Analytical Methods

# Direct Determination of Oxygen 18 Stable Isotope Ratio of In Situ Water Contained in Pasty Matrices.



| | |
|---|---|
| Journal: | *Analytical Methods* |
| Manuscript ID: | AY-ART-01-2015-000038.R1 |
| Article Type: | Paper |
| Date Submitted by the Author: | 10-Feb-2015 |
| Complete List of Authors: | Guyon, Francois; Service Commun des Laboratoires,<br>Gaillard, Laetitia; service commun des laboratoires,<br>Sabathié, Nathalie; service commun des laboratoires,<br>salagoity, Marie-Helene; SCL, |
| | |

Analytical Methods

RSCPublishing

PAPER

# Direct Determination of Oxygen 18 Stable Isotope Ratio of *In Situ* Water Contained in Pasty Matrices.

Cite this: DOI: 10.1039/xoxxoooooox

Francois Guyon,* Laetitia Gaillard, Nathalie Sabathié and Marie-Hélène Salagoity

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Traditionally, oxygen 18 stable isotope measurement of water using $CO_2$ equilibration system is performed on a liquid solution that could be a limitation for various applications. This paper demonstrates that $\delta^{18}O$ measurements of *in situ* water contained in pasty matrices (dried fruits) can be performed directly on the matrix without previous water extraction using a commercial system of isotope ratio mass spectrometry (IRMS) with similar equilibration conditions used for liquids (6 h at 40 °C). The main difference is that instead of pouring a liquid in the equilibration vial, pasty matrix is spread on the vial walls using spatula. Water standard analysis is used for equilibration gas calibration. The increase in the amount of pasty matrix in the vial leads to a rise in $\delta^{18}O$ values, until a plateau indicating optimum sample mass that is found to be 200 mg for a paste containing 35% of humidity. The results are obtained with a repeatability of 0.27 ‰. A set of 37 prunes is used to illustrate the method application for the discrimination between 2 different kinds of product: $\delta^{18}O$ value allows a clear discrimination between the prunes re-hydrated ($\delta^{18}O_{VSMOW}$ range: -0.78 to +3.77 ‰) and not re-hydrated ($\delta^{18}O_{VSMOW}$ range: +8.97 to 14.86 ‰).

## 1. Introduction

Water oxygen 18 stable isotope ratio ($\delta^{18}O$) is determined using stable isotope mass spectrometry (IRMS) after equilibration between oxygen atoms of $H_2O$ molecules of a liquid solution and oxygen atoms of added $CO_2$ equilibration gas, according to:

$$C^{16}O^{16}O_{(g)} + H_2{}^{18}O_{(l)} \iff C^{16}O^{18}O_{(g)} + H_2{}^{16}O_{(l)}.$$

This equilibration step requires an excess of water molecules compared to $CO_2$ gas to ensure a complete transfer of $^{18}O$ atoms from the water to $CO_2$. Isotopic variations are reported as delta values ($\delta$), expressed in per mil (‰), providing the deviation of the ratio of rare isotope to the most abundant isotope, relative to the internationally accepted standard V-SMOW (Vienna Standard Ocean Mean Water). Therefore, added $CO_2$ gas is calibrated against certified material, V-SMOW-2 and BCR660 to quantify the relative isotopic variation. The knowledge of $\delta^{18}O$ value in water is an important information for geographical localization [**1**]. As a result, this parameter is widely used for environmental studies,[**2**] forensic applications [**3, 4**], and beverage adulterations and authenticity controls [**5-7**]. For beverages, $\delta^{18}O$ value can be used to investigate water addition, geographical origin and, for wines, vintage authentication [**8**]. All these applications are exclusively performed on a liquid solution i.e. on the beverage or on the an aqueous solution extracted from a particular matrix. Despite its critical importance, this technique presents two main limitations: an exclusive application to liquid matrices and a relative high amount of liquid (200 µL) usually recommended for analysis by the manufacturers [**9**]. Therefore for routine analysis, these limitations exclude compounds from which, water extraction is extremely difficult and require specialized equipment (dried tomato paste as an example [**10, 11**]). For some food products, like dried fruits, the commercial price is strongly related to the preparation step, therefore it was important to develop a new water analysis method allowing the distinction between the different kinds of products.

In this study, we report for the first time, $\delta^{18}O$ measurement performed directly on a pasty matrix without any water extraction and using automated $CO_2$ gas equilibrium process. In order to develop this new application, prune was selected as a perfect working sample. It is easily available at the grocery store and conditioned under two different kinds of products: “regular” prunes are produced through a steam rehydration of dried plums to reach a final humidity of 35 % while “mi-cuit” prunes are produced through a plums dehydration to 35% of humidity. Therefore, this process difference, i.e. addition of exogenous water, is expected to modify $\delta^{18}O$ ratios. A set of 16 different prunes have been used.

The first part of this paper is dedicated to the optimization of analytical conditions, i.e. defined the amount of paste required to be spread out the vial walls to obtain an excess of water molecules in contact with the equilibrium $CO_2$ gas. The second part is an illustration of the possible application of this new method to the

Service Commun des Laboratoires; 3 Avenue du Docteur Albert Schweitzer; F-33608 Pessac, France.
**Phone** : 33-(0)5.56.84.24.37, **Fax**: 33-(0)5.56.84.66.71
**E-mail:** labo33@scl.finances.gouv.fr ; guyonfra@gmail.com

discrimination between the two types of commercialized prunes using $\delta^{18}O$ ratios.

## 2. Experimental

### 2.1 Reagents

Carrier gas (Helium, He, 5.6 grade), reference gas (carbon dioxide, $CO_2$, 4.5 grade) and equilibration gas $CO_2$/He (5%) are provided by Linde (Bassens, Bordeaux, France). Oxygen 18 standards used are BCR 660 (Institue for Reference Materials, Geel, Belgium) and V-SMOW-2 (IAEA, Vienna, Austria).

### 2.2 Samples

All prune samples were commercially available. Before analysis, all the samples were stoned and crushed using an analytical mill to obtain a homogeneous paste. Seventeen of the 37 samples are regular prunes i.e. dehydrated and subsequently re-hydrated prior commercialization. The other 20 samples correspond to "mi-cuit" prunes: plums dehydrated but not submitted to re-hydration.

### 2.3 Samples analysis

For each sample, the paste is manually spread out the LabCo Exetainer™ (Labco limited, UK) vial wall (characteristics: high: 5.0 cm; diameter: 0.9 cm) with a spatula with the objective of making a thin layer by distributing the paste on the maximum space available. Then, the deposed amount of product is controlled by weight and the vial sealed using a screw cap with a pierceable rubber septum. For each samples, three vials are prepared and all the isotope ratios given in the following correspond to the average value of the three measurements. The vials are then placed in a dry thermostated system (40 °C). After a helium purge, calibrated equilibrium gas $CO_2$/He is automatically injected in each vial and left during 6 hours for the equilibration step [**9, 12-13**]; then, the gas is collected and transferred for isotopic analysis to the mass spectrometer. Experimental condition set up was performed using a "mi-cuit" prune at different masses in the range 6-470 mg; after spreading the prune on the vial wall and weighting the amount of paste (approximately 3 min by samples), the vial is sealed to prevent from any evaporation. Then the series (samples + water standards) is analysed overnight.

### 2.4 Apparatus

Experiments were performed on a commercial Elementar system (MultiFlow + IsoPrime IRMS). The MultiFlow system consist in a 60 positions temperature regulated (40 °C) tray allowing the fully automated preparation system for online headspace sampling followed by IRMS analysis. It performs automatically the following steps using a triple holes needle: helium purge, 5% $CO_2$/He injection, extraction of the gas mixture in the headspace of the sample vial after equilibration step. The volume of gas of interest, sent to the gas chromatography column (Hayesep Q 60-80 mesh, 2.5m, ID: 2.0 mm, T 80 °C), is controlled by an injection loop of 50 µL. Then the gas is collected by the IRMS at the outlet of a Nafion® membrane, used for gas drying, via an open split system [**14**]. The system set up is the following: helium flux 15 ml $min^{-1}$, Nafion membrane external He flux 30mL $min^{-1}$, IRMS current trap 150 nA.

### 2.5 Isotope ratio computation

Measured masses are *m/z* 44 and 46 corresponding to $^{12}C^{16}O_2$ and $^{12}C^{16}O^{18}O$. In the following, all oxygen 18 sable isotope ratios, $\delta^{18}O$, are expressed against the international standard V-SMOW (Vienna-Standard Mean Ocean Water) according to

$$\delta^{18}O(‰) = ([(^{18}O/^{16}O)_{sample} / (^{18}O/^{16}O)_{standard})]-1)x1000.$$

## 3. Results and discussion

### 3.1 Isotope ratio quantification method

The first step of this study was focused on the determination of optimal conditions to obtained accurate and reproducible results. Effectively, the matrix amount (liquid or solid - this study) is critical with the equilibrium step: oxygen atoms of the $CO_2$ gas brought (via the equilibrium mixture $CO_2$/He, 5%) in the vial will exchange with oxygen atoms of *in situ* water present in the matrix. This exchange will reach an equilibrium after 6 H at 40 °C [**9, 12-13**]. Nevertheless, if the sample amount is too low, the equilibrium will not be characteristic of the oxygen 18 isotopic composition of the *in situ* water in the pasty matrix. Thus, the measured $\delta^{18}O$ value will partially correspond to oxygen 18 contained in the exogenous $CO_2$ gas.

Therefore, in order to fix the optimal amount of pasty matrix necessary to reach a $\delta^{18}O$ value reproducible and fully related to the matrix isotope composition, the quantity of "mi-cuit" prune paste spread out the vial walls was increased from 6 to 470 mg (**Fig. 1**). Results plotted in **figure 1** show an increase in the $\delta^{18}O$ values indicating the growing importance of isotopic exchange with water contained in the fruit. The $\delta^{18}O$ value is increasing as far as the critical mass is not reached, then a plateau is observed. The zero point corresponds to the measurement performed on a vial that does not contain prune paste and only filled with $CO_2$/He gas. Thus the $\delta^{18}O$ value of the equilibrium $CO_2$ gas is found to be -12.77 ‰. The measured values increase up to + 12.13 ‰ for a deposit of 470 mg of prune paste on the vial walls. From line shape drawn in figure 1, the

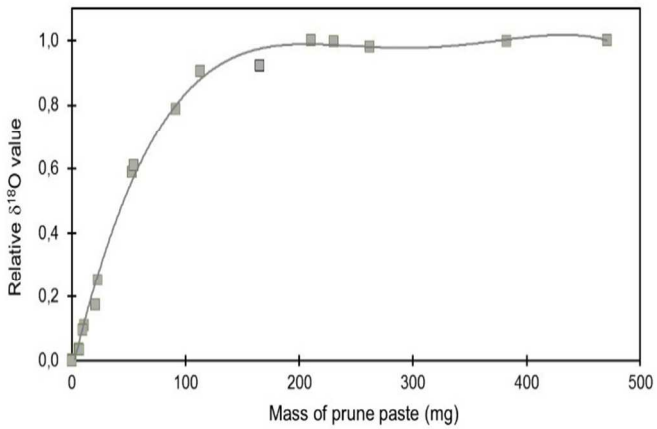

**Fig. 1** Effect of prune paste weight on the relative intensity of the relative $\delta^{18}O$ value computed according to $R=(\delta^{18}O_m-\delta^{18}O_i)/(\delta^{18}O_f-\delta^{18}O_i)$ were $\delta^{18}O_m$ corresponds to the isotopic value measured at a specific weight, $\delta^{18}O_f$ , the final isotope value (+12.13 ‰ for m=470 mg, in this example) and $\delta^{18}O_i$, isotope value of equilibration gas (-12.77 ‰).

optimal mass is estimate to be 200 mg for a prune paste containing 35 % of humidity.

As a first observation, it appears that, for a similar amount of the same matrix, it is better to spread it, at the maximum, out of the vial walls than to make a product pack. A discrepancy of -4 ‰ in $\delta^{18}O$ ratio is observed between $\delta^{18}O$ ratio of product layer and the ones of the same product but settled as a pack in the vial. A first assumption can be advanced to explain this phenomena: in a product layer, *in situ* water is more available (ie. proportionally a higher water volume) for equilibration with $CO_2$ gas. As a result, for the paste pack, the measured $\delta^{18}O$ value was not representative of the *in situ* water. Future studies will focus on confirming this hypothesis.
A set of nine different prunes was analysed in triplicate and for each sample, one of the three preparations was performed by a different laboratory assistant; the results are listed in **table 1**. These data allowed the computation of the method repeatability that is found to be equal to 0.27 ‰, value in the same order of magnitude as other methods in food product area. [**15**] This value demonstrates the good repeatability of the measurements and the possible application of this method in routine analysis for food control process, as an example.

**Table 1**: Reproducibility study of oxygen 18 isotope ratio measurements (in ‰ vs V-SMOW) on 9 different prunes analyzed in triplicate and the standard deviation (σ, ‰) for each set of measurements. Experimental condition: 200 mg of prune spread on the vial walls.

| Sample | $\delta^{18}O$ | $\delta^{18}O$ | $\delta^{18}O$ | σ |
|---|---|---|---|---|
| 1 | 14.2 | 14.7 | 15.0 | 0.40 |
| 2 | 12.9 | 13.1 | 12.8 | 0.15 |
| 3 | 5.8 | 6.2 | 5.4 | 0.40 |
| 4 | 14.2 | 14.7 | 14.4 | 0.25 |
| 5 | 7.2 | 6.8 | 7.1 | 0.21 |
| 6 | 1.7 | 1.5 | 1.7 | 0.12 |
| 7 | 0.3 | 0.4 | 0.7 | 0.21 |
| 8 | 12.8 | 12.9 | 12.9 | 0.06 |
| 9 | 5.8 | 6.2 | 5.4 | 0.40 |

### 3.2 Application to prune matrices

The natural distillation of water under the sun systematically creates an isotope fractionation as the "light" molecules of water ($H_2{}^{16}O$) evaporate first and, as a result, a concentration of "heavy" water ($H_2{}^{18}O$) is observable in the remaining water, characterized by a $\delta^{18}O$ value increase [**16**]. Similar phenomenon should happen with fruits during their drying step, i.e. an enrichment of oxygen 18 in water molecules remaining in the fruits must be observed. Moreover, re-hydration of dried fruit corresponds to an addition of water with much lower concentration in oxygen 18, therefore, a discrepancy in $\delta^{18}O$ ratio must be detected by IRMS. This method was applied to commercialized prunes in order to confirm these suppositions as well as to envisage this new analytical technique as a potential tool for the verification of prune label allegations. Effectively, two types of prune are commercialized: a "mi-cuit" prunes, resulting only from the dehydration of plums that contain approximately 82% water to 35% humidity, and regular prunes, resulting from plums dehydration to 20-22 % humidity followed re-hydration with steam to 35% humidity.

Thirty-seven samples, bought at the grocery store, were analysed. Among them, 20 samples were labelled as "mi-cuit" prunes and 17 as "regular" prunes. The samples were firstly manually stoned then crushed with an analytical mill to obtain a homogeneous paste. Then, each sample was analysed in duplicate according to the described experimental protocol. The results plotted in **figure 2** are the average $\delta^{18}O$ measurements of each prune. Two distinguishable clouds of points are observable. One set of $\delta^{18}O$ values is in the range +8.97 to +14.86 ‰ corresponding to the "mi-cuit" prunes while re-hydrated prunes shape a cloud of $\delta^{18}O$ values in the range -0.78 to +3.77 ‰.

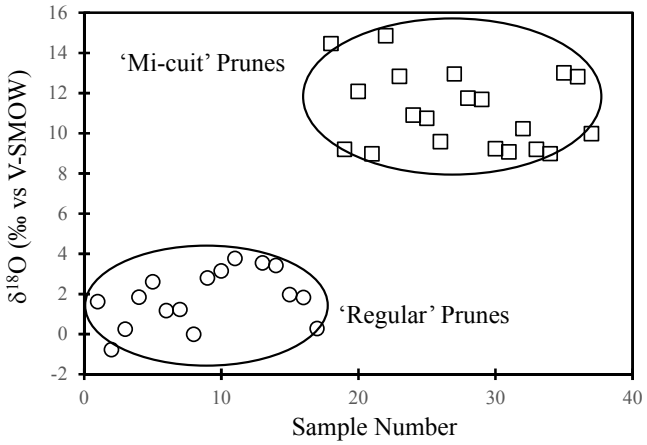


**Fig. 2**: Differences in oxygen 18 isotope ratio between commercial 'regular' and 'mi-cuit' prunes.

This discrepancy was a results of water added to regular prunes as tap waters usually used for re-hydration present $\delta^{18}O$ values in the range -10.0 to -8.0 ‰. **[6, 15]** These results confirm the oxygen 18 fractionation expectation during plum drying step, i.e. high $^{18}O/^{16}O$ ratio values for dried fruits and low values for re-hydrated fruits. The smallest difference between this two clouds is 5.0 ‰, and the difference between the two clouds data can be considered to be extremely statistically significant ($P<0.001$). Therefore, $\delta^{18}O$ ratio is a discriminant parameter to characterize these two types of prunes. Finally, the plot on **figure 2** confirms the good labelling of the purchased prunes.

A possible concern for the food controllers may be the detection of dry fruit mixtures. Two set of 'regular' (RP) and 'mi-cuit' (MCP) prunes were used to prepare various mixtures RP/MCP (**Fig. 3**). The initial $\delta^{18}O$ ratio of MCP was 10.23 and 11.82 ‰ and the RP $\delta^{18}O$ ratios of added RP was 2.8 and 3.44 ‰, respectively. Mixtures RP/MCP were realized by grinding, in an analytical mill, the two types of prune in various proportions. Once homogenized, 200 mg of paste was spread out the vial walls and analysed. The results, presented in **figure 3**, show a discrepancy in the $\delta^{18}O$ ratio related to the increasing percentage of RP in the mixture. Therefore,

it seems possible to detect fraudulent mixture in a MCP commercial bag. A roughly estimation of the detection limit, considering the lowest value of MCP $\delta^{18}O$ ratios, gives a 30% using a bulk mixture. The detection can be improved by the individual IRMS analysis of each prune in the commercial bag.

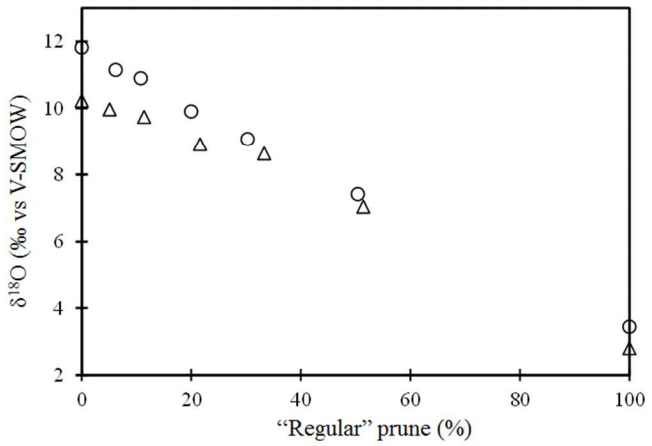


**Fig. 3**: Variation of oxygen 18 isotope ratio as a function of the mixture 'regular' prune (RP) / 'mi-cuit' prune (MCP). Experimental conditions: 200 mg of mixture paste spread on the vial walls; initial prunes characteristics: ○ (RP= 2.8 ‰, MCP= 10.23 ‰), △ (RP = 3.44 ‰, MCP= 11.82 ‰).

## 4. Conclusions

This study demonstrates, for the first time, the possible quantification of oxygen 18 ratios of *in situ* water contained in a pasty matrix without any water extraction step. This new development in IRMS applications is easy to set up and provides a good reproducibility in the results. The optimum amount of product needed to be spread out the vial walls is found to be 200 mg for a dried fruit that contains around 35 % of humidity. This method was used to quantify $\delta^{18}O$ ratios of *in situ* water contained in two different kind of prunes commercially available. A significant difference of at least 5.0 ‰ in $\delta^{18}O$ ratios between "mi-cuit" and "regular" prune is observed. This difference can be potentially attributed to re-hydration evidence of regular prunes. Therefore, this technique can be applied to verify the authenticity of prunes labels.

Beside the fact that this new development opens a new possibilities in food control authenticity and of dried fruits in particular (dates, apricots, dried figs, tomato pastes, etc.) for product regulation compliance, this study could also be a starting point of fundamental research to understand the equilibrium process with encapsulated water. Moreover, this experimental procedure could find some applications in areas where the analysis of small amounts of humid matrix is be useful like for forensic applications (small pieces of flesh, etc.), and/or in the industry for internal quality control purposes.

## Acknowledgements

The authors gratefully acknowledge the Bureau Interprofessionnel du Pruneau (B.I.P.) for its financial support.

## References

1 H. Craig, *Science* 1961, **133**, 1702.
2 P. Ghosh, WA. Bran, Inter. *J. Mass Spectrom*. 2008, **228**, 1.
3 JR. Ehleringer, TE. Cerlinf, JB. West. Forensic analysis on the cutting edge: new method for trace evidence analysis. Editor: RD. Blackledge, John Whiley & Sons, Inc. **2007**.
4 W. Meier-Augenstein. Stable Isotope Forensics: An Introduction to the Forensic application of Stable Isotope Analysis. Ed. John Wiley & Sons, **2011**.
5 E. Jamin, R. Guérin, M. Rétif, M. Lees, GJ. Martin. *J. Agric. Food Chem*. 2003, **51**, 5202.
6 F. Guyon, Ch. Douet, S. Colas, MH. Salagoity, B. Medina, *J. Agric. Food Chem*. 2006, **54**, 9918.
7 B. Medina, MH. Salagoity, F. Guyon, P. Hubert, F. Guillaume. New analytical approaches for verifying the origin of food. Ed. Brereton P., Woodhead publishing limited, **2013**
8 F. Guyon, L. Gaillard, N. Sabathié, B. Medina, F. Guillaume, MH. Salagoity, *Annal. Fals. Exp. Chim. & Toxicol*. 2014, 981, 33.
9 Isoprime Multiflow User Guide, p 35, http://www.isoprime.co.uk.
10 L Bontempo, F. Camin, L. Manzocco, G. Nicolini, R. Wehrens, L. Ziller, R. Larcher, *Rapid Commun. Mass Spectrom*. 2011, **25**, 899-909.
11 L. Bontempo, FA. Ceppa, M. Perini, A. Tonon, G. Gagliano, RM. Marianella, M. Marega, A. Trifiro, F. Camin, *Food Control* 2014, **35**, 413-418.
12 Resolution OIV/OENO 353/2009. www.oiv.int.
13 D. Mattey, D. Lowry, J. Duffet, R. Fisher, E. Hodge, S. Frisia, *Earth & Planetary Sci. Lett.* 2008, **269**, 80-95.
14 L. Gaillard, F. Guyon, MH. Salagoity, B. Médina, *Food Chem* 2013, **141**, 2103-2107.
15 New approaches for stable isotope ratio measurements, IAEA-TECDOC-1247, **2001**.
16 Tchekez G. (2010) in "Isotopie biologique", Editions Tech & Doc, Lavoisier