}C measurement. Since both ^{14}C and ^{3}H form in the atmosphere, and both being radioactive, experience in radioactive decay (however with very different half-life times), it is expected that the methodology to use the ^{3}H for the determination of naturally formed fractions of H in the products can be similar to what is used in the ^{14}C methods. However, at the current stage there are no developments of this matter and this subject needs more research.

5 Bio-based content determination

This paragraph will focus on already known and standardized bio-based content determination methods. The methods that will be described in this paragraph, combine both measurements and calculations for the final determination of the total bio-based content. These are the radiocarbon analysis method for the bio-based carbon determination (CEN/TS 16640) and the radiocarbon analysis supplemented with the elemental analysis for the total bio-based content determination (EN 16785-1).
Among international standards, American standard ASTM D6866 (Standard Test Methods for Determining the Biobased Content of Solid, Liquid and Gaseous samples Using Radiocarbon Analysis) is also used for the bio-based content determination. However compared to CEN/TS 16640 that is written in terms of total carbon (TC), ASTM D6866 is written in terms of total organic carbon (TOC). This can introduce some errors since during pre-treatment it is difficult to separate organic and inorganic carbon in a sample. Furthermore, the bio-based content of a product accordingly to ASTM D6866 is identical to the bio-based carbon content, while EN 16785-1 goes beyond this and incorporates contributions from other elements (oxygen, hydrogen) that can also fully or partly originate from biomass.
As example, a product with the bio-based carbon content that is lower than the total bio-based content can be mentioned, thus indicating that total bio-based content calculated only based on bio-based carbon, can lead to underestimation of actual total bio-based content in the product. As a real example, Sample 9 from Deliverable 3.1 of Open-Bio (report on the results of the round robin assessments), can be mentioned. The product was multilayer packaging film with measured bio-based carbon content 14% while the total bio-based content was stated and was verified to be 22%. More extensively this will be reported in Deliverable 3.3 of Open-Bio.
As it was already mentioned in the introduction, biomass fractions in the production streams can be also determined by material balance calculations. Material balance calculations take into account material input, material output and material losses during a production process. Material balance method can provide information about the proportion of bio-mass that is used in the production of the bio-based product. Total bio-based content then is proportional to the amount of biomass that is found at output stage. However, since material balance methods are not based on actual measurement but rather on the information about the biomass input into the production process, and that no standardized method is available at the moment, these methods will not be discussed in the current report. Material balance methods are described in details in Deliverable 4.5 of KBBPPS (University of York).
An overview of the different definitions leads to:

- Bio-based carbon content (CEN/TS 16640) – ratio between the total bio-based carbon and total carbon content of a product. This is mentioned biogenic carbon content in ASTM D6866.

- Bio-based content (ASTM D6866) – ratio between the total bio-based carbon and total organic carbon content of a product.
- Bio-based content (EN 16785-1) – ratio between the total bio-based mass (carbon, oxygen, nitrogen and hydrogen) and the total mass of a product.

5.1 Bio-based content determination based only on ^{14}C analysis

The radiocarbon method is used for the bio-based carbon content determination and can subsequently be extended for the total bio-based content determination. CEN/TS 16640 gives detailed description of the method and of the pre-treatment of the samples in order to apply the method. According to CEN/TS 16640, before the actual 14C analysis, every sample has to be converted to the form (usually CO_2) that is suitable for the 14C determination. Such conversion is normally achieved by the combustion of the sampled material and collection of CO_2 that is released during the combustion process. Representativeness of the sample and complete combustion (at least 90% recovery rate) are necessary in order to have reliable results on the bio-based carbon content. Combustion can be done in an elemental analyser, in a bomb calorimeter, or in a combustion furnace. More details are provided in CEN/TS 16640.

For the ^{14}C analysis, a number of ways can be used:
- AMS (accelerated mass spectrometry)
- LSC (liquid scintillation counting)
- Direct LSC

AMS technique

The AMS method determines the presence of ^{14}C directly: the atoms in the sample are converted into a beam of ions, then the formed ions are accelerated in an electric field, deflected in a magnetic field and detected in ion detectors resulting in the determination of the relative isotope abundances of these ions. As the ^{14}C is determined in graphite (carbon), all the carbon in the sample has to be converted into graphite before analysing. With AMS, the modern fraction in the carbon, present in the sample, is determined. The total carbon content is not determined with this technique and shall be determined separately.

Principle

$^{14}C/^{12}C$ and $^{13}C/^{12}C$ isotopic ratios are determined using AMS. The AMS method determines the presence of the ^{14}C isotope directly. The atoms in the sample are converted into a beam of ions. The ions formed are accelerated in an electric field, and subsequently deflected in a magnetic field, and finally detected in ion detectors, resulting in the determination of the relative isotope abundances of these ions. AMS uses a high potential electrostatic field, which serves not only to accelerate but also to specifically form only C^{n+} ions (n = 1 ... 4) that are allowed into the spectrometer, excluding all other ionic species (see **Figure 2** for schematic

illustration). This enhances sensitivity without compromising selectivity. In most AMS systems, the ^{14}C is currently determined from graphite (carbon) sample targets. To obtain graphite sample targets, it is necessary to convert the CO_2 from each sample into graphite before analysing.

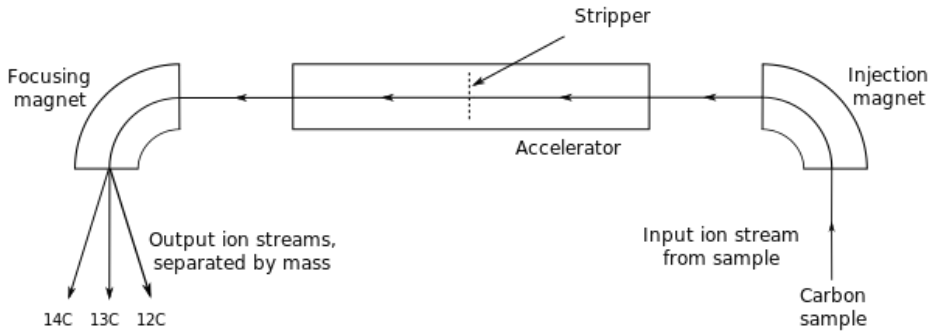

Figure 2. Schematic picture of working principle of the AMS technique.

With AMS the amount of ^{14}C atoms is measured relative to the amount of (one of) the more abundant carbon isotopes ^{12}C and/or ^{13}C. This measured $^{14}C/^{12}C$ or $^{14}C/^{13}C$ ratio is calculated relative to the measured isotope ratio in a reference material with standardized ^{14}C amount, to obtain standardized and normalized ^{14}C content (in pMC) for each sample.

Sample analysis

A zero percentage of ^{14}C represents the entire lack of ^{14}C atoms in a material thus indicating a fossil (for example petroleum based) carbon source. One hundred percentages ^{14}C, after correction for the post-1950 bomb injection of ^{14}C into the atmosphere, likewise indicates an entirely bio-based carbon source. The percentage modern carbon can be slightly greater than 100 % due to the continuing, but diminishing, effects of the 1950s nuclear testing programs (see **Figure 3**)

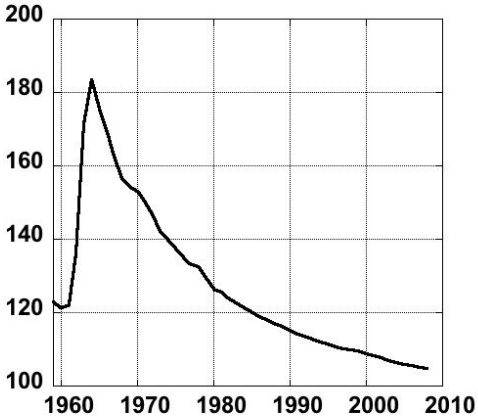

Figure 3. — Decrease in ^{14}C value atmospheric air CO_2 (in pmC), measured at High Alpine Stations Vermunt (Austria) and Jungfraujoch (Switzerland).Data has been published in [8]

However, all certified laboratories refer to pMC value determined from the atmosphere every year. Because all recent biomass is harvested within three years this yearly adopted value is used. All percentage modern carbon (pMC) values obtained from radiocarbon analyses must be corrected for isotopic fractionation using stable isotope data ($^{13}C/^{12}C$ ratios) obtained on CO_2 derived from combustion of the sample.

LSC methodology

The LSC method determines the isotope abundance of ^{14}C indirectly, through its emission of beta-particles due to the radioactive decay of the ^{14}C atoms. The beta-particles are detected through their interaction with scintillation molecules. The number of scintillations is counted and is proportional to the ^{14}C amount in a sample.

Principle

There are three methods that can be used for the preparation of the collected CO_2 for activity measurement

CO_2 extracted from a gas is converted to benzene. This benzene is mixed with an organic solution containing a scintillator. The ^{14}C activity of the mixture is measured in a liquid scintillation counter.

CO_2 extracted from a gas is trapped in an amine solution thus forming carbamates. This solution is mixed with the organic solution containing the scintillation reagent. The ^{14}C activity of the mixture is measured in a liquid scintillation counter in Bq.

Direct LSC measurements. These measurements are possible when a liquid sample can be directly mixed with the scintillation liquid without prior combustion.

In all cases, the measured activity of the sample is calculated relative to the known ^{14}C activity of a standard reference material to obtain standardized and normalized ^{14}C content (in pMC) for each sample.

Sample analysis

Significant ^{14}C counts indicate the presence of ^{14}C carbon. The lack of any ^{14}C counts in a material indicates a fossil (for example, petroleum based) carbon source. A sample that has the same ^{14}C activity level (after correction for the post-1950 bomb injection of ^{14}C into the atmosphere) as the oxalic acid standard is 100% bio-based and signifies an entirely modern carbon source.

Direct LSC measurements with the LSC technique are possible only for products that are homogeneous liquids. Direct LSC analysis can be performed when a liquid sample can be directly mixed with the scintillation liquid without prior combustion. This option is only allowed if equivalence with the methods with conversion to CO_2 can be demonstrated. This will in general be the case if no quenching is observed, or if correction for quenching is performed using standard addition technique using the same, ^{14}C labelled, bio-based product with known ^{14}C activity. When the liquid is not completely clear, but contains (very) small particles, the direct LSC measurements are not possible.

When using the ^{14}C method for the determination of total bio-based content, the final number that represent the bio-based content of a product is calculated as a fraction of the measured ^{14}C with respect to the total carbon in the product.
The bio-based content determined by the radiocarbon method is derived only from the carbon that is present in a product.

5.2 ^{14}C and elemental analysis – EN 16785-1

The method that is described in EN 16785-1 combines the radiocarbon and the elemental analysis in order to define the total bio-based content of a product. Usually an element analyser is used for the determination of the CHN-O composition of a product.

When following EN 16785-1, the radiocarbon analysis can be performed as described above. Contrarily to the ^{14}C method that reports the bio-based content based on the carbon atoms that are present in a product, the combination of the ^{14}C analysis and the elemental analysis returns the bio-based content that includes also nitrogen, hydrogen and oxygen contributions. Atoms connectivity and the rules of allocation of elements are used to decide which fraction of H, N and O originates from bio-mass. H, N and O that are connected to a ^{14}C, are considered as bio-based. The total bio-based content of a product is then calculated including contributions of bio-based fractions of all relevant elements and is expressed as a percentage of the total mass of the product. In reality, however, it is most often very difficult to distinguish what fraction of O, H or N is bound to the biogenic fraction of carbon. Therefore,

in practice, knowledge of the formulated product and knowledge about the production process are necessary to determine the total bio-based content accordingly to EN 16785-1. This implies then that EN 16785-1 can only be used in production environment and cannot be used when a bio-base product without any background information has to be analysed.

5.3 Stable isotopes

Currently, stable isotope analysis is a supplementary method to the previously described two methods. Stable isotope analysis alone cannot reveal a definitive result on the total bio-based content due to difficulties in differentiating those isotope fractions that can be considered as contributing to the bio-based and to the fossil parts of a product. But instead, stable isotopes of many elements (for example of C, N, O) behave differently at different environmental conditions (that is usually presented by isoscapes of a given element) thus making it possible to trace the geographical origin of the products or of the bio-based feedstock.

Stable isotopes are determined by using mass spectrometry techniques such as IRMS (ion ratio mass spectrometry).

Single isotope analysis: examples

In case of stable isotopes of carbon (^{13}C), the correlation between the ^{14}C and the $\delta^{13}C$ content is observed in a very limited number of products. As a single example, **Figure 4** below shows the correlation between $\delta^{13}C$ and ^{14}C content (bio-based content) in rubber samples. In such cases, the isotopic analysis results in the same bio-based content as the radiocarbon analysis.

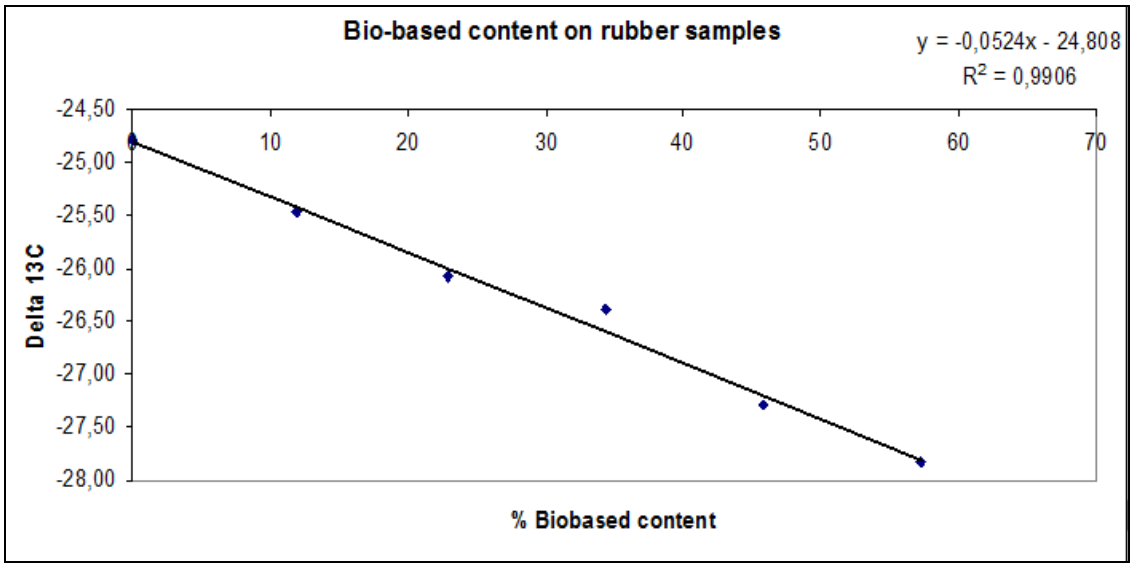

Figure 4. First investigations on bio-based components: assessment of natural polyisoprene in rubber (data provided by ISA, Lyon)

The above mentioned example is one of the few very limited cases where the correlation between $\delta^{13}C$ and the ^{14}C (bio-based) content was experimentally established. In most cases (as it will also be shown in later paragraphs where sun lotion and its components were tested), this correlation is not observed or does not exist. As example of this, **Figure 5** from Ref.3 can be referred to. Three different types of plastic were analysed in Ref. 3: they are petroleum-derived plastic, plastic from C3 plants, and plastic from C4 plants.

In general, three different types of plants are distinguished: C3, C4 and CAM plants. They are recognised by the different ways they undergo the photosynthesis, i.e. when carbon dioxide is converted into sugars in a process calledcarbon fixation. C3 plants undergo C3 photosynthesis, when carbon dioxide – sugar conversion becomes a molecule made up of three carbon atoms. C4 plants undergo C4 photosynthesis and CAM plants undergo CAM photosynthesis. C3 plants are the most common and the most efficient at photosynthesis in wet climates. They keep their stomata open during the day. In hot, dry areas, it is dangerous to leave stomata open during the day because the plants lose water through the stomata. Therefore in hot climates C4 plants are most efficient in photosynthesis since they perform photosynthesis even when their stomata are closed. CAM plants are known to avoid water loss during photosynthesis so they are best in deserts. About 95% of the plants on the earth are C3 plants. Rice is typical example of C3 plants, while maize and sugar cane are typical representatives of C4 plants [1,2].

As it can be seen from **Figure 5**, plastic derived from C4 plants has different $\delta^{13}C$ values (less negative) than plastic derived from C3 plants or petroleum derived plastic. Thus, in this specific case, the single $\delta^{13}C$ analysis can make it possible to distinguish between plastic derived from C4 plants and petroleum-derived plastic. However, it remains impossible to differentiate between plastic derived from C3 plants and petroleum derived plastic by only performing the $\delta^{13}C$ analysis. In such cases, multiple isotope analysis can be of help, as will be illustrated by the next example.

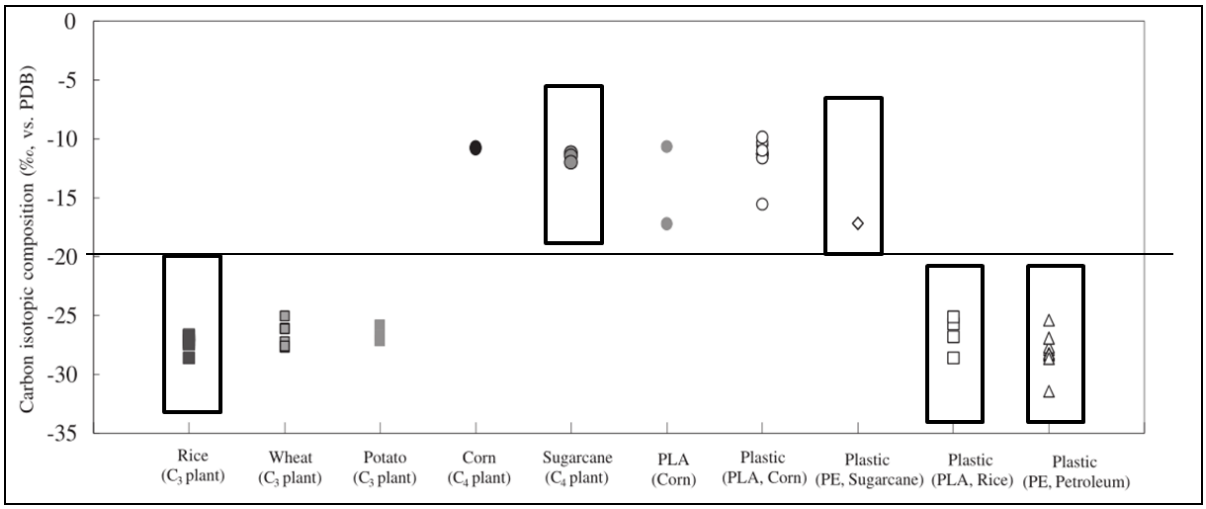

Figure 5. Discrimination between petroleum and plant-derived plastic (data from [3])

Multiple isotopes analysis: example

In general, multiple isotope analysis gives more information and helps to perform better differentiation between geographical origins or between different feedstocks.

Figure 6 illustrates how multiple isotope analysis, $\delta^{13}C$ in combination with $\delta^{18}O$, can be used for distinguishing the origin of alcohol samples [4]. The figure legend can be referred to for the geographical location. The samples were analysed by IRMS method. As can be seen from the figure, multiple $\delta^{13}C$-$\delta^{18}O$ isotope analysis allows distinguishing between the different geographical origins, but also between the synthetic and natural samples. Only the use of $\delta^{13}C$ would not be conclusive for the synthetic alcohol samples (black filled squares) and “Tapioca China” alcohol samples (black filled triangles).

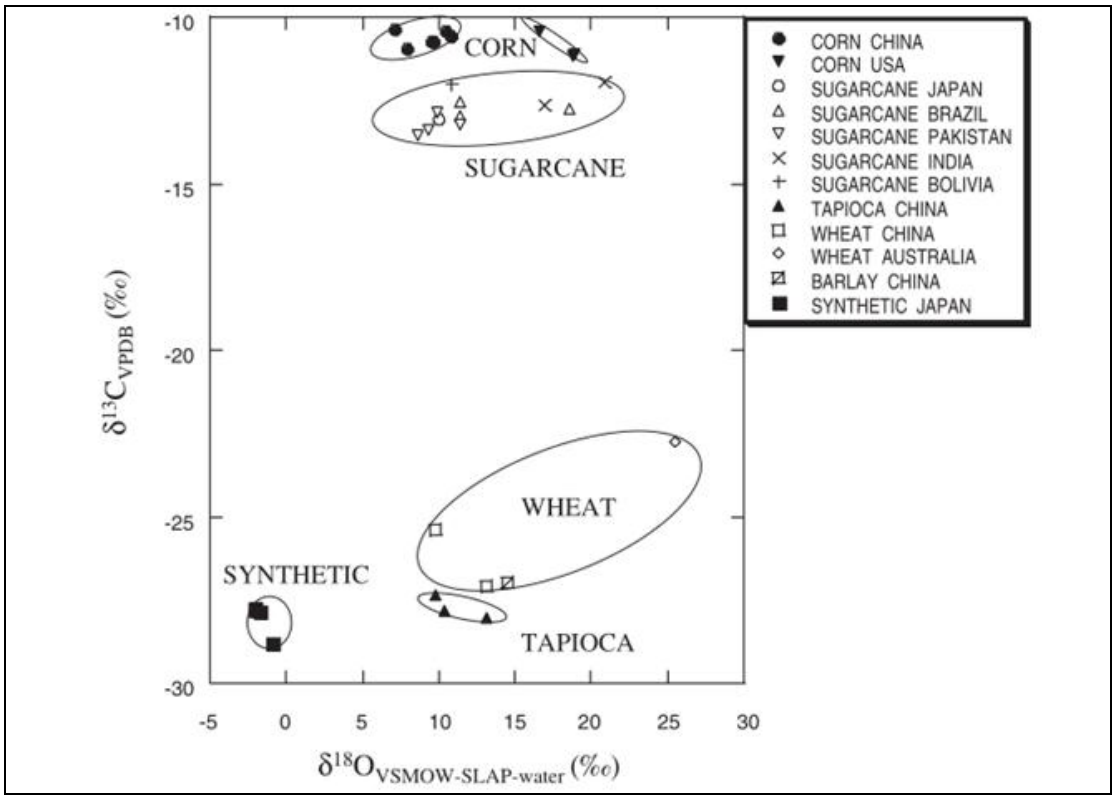

Figure 6. Combined analysis of $\delta^{13}C$ and $\delta^{18}O$ for alcohol samples with known origin. The samples were analysed by IRMS method (data from [4])

6 Analysis of selected product: results and discussions

As it was mentioned in the introduction, a sun lotion sample was selected for testing. The idea of the investigations was to measure the elemental composition, stable isotopes content and ^{14}C (named A6 in Table 2) and in all sun lotion components (named A1, A2, A3, A4, A10, A7, A8 and A9 in Table 2). The aim is to see whether the stable isotope analysis can be used to arrive to the same bio-based content that was determined from the ^{14}C measurements in the final product and calculated in the final product based on the ^{14}C measurements in each of constituents. Also the differences between the bio-based carbon content and bio-based content will be shortly discussed.

Table 2. Sun lotion (A6) and its components: elemental analysis (C,H,O), stable isotopes analysis ($\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$) and radiocarbon measurements (^{14}C).

Component	Amount in the end product A6 , %	C content, %	14C, %, Groningen	delta 13C, ‰, Lyon	delta13C, ‰, Groningen	H content, %	delta2H, ‰	O content, %	delta18O, ‰
A1	**5.00**	38.85	100.00	-32.975	-33.166	8.99	-39	53.18	28.75
A2	**3.00**	77.20	87.81	-30.055	-30.066	12.79	-162	9.79	19.4
A3	**3.50**	73.06	100.00	-29.095	-29.140	11.38	-153	16.23	23.15
A4	**1.00**	71.57	100.00	-28.89	-28.917	10.96	-183	18.1	28
A10	**18.00**	42.42	99.65	-31.87	-31.913	6.93	-214.5	14.8	15.25
A7	**6.00**	76.93	100.00	-30.735	-30.766	12.34	-231.5	9.81	27.6
A8	**1.00**	77.22	100.00	-30.545	-30.551	11.86	-177	10.58	21.7
A9	**3.00**	85.84	100.00	-28.49	-28.426	14.87	-161.5	0.35	0
	40.50								
Water	59.50					11.11	-111.58	88.89	9.62
A6	**100.00**	24.80	96	-30.42	-30.754	10.38	-139.5	47.88	13.45
Calculated in the final product, wet basis		23.13				10.50	-180.52	59.96	
Weighted average, in dry product		57.11	98.94	-31.11		9.61	-180.52	17.46	19.08

Short description of the product. The sun lotion was a white emulsion that contained 59.5% of water, accordingly to the data sheet that was provided by the supplier of the product. The fraction of components A1, A2, A3, A4, A10, A7, A8 and A9 are mentioned in the second column of Table 2 and in total they make 40.5% of the product (data from the product supplier).

Measurements. The CHN-O composition was measured with an elemental analyser and is also presented in Table 2. The CHN-O measurement were done on "as received" products, with no prior drying of the product and no special pre-treatment of its components. The N content in the product and in each component was measured to be very low (less than 0.5%) and therefore is not incorporated in the analysis. The ^{14}C measurements were performed for the final product and for each component and are presented as a fraction of biogenic carbon to the total carbon (reference value for 100% bio-based material was 102 pMC). Stable isotopes measurements were done by the IRMS to determine $\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$ ratios.

Analysis of the measurements. The following observations are made based on the results of the measurements:

1. There is a good agreement between carbon content measured in the end product (24.8%) and calculated carbon content (23.13%). The calculated value was determined based on the amount of carbon that was measured in each of the components of the product. If water was excluded from the product, the total carbon fraction would increase to 57.11%.
2. There is a good agreement between the ^{14}C content measured in the final product (96%) and the ^{14}C fraction calculated in the final product based on the measured ^{14}C in each of components (99%).
3. $\delta^{13}C$ values for each component and for the final product were measured by two independent laboratories and show good agreement as well. Measured $\delta^{13}C$ values for the components of the product do not show much scattering and are very close to each other ranging from -32.975‰ for component A1 to -28.49‰ for component A10. Measured $\delta^{13}C$ of the final product (-30.42‰) is in the same range thus indicating that no carbon is lost during the production of the product from its constituents. Measured $\delta^{13}C$ values in the final product and in its components correspond to the $\delta^{13}C$ range known for C3 plants (from -33‰ to -20‰), thus possibly indicating that the sun lotion that was analysed can be a C3 plants-derived product. Calculated $\delta^{13}C$ value in the final product (not altered by the presence of water) is -31.11‰ that is also in very good agreement with $\delta^{13}C$ value measured in the final product (-30.42‰).
4. Large scattering is observed for $\delta^{2}H$ values that were measured for the components of the sun lotion: $\delta^{2}H$ values range from -231.5‰ for component A7 to -39‰ for component A1. Measured $\delta^{2}H$ value in the final product A6 is -139.5‰. If the value for $\delta^{2}H$ for the final product had to be calculated only based on the $\delta^{2}H$ values that were measured for each component of the product, than the calculated value of $\delta^{2}H$ in the

final product would be -180.52‰ thus being lower than it was measured for the final product. This can indicate that there can be/is a loss of hydrogen in the production process due to synthesis, or exchange with atmospheric hydrogen, or any other chemical interaction during the process. For δ^2H values, it is difficult to identify whether any correspondence can be found between the measured values and the values that can be observed in C3 or C4 plants. Currently this correspondence has not been established/observed mainly due to the lack of data on the δ^2H in plants and plant-derived materials, but also the lack of data on the δ^2H in fossil materials.

5. Similar observation is made for the $\delta^{18}O$ values, where the measured value of $\delta^{18}O$ in the final product (13.45‰) is lower than if it would be calculated based on the measured $\delta^{18}O$ for all components (19.08‰). This could lead to the conclusion that there is a loss of oxygen due to possible chemical interactions during the production or due to exchange with the atmospheric oxygen. Currently, there is no sufficient data to conclude whether the measured $\delta^{18}O$ values can be related to C3 or C4 plant-derived materials.
6. In summary, since there is no (or very limited) data about the δ^2H and $\delta^{18}O$ values in fossil materials, the only $\delta^{13}C$ values of the tested product are not sufficient to conclude whether the product is derived from the C3-plants or possibly can be derived from fossil feedstock. To make quantitative predictions on the bio-based fraction in a similar way as it is done using the radiocarbon analysis at the current stage is not possible. At the current stage, the most reliable and accepted application of the stable isotopes analysis remains geographical origin tracing, in food authentication and in forensic analysis.

Measured stable isotopes data for the sun lotion can be mapped into the figures from Ref. 4, in order to have an indication how multiple stable isotopes analysis can be used. This is illustrated in **Figure 7** and **Figure 8**. However, this mapping of measured data for the sun lotion to the data from Ref. 4, can be used only for indication, but cannot be conclusive since it may be not appropriate to make a direct comparison of samples that belong to the different groups (alcohols and sun lotion). Nevertheless, even in such “rough” comparison, the sun lotion sample falls closer to the C3 and C4 plants rather than to synthetic samples (see **Figure 7** and **Figure 8**). Ideally, to make a proper comparison, more data from different sun lotion samples would be needed, both for the end products and for raw components.

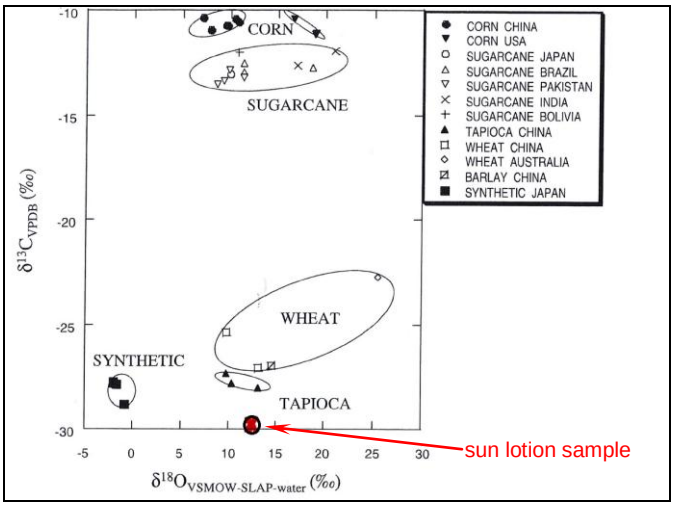

Figure 7. An indicative comparison of $\delta^{13}C$ versus $\delta^{18}O$ values from Ref.4 for alcohol samples and for the sun lotion tested in the current study.

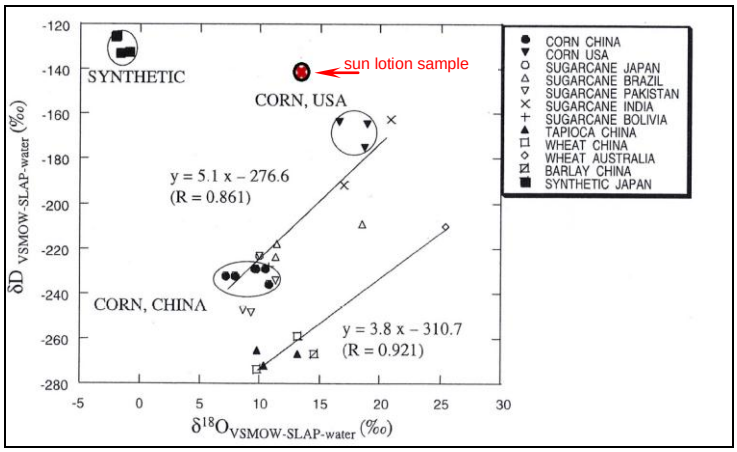

Figure 8. An indicative comparison of $\delta^{2}H$ versus $\delta^{18}O$ values from Ref.4 for alcohol samples and for the sun lotion tested in the current study.

7 Conclusions: comparison between the methods

Initial information needed for each method.

In order to determine the bio-based content of a product using the radiocarbon method (CEN/TS 16640 can be referred to), the knowledge of total carbon and the ^{14}C are needed. The bio-based carbon content is then represented as a fraction of the radioactive carbon to the total carbon in the product. Because this is a direct measurement no information on the product is needed.

For determining the total bio-based content of a product following EN 16785-1, both the radiocarbon analysis and elemental (CHN-O) analysis are needed. Accordingly to EN 16785-1, atoms connectivity and the rules of allocation of elements is used to decide which fraction of H, N and O originates from bio-mass. Normally these fractions of H, N and O that are connected to the ^{14}C, are also considered as bio-based. The total bio-based content of a product is then calculated including contributions of bio-based fractions of all relevant elements and is expressed as a percentage of the total mass of the product. To perform this determination additional information on the product has to be supplied by the producer in order to verify the statements on the bio-based content. Therefore this method can only be used by producers and cannot be used on individual products by product consumers.

In general, stable isotopes analysis cannot be used for the determination of bio-based carbon content or total bio-based content except the very limited cased where the correlation between $\delta^{13}C$ and ^{14}C was established (example of rubber in this report). The most common use of stable isotopes still remains tracing the geographical origin of products, in food authentication and in forensic analysis.

Applicability and (dis)advantages of each method.

The limitation of the radiocarbon method is that it can be only used for carbon-containing products that are very good (at least 95%) combustible. For products that do not contain carbon, tritium (^{3}H) can be used to date very recent (not older than 12.3 years, the half-life time of ^{3}H) H-containing materials. However, this application of ^{3}H measurements hasn't been investigated yet.

When following EN 16785-1 for the total bio-based content determination, the challenge is to determine what fraction of hydrogen, and/or oxygen and/or nitrogen is linked to the biogenic carbon in order to be considered as derived from biomass, accordingly to EN 16785-1.

As it was already mentioned in this report, material/mass balance calculations can be performed for the determination of the bio-mass fraction in the production streams. Mass balance calculations take into account material input, output and material losses during a production process. Mass balance method can provide information about the proportion of biomass that is used in the production of the bio-based product. Total bio-based content then is proportional to the amount of biomass that is found at output stage. The difficulty using this approach can be related with the necessity to control the material/mass flow at each production step in order to have a realistic estimation of the total biomass fraction in the final product.

The table below summarizes the applicability and the requirements of each method for a random product and for a random process.

Table 3. Comparison between different methods for a random product and for a random process (that can be either stable or variable)

	Random product (not directly from production)	**Random process (not directly from production)**
Only ^{14}C (CEN/TS 16640)	limited to carbon containing products	limited to carbon containing products
^{14}C and elemental analysis (EN 16785-1)	not applicable	limited to carbon containing products, minimum amounts can be determined
Stable isotopes	in general not applicable	only for sustainability research (geographical origin verification, etc.)
Mass balance	not applicable	minimum amount can be assigned to a final product

Conclusions with respect to the analysed product

With respect to the product (sun lotion) that was selected for testing, only the radiocarbon method (CEN/TS 16640) could be used for the determination of the bio-based carbon content. The ^{14}C analysis of the final product showed that its carbon is 96% bio-based. Since no CHN-O composition was provided by the supplier of the product, and no bio-based content was stated by the supplier, it is not possible to apply EN 16785-1 for the verification of the bio-based content. However, it can be useful to note that the calculated bio-based carbon content (based on the measured ^{14}C in each of the components of the product) of the final product was 99%, indicating a very good agreement between the measured and the calculated values for the bio-based carbon fraction.

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