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

Work package 3

Biomass Content

Deliverable N° 3.8:
Report on the use of isotopes

Public report

Version: 2

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1 Summary

The Open Bio project (www.open-bio.eu) carries out researches on standardization, labeling and procurement for helping the development of the biobased market. Test methods used for biobased content determination have been investigated, firstly based on 14C and organic elemental analysers. Others methods need to be evaluated. The automated stable isotopic approach method can be relevant in this frame. Stable isotopic approach has been previously undertaken for many years in the authenticity of natural components. This has been done thanks to the natural occurring variations of stable isotopes.

Following the *Direct Biobased automation* (**OPEN BIO Deliverable 3.7)** based on the different technical specifications, this deliverable presents isotopic test reports carried out on biobased feedstocks and products.

Multi isotopic investigations have been applied in various scopes: bio-plastics, bio-rubbers, biofuels and bio-solvents, bio-surfactants, etc. A wide range of components from raw-materials, to semi-finished product and commercial components have been taken into account, and the isotopic data were collected and evaluated.

On the whole, the variation of the botanical origins and the overlap among biomass and non-biomass isotopic values are significant drawbacks for the development of isotopic method to be used as biobased automation method. However our study presents interesting starting points for determination purposes when some conditions are fulfilled:

- Products belonging to C4 plants cycle with small natural isotopic variations and significant gap between biobased and non-biobased origins.
- Components with few origins (biomass and synthesis) including little isotopic intra-variations.
- Multi isotopic approach: in some cases the determination of origins can be carried out using the three main isotopes ^{2}H, ^{13}C, and ^{18}O and can be efficient to discriminate the different origins.

In all cases isotopic databases must be monitored and regularly updated with the knowledge of isotopic limits. These data are necessary to furnish the better uncertainty given to the biobased content assessment.

There is also another methodology which can be used to check products on production lines. When the raw-materials incorporated in an industrial process are always the same and isotopic values of every component and amount of components well known, the biobased content of the final product can be directly connected to isotopic values. This approach permits the manufacturers to check the biobased content. In this case no database needs to be monitored.

Investigations also show that isotopic values can be connected to the (botanical) origins of biomass. These measurements might be relevant a criteria for sustainable or economic issues. They can then be used to check a claim on the sourcing of the biomass.

This report is delivered to the relevant standardization groups in Europe (under CEN). They might use it to their benefit.

2 Introduction

2.1 Task description

Deliverable *Direct bio-content automation* **OPEN BIO Deliverable N° 3.7** and this report present the results of the investigations carried out in the frame of Task 3.5 *Direct methodologies for sustainability of bio-based products.* This concerns the development of complete bio-based content methodologies using stable isotopes and isoscapes.

The development of automation techniques for sample preparation and investigation into isotope measurement techniques for direct bio-based content analysis are included, while the sample preparation automation methodologies need to build upon the conclusions of the assessment from KBBPPS. The assessment includes examples of the use for bio-solvents, bio-lubricants, bio-plastics and bio-surfactants. The original idea was to prepare an overview of all stable isotopes in relation to biomass and fossil content; stable isotope measurements together with isoscapes could thus be used in order to define sustainability of bio-based products. As that appeared to be difficult, a database for feedstocks and products, with isotopes, fingerprint and sustainability aspects seemed neither interesting for the bio-based products' market nor presenting additional value to the already existing information publicly available. The project partners felt that it should be a good follow-up to develop a more generic report on how the isotopes could be used.

2.2 This report

Plants absorb CO_2 during the photosynthesis cycle ensuring their growth and create biomass material. The development of a green industry based on biobased products, compounds made wholly or partly with biomass, will continue in the upcoming future due the reduced availability of renewable resources.

It has been also important to ensure with confidence the content of the so-called products. To this goal, European partners have developed a methodology based on ^{14}C determination *Biobased products –determination of the bio-based carbon content of products using the radiocarbon method* and a second methodology based on ^{14}C added with elemental organic quantification (% C + H + N + O): *Determination of the bio based content using radiocarbon analysis and elemental analysis (EN 16785-1 part 1).*

In order to develop routine controls on biobased feedstock and products for commercial trading, supplementary alternative methods would be required.

Biomass is mainly made by carbon, hydrogen and oxygen and these elements own their proper isotopes (Isotopes are defined as atoms of the one element that have the same number of proton and differ in the number of neutrons present in their nucleus).

Due to the natural occurring variations of organic stable isotopes, isotope ratio mass spectrometry could be an interesting supplementary method. As a matter of fact the $^{13}C/^{12}C$ ratio depends on the photosynthesis pathways creating distinct 3 classes (C3 plant or Calvin cycle, C4 plant or Hatch and Slack cycle and CAM – Crassulacean Acid Metabolism) and the $^{2}H/^{1}H$ and $^{18}O/^{16}O$ isotopic composition related to geographical location, climatic conditions … which can be different to the isotopic composition of synthesis compounds originated fossil raw materials.

Previous studies, mainly based on published data, have investigated the potential of stable isotopes used for the determination of Biobased content. Some limitations due to significant naturally variations for feedstock and the overlapping of isotopic values between Biobased and non Biobased compounds have been already shown in the deliverables *Sample Preparation Techniques for Total Biomass Content Determination* **KBBPPS Deliverable N°4.3** and *Direct biomass content assessment: Assessment study report of analytical techniques for measurement of the biomass content including elements other than carbon* **KBBPPS Deliverable N°4.4.**

Technical performances and the evolution of the implementation have been recently presented in the *Direct Automation* **OPEN BIO Deliverable N°3.7.** Modern isotope analyses encompass mainly instruments connected to isotope ratio mass spectrometer (IRMS) for Bulk Stable Isotope Analysis (B.S.I.A.) or to investigate targeted molecules in mixture for Compound Specific Isotope Analysis (C.S.I.A.). Development of Position-Specific Isotope Analysis (P.S.I.A.) is a new tool and brings intramolecular data in addition to the original molecules results.

However the development of stable isotope approach dedicated to the authenticity of natural product has been going on since few decades. This methodology is currently used for checking feedstock and commercial components and some analytical methods are registered or recognized as official methods. But to obtain this result many years of investigation have been necessary. Public and private isotopic laboratories developed step by step new methods following the evolution and the improvement of instrumentation.

The investigations on isotopic approaches performed during the period of time of Open Bio project brought a large amount of isotopic data on biobased products, a sort of data bank, which can be the base for the development of new methods adapted to each sector of activity or each type of products. If we compare it with the knowledge accumulated during 30 years of investigation on natural authenticity assessment, we can imagine to be at the start of a new field of investigation.

Similarly to the natural authentic market, these new approach to develop methods can help to create the biobased products of the future. This report focusses on the opportunities and limitations of investigations of isotopic method initiated in different fields of the Biobased industry.

3 Assessment of the authenticity of natural products

As previously described in *Direct Automation* **OPEN BIO Deliverable N°3.7**, isotopic measurements are performed using an Isotope Ratio Mass Spectrometer (IRMS). In order to test various samples (solids, liquids), automatic elemental analysers are connected to isotope ratio mass spectrometer for whole sample material for Bulk Stable Isotope Analysis (BSIA) or using separate methods for Compound Specific Isotope Analysis (CSIA).

Stable isotope methodology is easy to operate, carries out fast analyses, and has relative low cost enabling multi isotopic determinations. This approach is extremely appropriate for regular authenticity control of natural products (flavour, essential oil…) and is well used in this scheme. Usually isotopic instruments are able to give the isotopic values of all organic elements contained in the samples.

Isotopic composition is reported in Delta notation (δ):

$$\delta(^{13}C/^{12}C) = \left[\frac{R\,(^{13}C/^{12}C)sample}{R\,(^{13}C/^{12}C)standard} - 1\right] * 1000$$

During the last decades the measurement of isotope ratios has acquired increasing importance in quality control, in the authenticity assessment of natural flavours, proof of authenticity of various food products. Although chemical methods can be used to detect contamination they are limited when looking at the geographical origin or to bring proof of authenticity. A high precision was developed for methods used to detect adulteration of natural products and particularly the addition of synthesis molecules. Methods based on the determination of $^{13}C/^{12}C$ ratios were first applied on molecules previously isolated and measured on offline combustion instruments.

The stable isotopic composition of plants depends on the carbon dioxide assimilation and the fixation of carbon. As a matter of fact plant could be divided in three classes according to their metabolism assimilation. For most of the plants (so called C3 plants) the first intermediary molecule elaborated is the phosphoglycerate (molecule with 3 carbons atoms) and $\delta^{13}C$ generally range from -20‰ to -33‰. For the second class (C4 plants) the first intermediate molecule is a malate (molecule with 4 carbons) and $\delta^{13}C$ are generally in the range from -10‰ to -12‰ .Two plants are mainly representatives in this class: corn and sugarcane. Finally the third class concern plants which can process with the 2 pathway phosphoglycerate and malate (CAM plant) and $\delta^{13}C$ range from -10‰ to -24‰. In this last category we can find vanilla and pineapple.

Among food flavours, vanilla has been probably the most investigated. Vanillin is the principal flavouring constituent of vanilla beans an orchid which operates according to the CAM pathway. $\delta^{13}C$ of vanillin origin beans is close to -20‰ where the $\delta^{13}C$ of synthesis origin are close to -28‰ to wood lignin (C3 plant) and -29‰ to guaiacol (1).

The development of a reference method about the detection of C4 plant sugars in honey by Jonathan White was a significant progress in the struggle of adulteration (2). The methodology compares $\delta^{13}C$ of protein extracted from honey and used as internal standard with $\delta^{13}C$ of honey. A difference exceeding one delta evidences the presence of C4 additional sugar.

At the beginning of 1990 the involvement of on-line coupling of high resolution gas chromatography (HRGC) with IRMS through combustion interface (HRGC-C-IRMS) has provided access to the analysis of individual constituents of complex flavouring by measuring in particularly $^{13}C/^{12}C$ ratios (3). For food authenticity assessment, it was a significant time saving to remove the required extraction steps necessary to isolate pure molecules. Several applied methods based on GC-C-IRMS appear: Authenticity of essential oils such as Coriandrum (4) mandarin oils (5) beverages such as whisky (6) oils such as olive oil (7).

Plant water is always enriched in the heavy isotopes ^{18}O and ^{2}H relative to the precipitation or groundwater, and this enrichment depends on plant transpiration as a function of assimilation type (C3, C4 or CAM plant). It can be assumed that the plant water ^{18}O enrichment relative to precipitation water decreases with increasing North latitude (8).

The development of the online gas chromatography pyrolysis isotope ratio mass spectrometry (HRGC–P-IRMS) technique used for the quantification of the ratio $^{2}H/^{1}H$ allows to acquire new data in the authenticity of natural flavours assessment of natural origin of the main flavour compounds: Decanal, linalool, linalyl acetate (9). All of these evidences results to the large variations in $^{2}H/^{1}H$ ratios in nature and the wide gap between natural and synthesis origins.

Furthermore the combination of ^{2}H and ^{13}C investigation using HRGC-C/P–IRMS has been a significant improvement in the knowledge of the assessment of natural molecules: citral (10) (11) ά and β ionone (12) (13) ϫ and β decalactone (14).

The last step has been to associate the pyrolysis interface for the determination of $^{18}O/^{16}O$ isotope ratios in complement to the others isotope ($^{2}H/^{1}H$ and $^{13}C/^{12}C$) in the aim to deliver a three-dimensional plot. New applications were demonstrated for the authenticity of natural compounds: linalyl acetate and linalool in lavender essential oil (15).

In 2004 the development of Liquid Chromatography coupled to stable carbon Isotope Ratio Mass Spectrometry via a Chemical Oxidation interface LC-Co-IRMS allowed new applications and perspectives in the authentication of origin. Twenty two amino acids were separated and the ^{13}C values determined. The results were similar to those extracted with chemical process and evaluated using an EA-IRMS approach (16). Cabanero et al (17) showed a method allowing the determination of glycerol and ethanol. The results obtained were in good agreement with those performed using EA-IRMS. Guyon et al. (18) improved this method leading the determination of $\delta^{13}C$ of glucose, fructose, glycerol and ethanol in the same run for wine authentication check.

The development of laser spectroscopy permits to determine the organic isotope ratios in gases with an inherent compound–specific. Analytes do not necessarily need to be isolated, separated or trapped. Keppler et al. (19) showed the interest of the determination of ^{13}C in methane from anaerobic digesters.

The development of the intra-molecular isotope ratio analysis is the control degradation of targeted molecules. The degradation techniques are usually based on partial pyrolysis using an additional thermogravimetric device or modification of the GC-pyrolysis- IRMS implement.

A large part of theses isotopic methods are regularly undertaken using fast analyses to check the authenticity of flavours, essential oils and natural products in trading process. For industrials involved in the fields of food, perfumes, essentials oils and cosmetics, stable isotopic approaches are definitively useful tools in the aim of validating the authenticity of natural products regarding synthesis adulteration, even if they know that the uncertainty could be quite important due to the variation of plants origins.

During the last decade stable isotope laboratories involved in authenticity assessment had to collect lots of isotopic data related to the different origins of molecules. This required work is important and must be regularly carried out for ensuring a steady evaluation of the data base collected.

Some isotopic methods have been also recognized as international methods. The list below gives some examples of the official methods elaborated in the naturalness authenticity assessment:
CEN Standards test methods:

- ENV 12140 Fruit and vegetables juices – Determination of the stable carbon isotope ratio ($^{13}C/^{12}C$) of sugars from fruit juices-method using isotope ratio mass spectrometry
- ENV 12141 Method for determination of stable oxygen isotope ratio ($^{18}O/^{16}O$) of water from fruit juices, using isotope ratio mass spectrometry
- ENV 12142 Method for determination of stable hydrogen isotope ratio ($^{2}H/^{1}H$) of water from fruit juices, using isotope ratio mass spectrometry

AOAC (Association of analytical communities) methods:

- Detection of C-4 Plant Sugars in Honey by $^{13}C/^{12}C$ analysis. Method 998.12
- Detection of addition of beet sugars in fruit juices. Methods 981.09 and 982.21 ($^{13}C/^{12}C$ analysis)
- Carbon isotope ratio mass spectrometric method for detection of corn syrup and cane sugar in maple syrup. Method 984.23
- Determination of sugar beet derived syrups in frozen concentrated orange juice- $\delta^{18}O$ measurements in water. Method 992.09
- Carbon stable isotope ratio of ethanol derived from fruit juices and maple syrups. Method 2004.01

OIV (International Organization of vine and wine):

- Resolution OENO/7/2001 Determination by isotope ratio mass spectrometry of $^{13}C/^{12}C$ of wine ethanol or that obtained through the fermentation of musts, concentrated must or grape sugar.
- Resolution OENO/7/2005 determination of the carbon isotope ratio $^{13}C/^{12}C$ of CO_2 in sparkling wines method using isotope ratio mass spectrometry (IRMS).

Isotopic analysis used for the assessment of natural product are undertaken to validate (or not) the authenticity of the target samples. In the field of biobased product, a compound could be biobased, non-biobased or partly biobased with a known level of biobased content. If the isotopic methodology would be used as an alternative method, it could bring this assessment of the biobased content with the higher acceptable uncertainty.

4 Overview of feedstock isotopic fingerprint

4.1 C4 plant

Sugarcane and maize have a specific isotopic ^{13}C fingerprint due to their belonging to the C4 photosynthesis pathway cycle and are among the major feedstock employed.

Sugarcane

Rodushkin et al. (20) present the results of an inter-laboratory program based on the multi elements and isotopic measurements of several sugar samples with different geographical origins: USA, Costa Rica, Argentina and Swaziland. The results obtained on these cane sugar samples were:

$\delta^{13}C$ Mean: -11.70‰
Standard deviation: 0.52‰
Upper $\delta^{13}C$: -10.5‰
Lower $\delta^{13}C$: -12.6‰

Two samples have been analyzed (see Table 1).

Table 1: measurement of sugarcane samples from various geographical origins

references	plant	origin	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
sugar	cane	Paraguay	-11.71	-3	35
		Reunion Island	-11.66	11	37

Corn

Several samples of starch have been analyzed. They are originated from the fields of production feeding the biobased industry factories.

Table 2: Measurements of corn samples from various geographical origins

references	plant	origin	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
starch	corn	China	-11.66	-28	30
		France	-11.44	-16	32
		Italia	-11.81	-19	32
		Spain	-11.49	-21	32
		France	-11.42	-2.5	33
		Brazil	-10.72	-21	29
		Brazil	-11.02	-25	29
		USA	-10.82	-17	29
		Turkey	-11.51	-22	31

$\delta^{13}C$ Values range from -11.81 to -10.72‰ .
$\delta^{13}C$ Average -11.34‰ SD 0.35‰

$\delta^{13}C$ measured on C4 plant origin sample were in good agreement with data published.

4.2 Other raw materials: C3 plants

Rodushkin et al (20) present, in the same draft previously cited, the results of an inter-laboratory program based on the multi elements and isotopic measurements of several sugar samples with different geographical origins: Moldavia, Poland, France, Netherlands, Germany, Hungary and USA. The results obtained on various cane sugar samples were:
$\delta^{13}C$ Mean: -24.98‰
Standard deviation: 0.75‰
Upper $\delta^{13}C$: -23.8‰
Lower $\delta^{13}C$: -26.5‰

Starch which is a significant raw-material could be originated from various origins. The table below present $\delta^{13}C$ of starch samples from C3 plants.

Table 3: Measurements of starches from various origins

references	plant	origin	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
	wheat	France	-26.91	-47	31
		Corby	-27.00	-43	32
		France	-27.21	-43	32
	pea	France	-27.38	-40.5	33
	potatoes	Denmark	-28.44	-102	27
		France	-26.51	-79	35
	tapioca	Brazil	-26.11	-82	29

$\delta^{13}C$ measured of C3 starch samples range from -26 to -28‰. $\delta^{18}O$ obtained on C3 and C4 starch plant origins seem to be in the same isotopic area.

5 Determination of Biobased content for feedstocks and products

5.1 Bioplastics

Current plastics products are composed of biobased synthetic polymers, fossil-based synthetic polymers, natural polymers and additives that can include biobased materials. “Biobased plastic” refers to plastic that contains materials wholly or partly of biogenic origin (21).

Polyethylene Terephthalate (PET)

In 2009 the Coca-Cola Company present their Plant Bottle® PET packaging innovation made from up to 30% biobased, sugar cane renewable materials. The new product encompasses a part of MEG (mono ethylene glycol) biobased origin in the PET final product.

In response, the Coca-Cola Analytical Science Team has developed a novel, patent pending analytical method to quantitatively determine the amount of biobased material in Plant Bottle® PET resin. This new approach has shown good reproducibility and accuracy, with excellent correlation to the conventional ASTM 6866 method using radiocarbon analysis. The new implement is made by an elemental analyser TOC (total organic carbon) connected to a cavity ring-down spectroscopy (CRDS) detector (TOC-CRDS). This equipment allows the determination of the delta ^{13}C value from sample after combustion. The correlation between the delta ^{13}C values and the ^{14}C measurements connected to the biobased carbon content were in good accordance allowing the ^{13}C method to be an efficient alternative method in this particular industrial process. Rapidity and relatively inexpensive test are also significant advantages of the alternative stable isotopic approach (22).

Suzuki et al. presented the ability of $\delta^{13}C$ method to discriminate between plant and petroleum derived plastics (23). The $\delta\ ^{13}C$ values of the plastics investigated range from -17.3‰ to -10.0‰ for corn derived plastics PLA (Poly-Lactic acid) , from -28.6‰ to -25.8‰ for sugarcane-derived plastics PE (polyethylene) from -28.6‰ to -25.8‰ for rice-derived plastics PLA and from -32.1‰ to -25.4‰ for petroleum derived plastics PE. The $\delta^{13}C$ results obtained suggest that plastics derived from C4 plants are clearly significant higher than the fossil origins.

In the aim to complete these data, several PET mineral water bottles have been collected from the French market for a multi-isotopic approach. PET is made by esterification reaction between terephthalic acid and ethylene glycol. Only ethylene glycol was biobased since it was made from cane sugar. The plastic elaborated has 31% of biobased content, and if recycled PET is added (35%), this biobased content part decreases to 20%.

Sample preparation has been directly carried out by cutting out small parts of the plastic bottles. Determination is done according to AE-IRMS method.

The results on the table below present different types of origins of the samples:

- Sample M1 and M2 called “green bottle” are partly biobased and the % of biobased content is the statement given by the manufacturer.
- Samples from M3 to M6 are fossil originated bottles

Table 4: Measurements of PET mineral water bottles

References	% Biobased	δ ^{13}C (‰)	δ ^{2}H (‰)	δ ^{18}O (‰)
Sugar cane	100	-11,7		
M1	30	-23,83	-89	9,6
M1	30	-23,54	-90	9,1
M2	20	-24.96	-99	8,7
M2	20	-25.00	-101	8,5
M3	0	-27,63	-70	21,6
M3	0	-27,79	-68	21,9
M4	0	-28,73	-58	12,9
M4	0	-28,69	-55	13,8
M5	0	-28,06	-80	21,1
M5	0	-27,86	-70	21,1
M6	0	-28,93	-56	15,5
M6	0	-28,91	-50	15,2

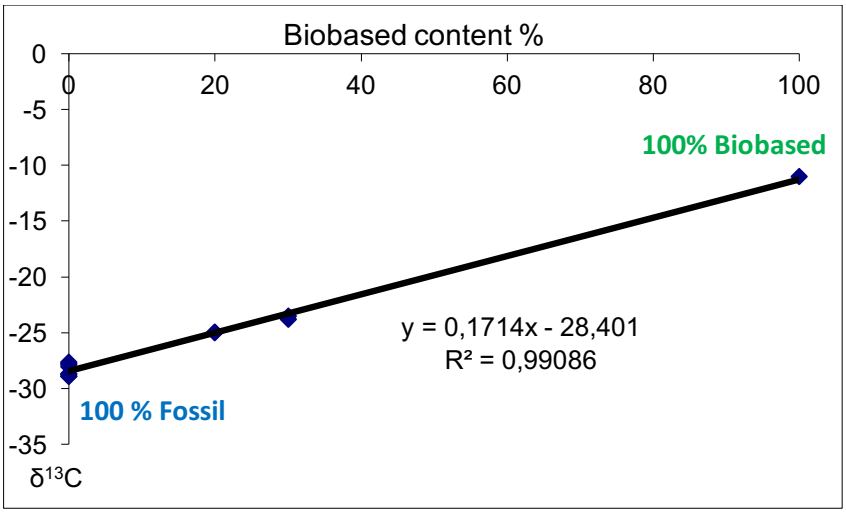


Figure 1: Representation of δ^{13}C vs % biobased content on PET plastic samples

A linear regression between $\delta^{13}C$ and biobased content % is clearly shown allowing measurement with an uncertainty lower than 5%. These results are in good agreement with the previous data provided by Coca Cola Company about the biobased sugarcane PET bottles. The arrow placed on the figure show the increasing direction of Biobased amount.

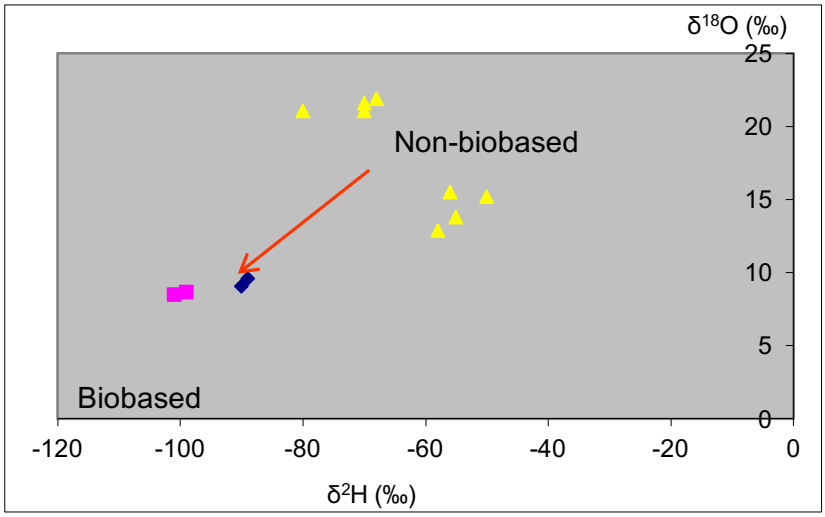


Figure 2: Representation of $\delta^{2}H$ vs $\delta^{18}O$ for PET plastic samples.

These results emphasize that multi isotope methodology could be discriminant for the assessment of Biobased content regarding PET biobased sugarcane plastics. The figure presents 2 groups of synthetic components.
But PET bio-plastic is currently withdrawn from the drinking water commercial market and no more investigation could be performed on this type of plastic.

Polyethylene furanoate (PEF)

Another plastic elaborated from sugar and interesting for it physical property is PEF (Poly-Ethylene Furanoate).

Table 5: Measurements of a sugarcane PEF sample

composition	Biobased	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
PEF sample	100	-11.57	-114	12

In this case $\delta^{13}C$ of the PEF sample analyzed is clearly in good correlation with the footprint of C4 plant. But if PEF was made from sugar either beet or cane, the $\delta^{13}C$ value would be probably limited for discrimination.

Poly-lactic acid (PLA)

PLA (Poly-Lactic acid) is a biodegradable polyester derived from renewable resources. However PLA made by fermentation of starch and condensation of acid lactic could be made by a mix of various starch origins (C3 or C4 plant) and the $\delta^{13}C$ obtained could be inappropriate to distinguish the origin due to high isotopic variations.

Measurements of various biobased plastics samples:

Table 6: Measurements of biobased plastic samples
(Samples ranking from lower to higher biobased content values)

composition	Biobased %	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
PLA-PBAT-fossil compound	10	-30.78	-181	20
Starch - PBAT fossil -plasticizer	18	-25.06	-155	23
Starch-PLA-co-polyester-plasticizer	20	-25.25	-100	25
Starch-PBAT-plasticizer	23	-32.21	-170	23
PLA-PBAT-mineral charge-plasticizer	25	-28.31	-176	23
PLA co-polyester	30	-23.70	-143	26
PLA-PBAT-fossil product	30	-27.24	-203	24
Starch-PLA-PBAT-plasticizer	31	-26.77	-145	23
PBAT –mineral charge	34	-29.77	-178	22
Co-polyester	50	-23.82	-129	26
Starch-PLA-Co-polyester	50	-24.61	-112	24

No tendency appears in this table due to the wide difference of origins of compounds and mixture of various products. The multi-isotopic methodology would only be efficient on plastic samples if the botanical origin of the products was known.

5.2 Bio-rubbers

Polyisoprene

Polyisoprene is a naturally occurring polymer mainly originated from rubber tree (Hevea tree). The product harvested is latex and the main producer in the word is Malaysia. Polyisoprene is widely used to produce natural rubbers.
It could be also synthesized by polymerisation of various petroleum monomers.

The assessment of the origin of polyisoprene using stable isotope has been previously undertaken. A Chinese pattern has been edited in 2015 related to polyisoprene synthesis using renewable raw material (24). The present invention more specifically discloses an isoprene monomer comprising repeating units derived from a polyisoprene polymer, wherein the polyisoprene polymer has $\delta^{13}C$ value of greater than -22‰. Such polyisoprene can be cis-1,4-polyisoprene homo-polymer rubber. Also provides a polyisoprene homo-polymer or copolymer having repeating units derived from isoprene containing from sustainable renewable non-petroleum derived source of isoprene method for authentication.

The investigations carried out in OPEN BIO project concern any natural polyisoprene wholly or partly biobased.

Table 7: Measurements of various polyisoprene samples

References	% Biobased	origin	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
Nat polyisoprene	100	Ivory Cost	-28.47	-118	19
Nat polyisoprene	100	Malaysia	-27.87	-152	17
Nat polyisoprene	100	unknown	-27.13	-154	17
Nat polyisoprene	100	unknown	-27.47	-148	18
polyisoprene	57.2		-27.83	-144	10.7
polyisoprene	45.8		-27.29	-132	9.5
polyisoprene	34.3		-26.39	-109	9.4
polyisoprene	22.9		-26.08	-95	8.2
polyisoprene	11.9		-25.48	-76	8.5
polyisoprene	0	synthesis	-24.80	-63	5
polyisoprene	0	synthesis	-31.30	-87	6

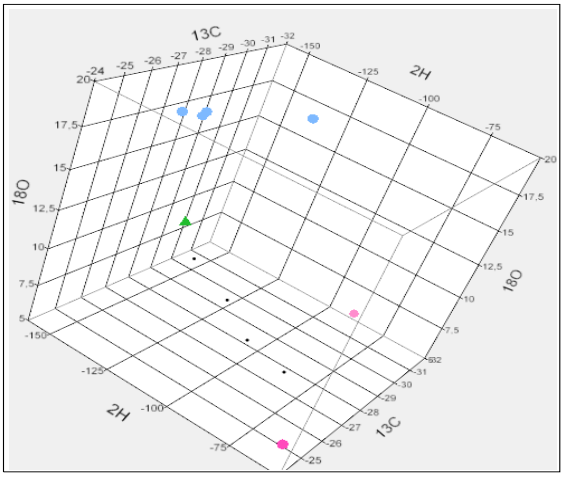


Figure 3: Three dimension representation of isotopic values of polyisoprene samples
Blue points: 100% Biobased rubber
Pink points: 0% Biobased rubber
Black points: Intermediary Biobased rubber

The multi isotopic representation shows distinction among the different origins. But the selection of samples is unfortunately too limited. Further investigations are required in order to validate isotopic approach.

5.3 Biofuels – Bio-solvents

The sustainable production of biofuels remains one of the major issues of the upcoming years. Two of the most common biofuels are bioethanol and biodiesel. Bioethanol is an alcohol resulting from the fermentation of the sugars in plants, such as corn or sugarcane, or from the cellulose in non-food sources, such as trees and grasses. Both types of bioethanol can be used directly as fuel for vehicles but they are typically mixed with gasoline, a fossil fuel, to increase the fuel's octane and improve vehicle emissions.

Ethanol which is widely used has been elaborated through fermentation process with many plant origins (sugar beet, sugar cane, corn, potatoes, barley, rice…. Ishida-Fuji et al (25) presented a multi- isotopic approach ($\delta^{13}C$, $\delta^{2}H$ and $\delta^{18}O$) elaborated on various ethanol samples. The different origins studied (corn, sugarcane, wheat, tapioca and synthetic) have been discriminated. However in order to determine Biobased amount of mixture, more investigations must be done mostly to determine uncertainty of the isotopic methodology.

Butanol and Propanol

Among the number of most desirable molecules to be produced, butanol and isobutanol deserve a prominent place. They have superior liquid-fuel features in respect to ethanol. Particularly, butanol has similar properties to gasoline (close energy density) and thus has the potential to be used as a substitute for gasoline in currently running engines.

Table 8: Measurements of butanol and propanol from various origins

References	#	Origins	plant	Biobased	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
Butanol	1	C4 plant	maize	100%	-14.71	-286	5
	2	C4 plant	maize	100%	-13.68	-269	6.5
	3	C4 plant	maize	100%	-14.26	-298	6.1
	4	C4 plant	maize	100%	-14.43	-287	
	5	C4 plant	maize	?	-13.45	-221	10.9
	6	C3 plant	manioc	100%	-30.77	-298	9
	7	C3 plant			-31.6	-278	8.8
	8	C3 plant			-29.62	-301	5.5
	9	synthesis		0%	-30.63	-148	19
Propanol	10	C4 plant		100%	-15.86	-407	16
	11	C4 plant		100%	-10.20	-403	9
	12	C4 plant		100%	-12.90	-409	7

The multi isotopic approach brings opportunities for the assessment of the origin of butanol and propanol especially $\delta^{2}H$ values. However one butanol sample (number 5) correlated to a $\delta^{13}C$ typical C4 fingerprint presents problematic $\delta^{2}H$ and $\delta^{18}O$ in comparison with the other samples. Further investigations must be undertaken especially to determine the isotopic limits of biobased field.

Farnesane

Farnesane is industrially produced by Amyris Company from sugarcane origin (see process in chapter 4.6.1). Farnesane is made through a fermentation process via an intermediary hydrocarbon: Farnesene. Farnesene is converted into Farnesane by hydrogenation. Farnesane burns cleaner than conventional jet fuel and could easily be added to the jet fuel. Farnesane normally will have the typical fingerprint $\delta^{13}C$ C4 plant but no sample have been analysed in this project.

5.4 Biosurfactants

Gaubert et al (26) elaborated a wide multi isotopic approach to discriminate plant and petroleum derived surfactants. Various origins of surfactants have been investigated.

Table 9: Measurements of various surfactants

reference	origin	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
Cationic surfactant				
Esterquat	Biobased	-30.0	-181	9.6
Quaternary ammonium	Hybrid	-29.0	-132	0.8
Quaternary ammonium	Synthetic	-30.4	-177	7.7
Amphoteric surfactants				
Cocamido propyl betaine	Biobased	-31.8	-153	20.9
Cocamido propyl betaine	Biobased	-31.4	-198	21.2
Betaine	Biobased	-30.4	-154	15.4
Betaine	Biobased	-30.9	-146	16.2
Sodium cocoamphoacetate	Biobased	-29.0	-125	14.6
Disodium cocyl glutamate	Biobased	-29.0	-152	18.9
Anionic surfactants				
Sodium lauryl ether sulphate	Hybrid	-29.4	-178	15.3
Sodium lauryl ether sulphate	Synthetic	-29.3	-185	11.7
Sodium lauryl ether sulphate	Synthetic	-28.5	-65	7.5
Sodium lauryl ether sulphate	Synthetic	-28.7	-61	6.5
Sodium lauryl ether sulphate	Synthetic	-28.4	-155	8.9
Sodium lauryl ether sulphate	Biobased	-28.0	-233	19.0
Sodium lauryl ether sulphate	Biobased	-28.0	-223	21.6
Sodium lauryl ether sulphate	Hybrid	-28.2	-200	12.9
Ammonium lauryl sulphate	Biobased	-28.7	-165	17.5
Non-ionic surfactants				
Fatty alcohol poly-ethoxylated	Synthetic	-30.8	-83	-4.8
Fatty alcohol poly-ethoxylated	Synthetic	-31.2	-73	2.6
Fatty alcohol poly-ethoxylated	Synthetic	-28.7	-175	2.6
Fatty alcohol poly-ethoxylated	Synthetic	-29.4	-129	-3.7
Alkyl poly-glucoside	Biobased	-22.9	-135	22.1
Alkyl poly-glucoside	Biobased	-21.8	-116	24.2
Alkyl poly-glucoside	Biobased	-23.6	-122	21.1
Alkyl poly-glucoside	Biobased	-23.8	-135	28.2
Poly-sorbate	Synthetic	-28.3	-103	2.3
Alkyl poly-glucoside	Biobased	-25.3	-119	16.1
Alkyl poly-glucoside	Biobased	-23.5	-156	22.7
Alkyl poly-glucoside	Biobased	-25.3	-133	28.6

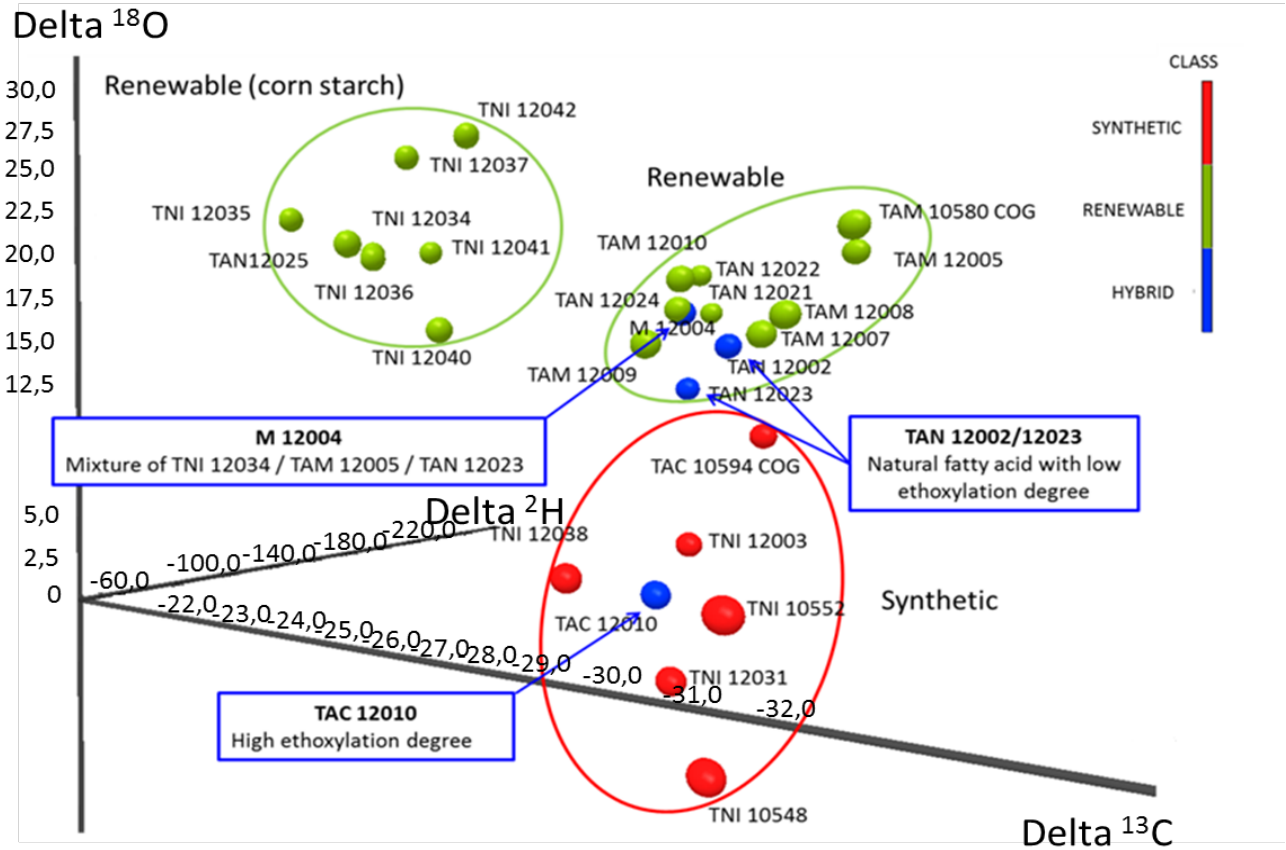


Figure 4: 3D representation of isotopic values of surfactants (Gaubert et al.)

Gaubert probably presented the first wide study carried out on different chemical families using multi isotopic approach.
This three dimensional figure discriminates several biobased and non biobased surfactants.
However this field encompasses a huge amount of different molecules and other investigation must be carried out to be sure of isotopic methodology in the goal of Biobased assessment.

5.5 Other bio products

5.5.1 Biocosmetics

Squalene and squalane

Squalane is a fully saturated hydrocarbon widely used in cosmetic for many years. It is excellent emollient oil, appreciated for the exceptional silky touch, hydrate epidermis.
Squalane is obtained on industrial scale by catalysis of the naturally occurring molecules: squalene which has 2 main origins: animal origin (shark liver oil) and plant origin (olive oil).
Squalane is considered as biobased by the cosmetic industry, but the animal component origin is banned and shark couldn't be assimilated to a renewable material.

In 2010 in the cosmetic fields, the knowledge of the origin of squalene and squalane among the two main origins animal (shark liver oil) and vegetal (olive) was demonstrated using $^{13}C/^{12}C$ stable isotope directly on pure squalene and pure squalane (27). This approach permitted to eliminate the addition of animal raw material incorporated in the commercial cosmetic cream. A second survey elaborated in 2011 for the determination of the ^{13}C of squalane extracted from on commercial cosmetic creams and analysed using GC-C-IRMS permitted to establish a new approach for cosmetic commercial products (28).

Table 10: Measurements of squalene and squalane samples

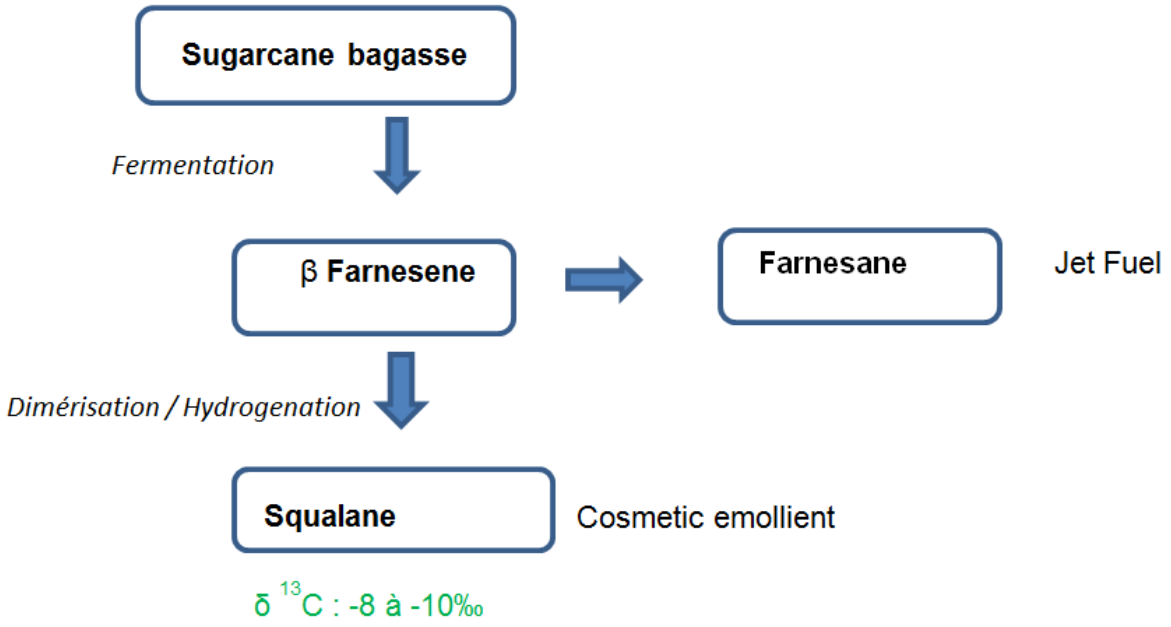
Reference	**Origin**	**$\delta^{13}C$ (‰)**
Squalene	Olive (plant)	-28.2
Squalene	shark (animal)	-20.4
Squalane	Olive (plant)	-28.1
Squalane	shark (animal)	-20.1

Isotopic method is capable to assess the origin of biobased squalane origin with an uncertainty lower than 5% (*Direct Automation* **OPEN BIO Deliverable N°3.7).**
Since 2010 the method has been regularly carried out for checking samples for the authentication of plant origin or to determine with a relative good efficiency the level of adulteration.

A recent process elaborated to produce squalane from cane sugar origin has been established from fermentation of bagasse to create an intermediary component β Farnesene prior to obtain squalane (Amyris company process).

$\delta^{13}C$ of sugarcane origin Squalane ranges from -10 to -8‰ in accordance to the $\delta^{13}C$ C4 fingerprint. Isotope method is able to asses this origin with about 10% of uncertainty and is currently used to validate origin of sample for commercial transactions.

If suppliers want to sell mixtures of 2 plant origin squalane (sugarcane and olive) the $\delta^{13}C$ obtained would be close to the area of shark squalane isotopic values, and the isotopic method would be totally inappropriate.
But currently industrials are sensitive to this problem and keep only one origin in theirs commercial batches to be sure to validate their feedstocks or commercial products for customers and consumers.



Glycerol

Glycerol or glycerin is generally obtained from plant and animals sources from triglycerides molecules. The hydrolysis of triglycerides produces glycerol and fatty acids. The typical origins are soybeans and palms, and tallow from various animals (beef, pork…).

Isotopic investigations have been previously undertaken on glycerol origin assessment by Webe et al (29): ^{13}C and ^{18}O isotopic ratios enable to determine several origins of glycerol. From vegetal origin (C4 plant C3 plant) regarding animal origin (cattle fat, butter, cheese) which depends of the diet (30).

Table 11: isotopic values of glycerol from various origins (Webe data)

Glycerol	Origins	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
	Corn	From -13.9 to -15.6	From 25.8 to 27.6
	Mustard	-30.5	
	sunflower	From -30.0 to -30.7	23.5
	soy	From -30.4 to -32.6	From 22.4 to 24.0
	Grape seed	From -28.6 to -31.6	
	Thistle	-31.6	
	Peanut	-29.1	
	Walnut	From -28.8 to -30.0	24.9
	Cotton	-29.3	
	Castor oil	From -29.5 to -31.4	
	Sesame	-29.5	22.6
	Wheat	-30.2	25.3
	Coconut	-27.6	
	Almond	-30.8	21.1

Glycerol	Origins	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
	Olive oil (Greece, Tunisia, Italy)	From -28.6 to -31.6	From 20.1 to 23.1
	Linen	-31.1	23.3
	Red wine	-31.6	
	White wine	-28.6	
	Fat of cattle	-22.6	15.1
	Bacon	-20.2	
	Pork fat	From -25 to -26	
	Lard	From -15.4 to -20.2	16.6
	Goat fat	-26.2	
	Butter	-23.5	17.2
	Fish oil	-21.4	23.5

The multi isotopic approach ^{13}C and ^{18}O couldn't distinguish every origins of glycerol but is able to separate the animal origins from plant origin. Limits on the different areas must be more investigated to be clearly known.

Pentylene glycol

Pentylene glycol is widely used as moisturizing agent. It is also used as preservative components against bacteria, molds in cosmetic formulations. Currently biobased pentylene glycol has been industrially produced from sugarcane bagasse or corn cobs.

Table 12: Measurements of Pentylene glycol from various origins

	Biobased %	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
Pentylene glycol	0	-35.26	-162	-14.3
	0	-35.65		
	100	-12.10		
	100	-12.89	-115	8.8
	100	-13.22		
	100	-12.18		
	100	-12.90		

$\delta^{13}C$ obtained on both C4 plant origin (corn or/and sugarcane) present small internal variations : Average $\delta^{13}C$ (C4 plant): -12.65 SD +/-0.5 ‰

$\delta^{13}C$ of synthesis origin is quite distant to the $\delta^{13}C$ plant origin (gap between both origins 23 ‰).

Isotopic method could be a relevant method for Biobased assessment in this precise case.

5.5.2 Bio-Flavours-Foods

Tryptophan

Tryptophan is an amino acid used in the synthesis of bio protein. Tryptophan is usually synthesised by microorganisms. Several samples have been collected:

Table 13: Measurements of tryptophan samples from various origins

References	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
C3 plant	-26,52	-3,37
C3 plant	-26,45	-3,29
C3 plant	-26,42	-3,43
C3 plant	-26,21	-3,76
C4 plant	-10,58	-3,95
C4 plant	-9,65	-3,47
C4 plant	-10,35	-0,08
C4 plant	-10,54	-0,51
Unknown origin	-20,83	9,21
Unknown origin	-25,45	6,79

Results show a significant difference among plants origin. However due to the limited number of samples, further investigations must be carried out to confirm these results.

5.5.3 Bio-pesticides

Methyl salicylate

Methyl salicylate is the primary constituent in Oil of Wintergreen, a naturally fragrant oil. It may be characterized with the distinct odour and taste of wintergreen or gaultheria (2 plant origin). Methyl salicylate can also be derived synthetically, and this synthetic variety of methyl salicylate is considered to be structurally and functionally equivalent to the naturally occurring Oil of Wintergreen. Methyl salicylate is manufactured from the steam distillation of macerated leaves from the low growing plant, Gaultheria Procumbens or from the Betula Lenta (Sweet Birch). Isotopic investigations were firstly undertaken by Culp et al. (36).

Methyl salicylate is used as repellent on terrestrial and greenhouse food crops and as an insect repellent when incorporated into a coating on the internal and outer surfaces of cartons used to store consumer products like human and pet foods, animal feeds and non-food items such as clothing and textiles.

Methyl salicylate can be produced by esterifying salicylic acid with methanol. The results presented below complete the former data.

The multi- isotopic approach enables to clearly distinguish the origins. $\delta^{18}O$ values will be probably the better solution to determine the level of Biobased content in mixtures of origin.

Table 14: Measurements of methyl salicylate from various origins

Origins	Reference	Biobased %	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
synthesis	1	0	-27.28	-45	19
synthesis	2	0	-28.86	-40	20
synthesis	3	0	-28.95	83	20
Gaultheria	1	100	-36.95	-116	1
Gaultheria	2	100	-34.54	-130	-2
Gaultheria	3	100	-33.88	-124	0
Gaultheria	4	100	-33.45	-108	0
Gaultheria	5	100	-34.15	-125	-2
Birch	1	100	-34.36	-118	-1
Birch	2	100	-34.11	-123	-1
Birch	3	100	-33.58	-118	0

6 Monitoring industrial process approach

Isotopic methodology previously described is based on isotopic variations correlated to the origins. This implies to manage a database of all of the feedstock origin and synthesis products.

The aim of the "monitoring industrial process approach" is only to consider the reactants involved and their proper isotopic values obtained. This methodology could only operate if the components introduced in the industrial process would be always the same.

Two examples of applications have been presented: synthesis of isosorbide (biobased product) and synthesis of a specific plastic (non biobased product).

6.1 Synthesis of Isosorbide

Table 16: Synthesis of isosorbide and isotopic approach

Products	Chemical reaction	$\delta^{13}C$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)	Comments
Grain corn		-11.4	-7	27.4	
	Crushing				
Starch		-11.5	-3	-30	Physical treatment (few isotopic variations)
$+H_2O$	Enzymatic hydrolysis				
Maize hydrolysate		-11.3	10	26	Variation $\delta^{2}H$ and $\delta^{18}O$ – H_2O added
$+H_2$	hydrogenation				Sorbitol $\delta^{13}C$ corn: -11.2
	Addition of sorbitol (wheat)				Sorbitol $\delta^{13}C$ wheat: -26
Sorbitol syrup		-20	42	25	$\delta^{13}C$ middle (mixture of two origins)
	Dehydration ($-H_2O$)				
Isosorbide		-20	-72	33	Variation ^{2}H and ^{18}O
	Addition of 2 fatty acids				Fatty acids $\delta^{13}C$: -35 / $\delta^{2}H$: -300
Isosorbide diester		-29	-225	25	

Synthesis of isosorbide is a linear process from corn grain to isosorbide. The final product is made by addition of different reactions and reactants are added one after the other. Accord-

ing to the data given by the industrial, it is possible to verify at each step the good agreement of the biobased process. Isotopic measurements obtained are typically related to the components added or released.

In the case where Isosorbide synthesis is only carried out by starch C4 origins, isosorbide diester will have a $\delta^{13}C$ C4 fingerprint. But due to addition of various starch origins, $\delta^{13}C$ will have an intermediary value. For $\delta^{2}H$ and $\delta^{18}O$ the variation would be limited and correlated to the value of fatty acids added.

6.2 Synthesis of a specific plastic

In this industrial process, a plastic is made by the addition of 5 reactants. In the Process I, all of the reactants are non Biobased. The industrial elaborate a new process by replacing a reactant with a new biobased reactant.

Process 1:

Commercial Compound I (final product) = Thermoplastic resin + **Primary plasticizer I (~30% of the formulation)** + Secondary plasticizer + Mineral load + Premix.

Process 2:

Commercial Compound I (final product) = Thermoplastic resin + **Primary Plasticizer II bi-obased (~30% of the formulation)** + Secondary plasticizer + Mineral load + Premix.

Isotopic investigations are done on all reactants introduced. The knowledge of the amounts and parts of every reactant and their isotopic ratio permit to calculate a theoretical isotopic value expected for the commercial final compound. This value is registered as “calculated value” The measurement is also performed on the commercial component

Process I and II Approach $\delta^{13}C$ (‰)

Table 17: $\delta^{13}C$ measurements of various reactants (process 1 and 2) and final products

Thermoplastic resin	**Primary Plasticizer I**	Primary Plasticizer II	Secondary Plasticizer	Mineral Load	Premix	Final product
Process I						
-39.24	-28.36		-30.59	-1.85	-28.25	
						-33,76 (calculated)
						-33.66 (measured)
Process II						
-39.24		-28.97	-30.59	-1.85	-28.25	
						-34,22 (calculated)
						-33,91 (measured)

Process I and II Approach δ^2H (‰)

Table 18: δ^2H measurements of reactants (process 1 and 2) and final products

Thermoplastic resin	**Primary Plasticizer I**	Primary Plasticizer II	Secondary Plasticizer	Mineral Load	Premix	Final product
Process I						
2.30	-139		-168	-48	-143	
						-71 (calculated)
						-73 (measured)
Process II						
2.30		-211	-168	-48	-143	
						-105 (calculated)
						-104 (measured)

Remarks

- Calculated isotopic value of the final compound (determined by calculation of the isotopic values and the amount of every reactant) is quite close to the result directly measured on the final product due to the relative good precision of the method.
- The $\delta^{13}C$ values don't' show any significant differences. But δ^2H final product change from -73‰ (or -71‰) to -104‰ (or -105 ‰) due to the addition of an amount of biobased reactant.

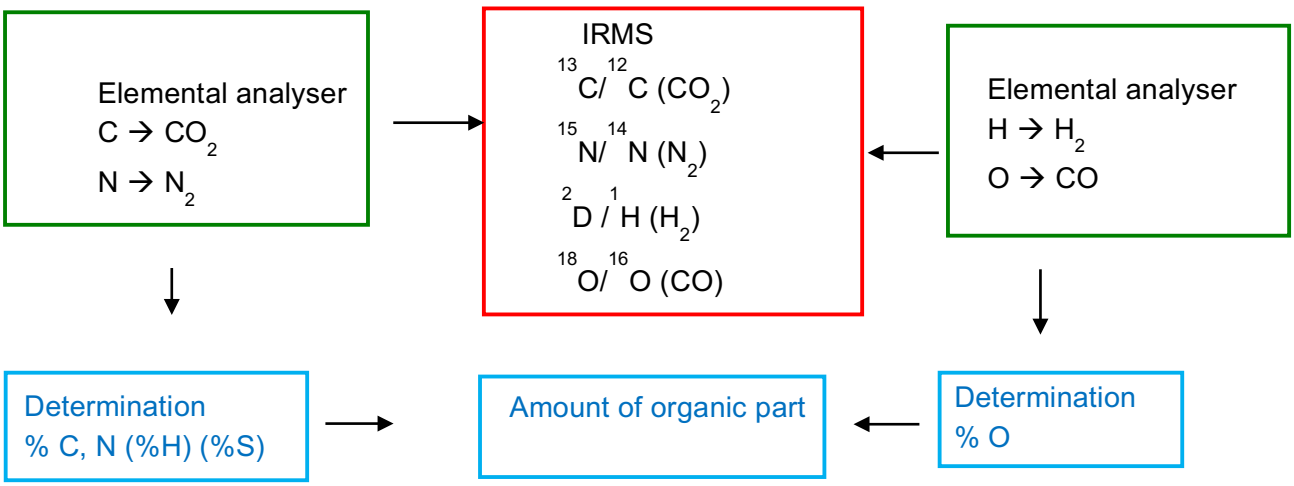
In conclusion, in some cases the biobased content on a final product can be directly linked to isotopic values and permit to the industrial to communicate on the biobased process.

7 Supplementary benefits

7.1 Technical impacts

7.1.1 Bulk Stable Isotope Analysis

As previously presented in the *Direct Automation* **OPEN BIO Deliverable N°3.7** organic stable isotope are performed on equipment encompassed an Elemental Analyzer connected to an Isotope Ratio Mass Spectrometer (EA-IRMS) for Bulk Stable Isotope Analysis.
Basically Elemental Analyzers are able to take into account the percentage of elemental analysis in the isotopic methodology.
For example the determination of% C and %N can be obtained in addition to $\delta^{13}C$ and $\delta^{15}N$ in the same analytical run.



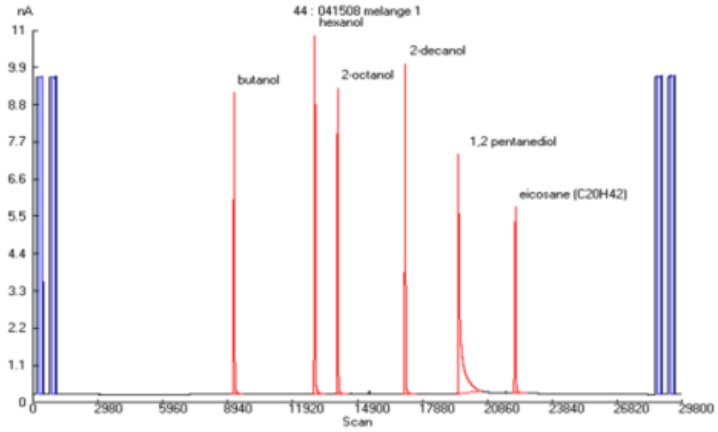
This implements present additional advantages:

If the isotopic approach is efficient in a specific field and combined with elemental analysis, the whole approach could be used to confirm a statement given by an industrial, similarly as the official method: pr EN 16785 -1 –Bio-based products – Bio-based content – Part 1*: Determination of the bio-based content using the radiocarbon analysis and elemental analysis.*

In another situation the determination of an element (N, S) could directly be connected with the abundance of a dedicated product, if this element is present in only one molecule.

7.1.2 Compound Specific Isotope Analysis

As previously presented in the *Direct Automation* **OPEN BIO Deliverable N°3.7,** gas chromatography-combustion/pyrolysis- isotope ratio mass spectrometry is capable to determine the origin a molecule in a mixture.



Molecule	origin	$\delta^{13}C$ (‰)	SD
butanol	fossil	-26,80	0,24
hexanol	fossil	-26,13	0,09
2-octanol	fossil	-28,76	0,13
2-decanol	fossil	-34,91	0,11
1,2 Pentanediol	**Biobased**	**-10,24**	**0,05**
eicosan	fossil	-32,78	0,07

In this example $\delta^{13}C$ allows to know the origin (biobased or not) and the amount of signal determines the part of each component in the mixture.

In complement

- GC-C/P-IRMS encompasses various sampling systems: liquid injection, gas injection, Head-Space Solid-Phase Micro-Extraction …
- GC-C/P-IRMS equipped with mass spectrometer is capable of determining the amount of the molecules in a mixture, traces or organic components revealing process or origin.
- A wide range of chromatic columns are available: for gases, enantiomeric applications: natural process give only one isomer where a racemic mixture is obtained in synthesis process.

7.1.3 Approach multi methods

Isotopic analysis also could be used associated with another analytical method to bring complete information. In the case of the assessment of ascorbic acid (Vitamin C) Albertino present a double investigation (IRMS and NMR) (37) to distinguish between the natural origin (Camu-camu, acerola) and the synthesis product.
Complementarity data obtained using a compound specific isotope analysis (CSIA) and position specific isotope analysis (PSIA) are relevant for the determination of origin of ethanol (38).

7.2 Sustainability criteria

The main advantage of isotopic methodology regarding ^{14}C method is probably due to the fact that isotopic could be able to link the results obtained to an origin of a plant or an animal. This information could be relevant in several fields.

7.2.1 Agricultural and social impacts

Any plant used as renewable raw-material for biobased industry could be also be feedstock for food industry and thus be in conflict: limitation of resources, increase of price, possibility of famine. Currently several materials, such as corn, sugars…are used in the two processes. In the future the knowledge of the originate material could have a huge impact for the development of a new biobased product.

7.2.2 Cosmetic issue

Cosmetic industry is determined to ban animal origin feedstock. The most important example is the emollient squalane widely used in commercial cosmetic products. The ^{14}C approach enables to distinguish animal or plant origin, and currently ^{13}C method is the only one capable to bring the information with good efficiency.

7.2.3 Religious impact

Several material based animal origins are incorporated in different processes. Glycerol is widely used in cosmetic, food, and pharmaceutical industry. For few religions swine is forbidden and as presented in this report any various origins for glycerol are available. The authentication of the animal origin could have a significant impact in its valorization.

8 Conclusion

Stable multi-isotopic determinations performed using IRMS are fully automated methods capable to create and monitor database for feedstocks and products. Since many years this approach has been undertaken in the frame of natural authenticity assessment.

In this report, different isotopic investigations are presented. They were carried out in different fields of the biobased industry: bio-plastics, bio-rubbers, bio-solvents bio-surfactants and on other specific bio products. From this first round of investigation we are concluding that the isotopic method can't be an overall relevant and applicable method for the direct determination of biobased content. The main drawbacks come from the isotopic variations of origins (bio-sources or chemical process) and the potential overlapping of both origins: biobased and non biobased.

However some results present several interesting opportunities, which should be further investigated:

a. Some biobased feedstocks are only coming from C4 plants origin. These components present a specific isotopic footprint ($\delta^{13}C$ close to -11‰). Few examples have been given in the fields of bioplastic, biosolvent or biocosmetic. The determination of the biobased content can be achieved either on pure materials (pentylene glycol) or on finish products (PET).
b. Components with few origins and little isotopic variations: in this report the best example is squalane which come from 3 main origins: shark ($\delta^{13}C$: -20‰), olive oil ($\delta^{13}C$: -28‰) or cane sugar ($\delta^{13}C$: -10‰). The differentiations between the origins are well established and the variations in each group are limited.
c. Multi isotopic approach: in some cases the determination of origins can be carried out using the three main isotopes ^{2}H, ^{13}C, and ^{18}O. Although the results presented in this report are not complete, the multi isotopic approach elaborated on polyisoprene permits to distinguish and determine the part of biobased product.
d. Products always made from the same raw materials (industrial process).
e. The principal advantage of this approach is that no database needs to be monitored. The biobased content of the product manufactured is clearly linked to the isotopic value and the input of raw material. The industrial process has to be stable (raw material origin and amount).

It has been evident that more investigations need to be undertaken. Lots of data need to be collected to determine limits, accuracy and isotopic variations. Moreover when created, the database will have to be regularly fed with isotopic values of the targeted molecules or targeted components.

Isotopic method permits also to link measurements to the sources of biomass (plant or animal species) and give supplementary data for sustainable criteria (social or economic is-

sues). These inputs will be useful and suitable to be integrated to the database developed under Open-Bio.

This report is delivered to the relevant standardization groups in Europe (under the European Standardization body, CEN). They will be asked to use it to their benefit, preferably to check if it can become a CEN Technical Report.

9 Acknowledgements

The authors would like to thank the standardization groups, institutional and economic supports, industrial partners, academic laboratories and research centers for providing advices, knowledge and samples regarding the biobased investigations carried out in this project.

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