IsoCamp
SIRFER @ Utah
2017
stableisotopes.utah.edu

Stable isotopes and food

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IsoForensics

There are three basic food types or food divisions in your* diet

*Note: I'm assuming "you" are an omnivore!

Animal-based food items

Plant-based food items

Beverages: Natural (bottled water, milk, juice)

Manufactured (beer, wine, soda)

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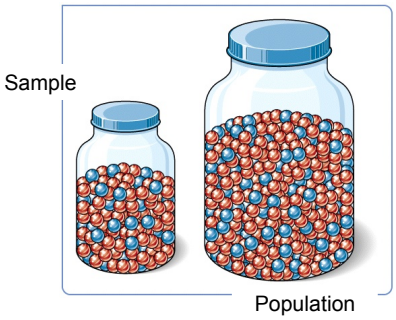
Stable isotope analysis of food often involves sampling

Why do we sample?

To get inferences applicable to the whole (food item, population, universe!) with minimum resources.

However, if you want to apply what you observe for a sample to make inferences about a population…



… you need to examine a representative sample.

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How did your (tasty) sample compare to the M&M's population?

13%
14%
13%
24%
20%
16%

(Data from 2008)

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EXAMPLE: Sampling prawns (or alternatively, shrimp)

Population A Population B

What is a representative sample?

In this example –

There are 70 ways to sample 4 prawns; only 24 samples will be representative.

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What considerations should you make while sampling?

1. Is your sample homogeneous at the scale of analysis (i.e., smallest amount tested)?
2. If not, how can you make it homogeneous?
   - Isolate specific components (or compounds)
   - Grind to fine powder



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CASE STUDY: Sampling scallops for stable isotope analysis

Initial questions:

Are the scallops homogeneous?
- Size?
- Shape?
- Color?
- Pattern?

What strategy will yield a representative sample for analysis?

1 kg or 24 scallops ➔ 0.500 mg sample
0.000005 % or 0.5 ppm of the original mass

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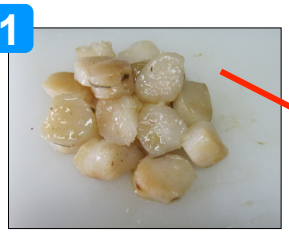
CASE STUDY: Sampling scallops for stable isotope analysis



1 Select a component that is visually homogeneous
− about 165 grams (16.5% of initial mass)

2 Crudely homogenize ~10 seconds

3 Select some visually homogeneous material
− about 100 grams (10% of initial mass)

Squash into a layer ~3 mm thick

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CASE STUDY: Sampling scallops for stable isotope analysis

4 Freeze the sample at −20°C
(slit open the bag)

Freeze dry for 24 hours at −10°C

5

6 Break up the dried material
Remove any obvious heterogeneities
− about 20 grams (2% of initial mass)

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CASE STUDY: Sampling scallops for stable isotope analysis

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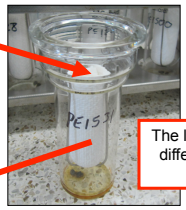
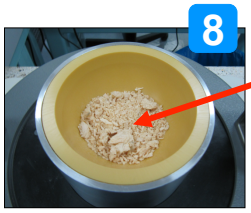
Remove lipid (fat) component:
reflux with 100 mL hexane, 2 hours



The lipid content may have a very different isotopic composition to the fat-free muscle.

8



Break up the defatted material
Remove any obvious heterogeneities
– about 5 grams (0.5 % of initial mass)

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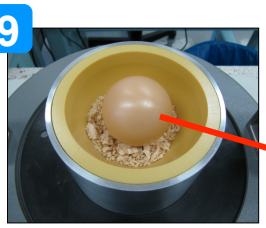
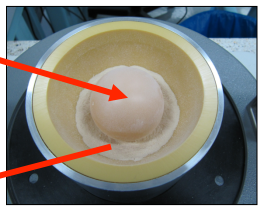
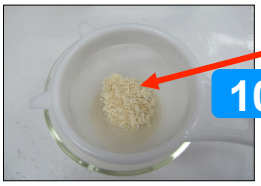
CASE STUDY: Sampling scallops for stable isotope analysis

9



Grind to <40 micron powder, using ball mill:
5 – 20 minutes (liq. N_2 optional)





10

Pass through a fine (metal-free) sieve
Remove residual shell / connective tissue

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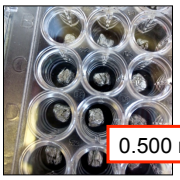
CASE STUDY: Sampling scallops for stable isotope analysis

Finally

Note: a sterile container is used to prevent microbial degradation.

Our food item is ready for analysis!
− about 3 grams (0.3% of initial mass)



0.500 mg sample

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Different isotope ratios of food record a variety of information

"Geo" elements (Sr, Pb, other trace elements & metals):

Soil or dust type & origin

Underlying rock formation(s)

The focus of this talk

"Bio" elements (H, C, N, O, S):

Biological processes

Environmental processes

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A brief reminder regarding…Carbon isotope ratios

- Measured $\delta^{13}C$ value of a food item is ultimately linked to photosynthesis.
- Plants using C_3 photosynthesis (e.g., wheat, barley, rice, most fruits): $\delta^{13}C = -30$ to -22 ‰.
- Plants using C_4/CAM photosynthesis (e.g., corn, sugar cane, millet, pineapple): $\delta^{13}C = -14$ to -10 ‰.
- Animal tissues reflect diet, with a (small?) “trophic shift” or fractionation effect.
- Bulk tissue $\delta^{13}C$ value = combination of different fractionation effects within separate components.

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A brief reminder regarding…Nitrogen isotope ratios

- Ecosystem nitrogen influxes and effluxes influence soil and plant $\delta^{15}N$ values.
- Inputs to ecosystem include: fertilization, N deposition, and N fixation.
- Outputs from ecosystem include: gaseous losses and hydrologic leaching.
- In general, $\delta^{15}N$ values in food web increase with each trophic level; magnitude of “trophic shift” impacted by protein quantity and quality.
- Bulk tissue $\delta^{15}N$ value = combination of different fractionation effects within separate components.

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A brief reminder regarding…Hydrogen & Oxygen isotope ratios

- Majority of water in the hydrologic cycle is found in the world's oceans.
- Globally averaged ocean water: δ^2H & $\delta^{18}O$ = ~0 ‰.
- Fractionation processes during evaporation and condensation result in predictable patterns of water isotopes across landscapes.
- These patterns can be presented graphically: *isoscapes.*
- Relationship between water H & O is known as the Global Meteoric Water Line (GMWL): $\delta^2H = 8 \times \delta^{18}O + 10$ ‰.
- Plants and animals record the isotopic composition of available water within their tissues, with some fractionation.

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No. 1: Animal-based foods (& a bit about data interpretation) Food forensics has two major focus areas: authenticity & origin

Likelihood-based data interpretation techniques used in ecology can be effective in food forensics:

*Discrete approach**, in which there are a limited number of groups to which an unknown sample may belong.

Continuous approach, in which an isotopic distribution across space is used to determine the likelihood of origin from any number of geographic locations.

*Perhaps unsurprisingly, the discrete approach is often more relevant to authenticity questions as opposed to origin questions and has been used more frequently in food forensics.

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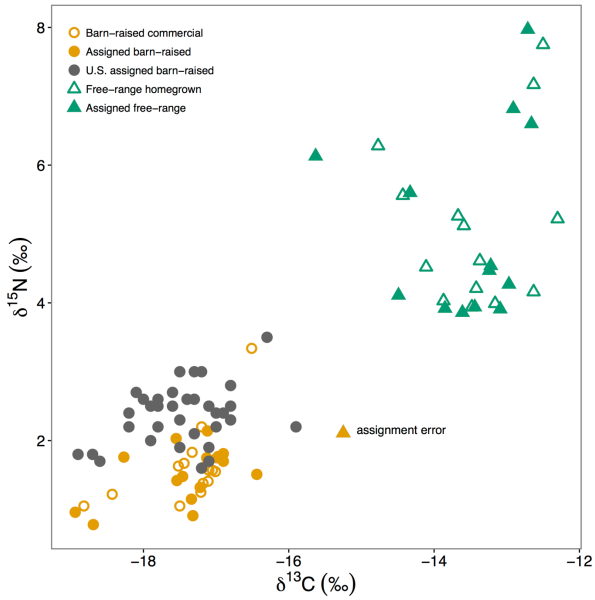
EXAMPLE: Interpreting chicken production w/discrete approach



Vander Zanden & Chesson 2017 *Food Forensics*

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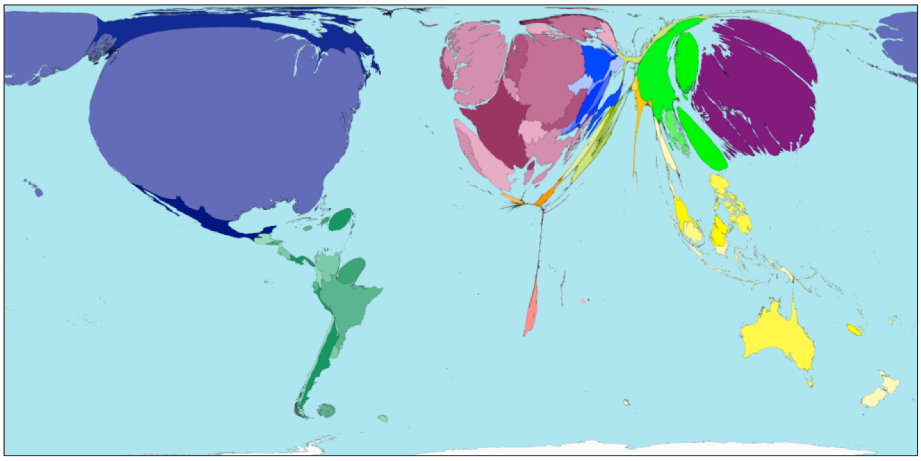
Is McDonald's Big Mac a representative sample of human diet?

Countries re-sized based on the prevalence of McDonald's restaurants.



Data from 2004; www.worldmapper.com, Map #364

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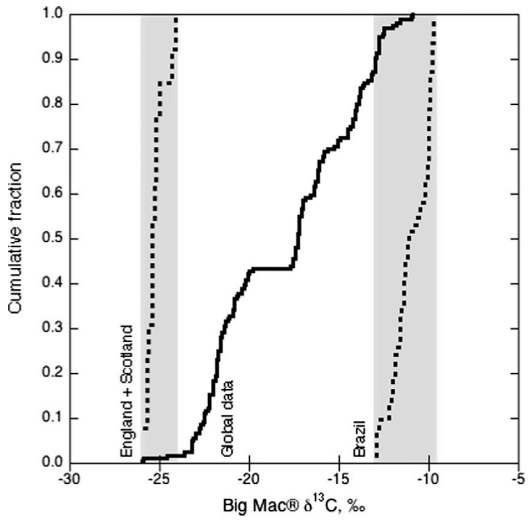
Carbon isotope ratios reveal beef cattle eat both C_3 and C_4 feed



- Big Mac® sandwiches purchased from across the globe
- Pure C_3 diet = England & Scotland (−25 ‰)
- Pure C_4 diet = Brazil (−11 ‰)

Martinelli et al. 2011 *Food Chemistry*

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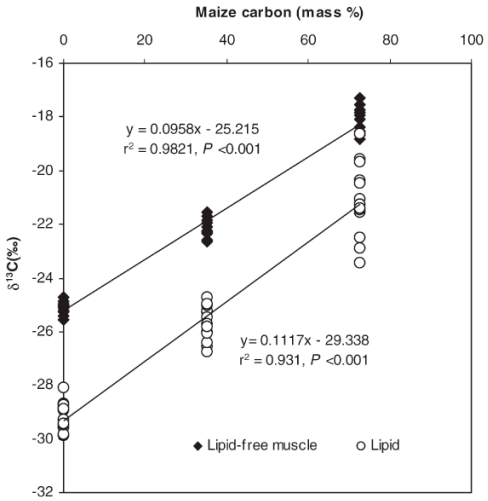
The amount of corn (maize) in cow diet is recorded in tissues



Bahar et al. 2005 *Rapid Commun. Mass Spectrom.*

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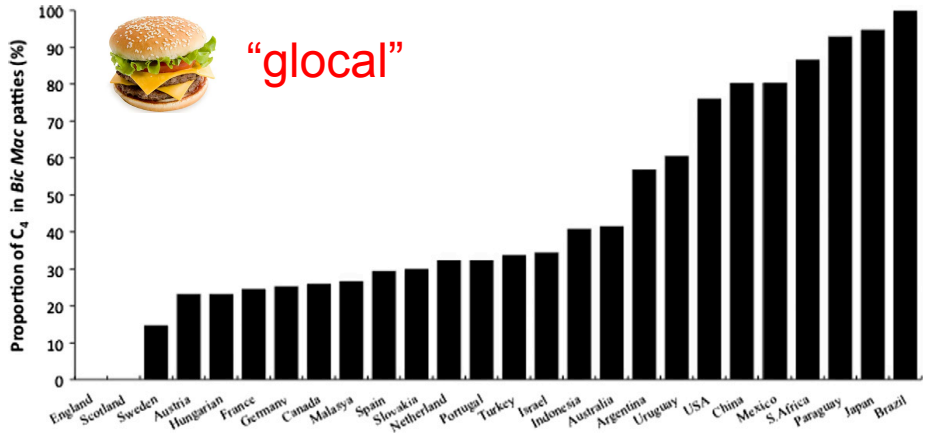
% C_4 varies by country, suggesting use of local meat sources



Martinelli et al. 2011 *Food Chemistry*

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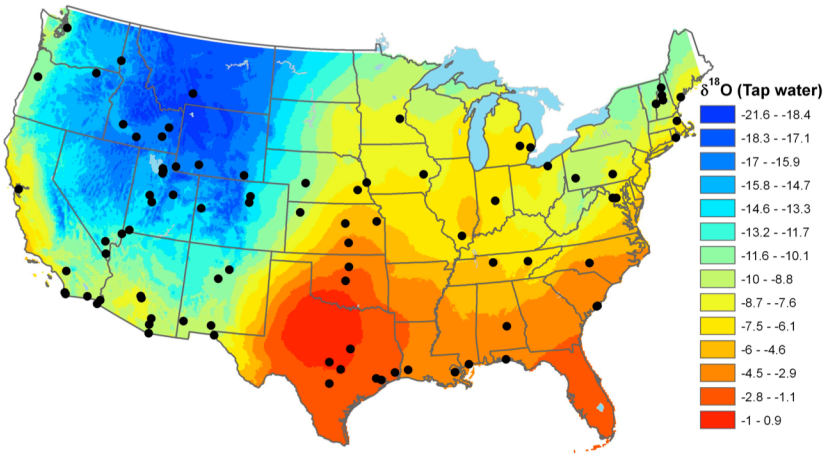
H & O isotope ratios of beef should relate to local precipitation



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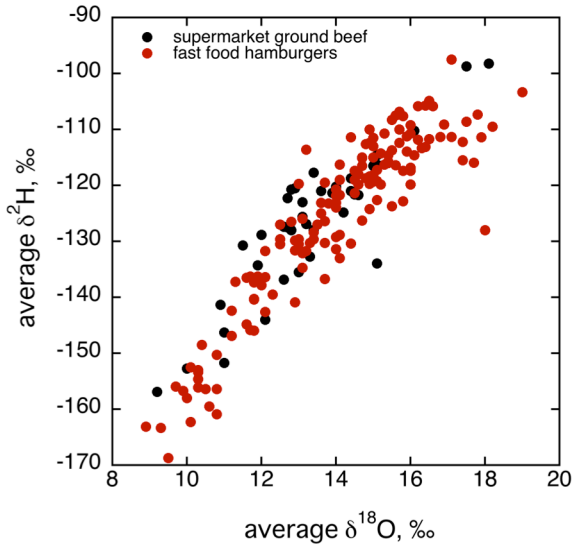
H & O isotope ratios of ground beef samples vary across the US



L. Chesson 2006-2009 (thesis data)

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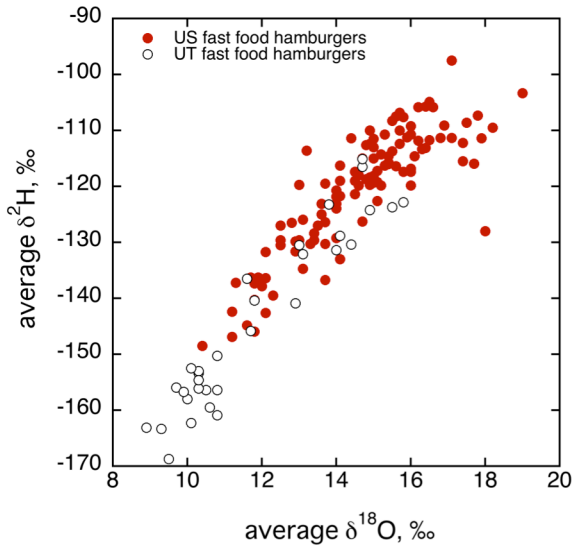
H & O isotope ratios of ground beef samples vary across the US



Chesson 2017 *Food Forensics*

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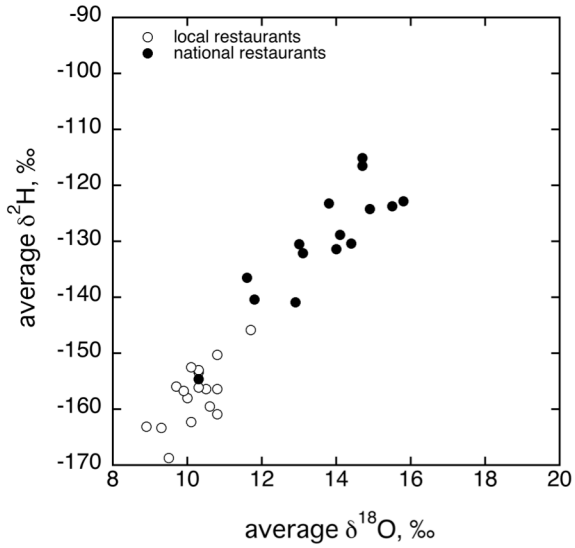

Chesson 2017 *Food Forensics*

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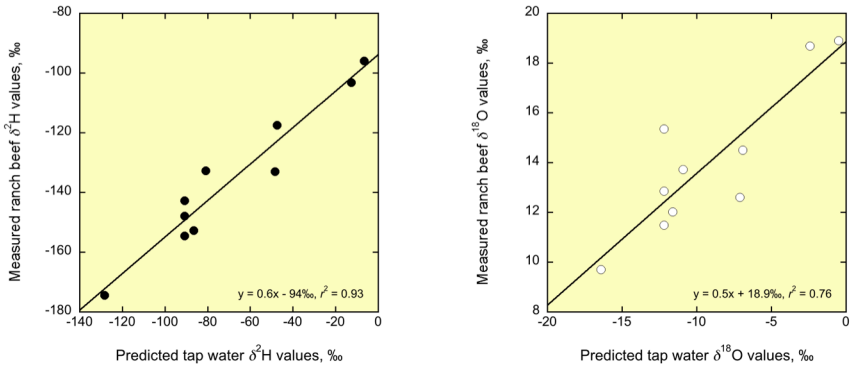
Isotopic link between tissue & water needed for origin prediction



Chesson et al. 2011 *Rapid Commun. Mass Spectrom.*

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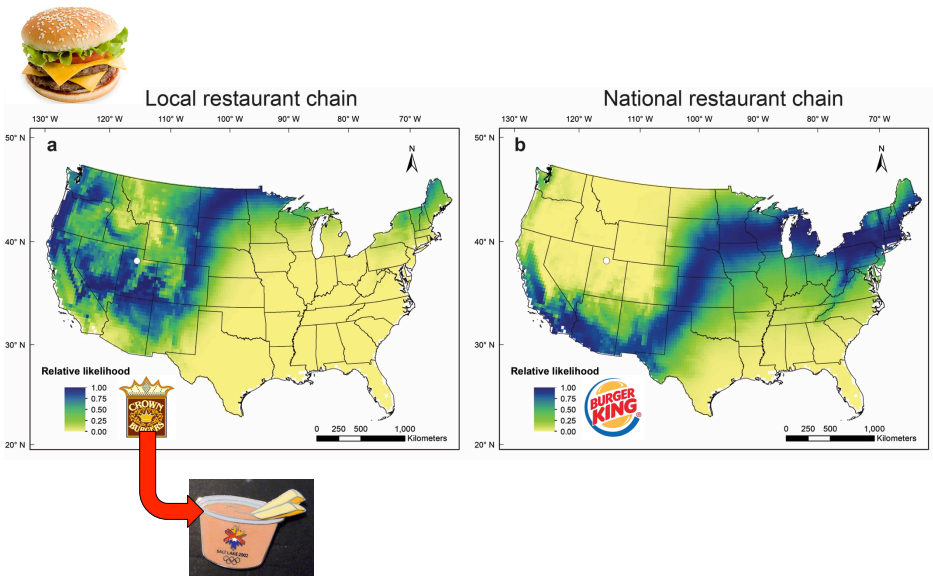
Ground beef origin can be interpreted w/continuous approach



Vander Zanden & Chesson 2017 *Food Forensics*

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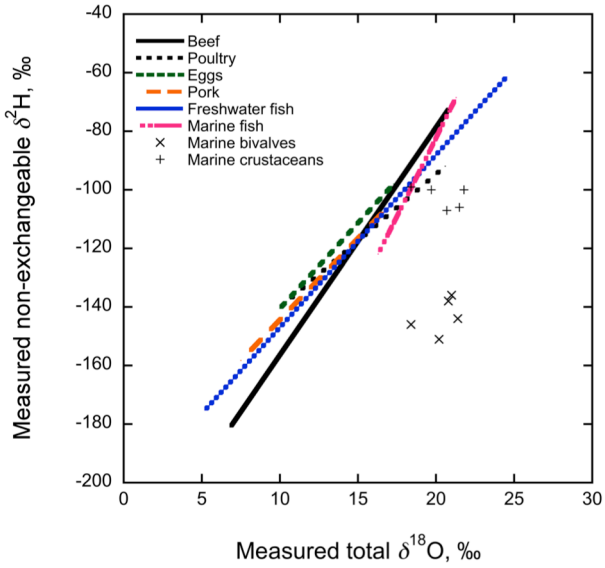
Co-variation in H & O isotopes is a ubiquitous feature of "meats"



Chesson et al. 2011 *Rapid Commun. Mass Spectrom.*

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Questions answered by isotope analysis of animal-based foods

- What did the animal eat?
- What did the animal drink?
- (**Spoiler alert:** Humans are animals, too – these questions have forensic implications…)

- Where was the animal raised?
- Am I really buying local?

- Up next: Can other isotopes provide additional information about protein production?

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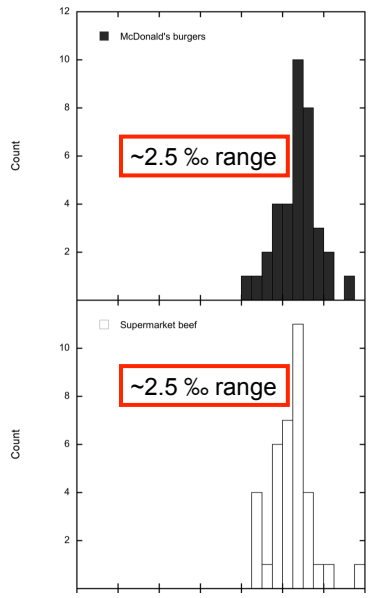
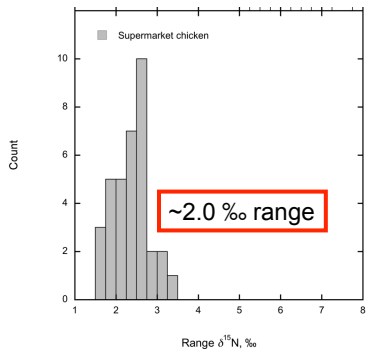
Nitrogen isotope ratios provide data on trophic level of US meats





Chesson et al. 2011 *Handbook of Environmental Isotope Geochemistry*

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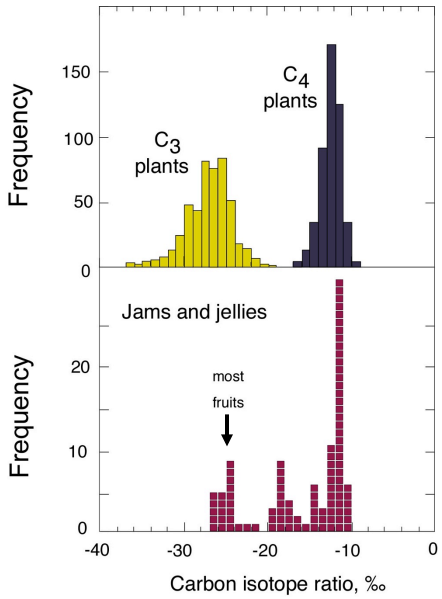
No. 2: Plant-based foods
Carbon isotope ratios can identify "real" fruit jams & jellies



J. Ehleringer, *unpublished data*

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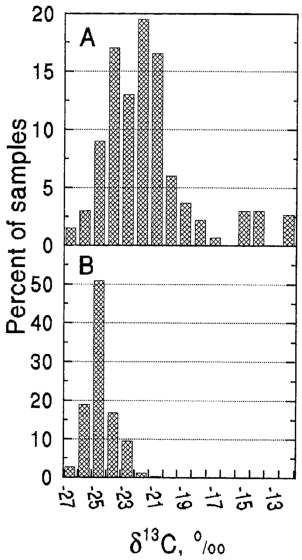
Carbon isotope ratios can also identify honey adulteration



- Top: Honey imports from China to the US *before* setting maximum $\delta^{13}C$ value of –23.5 ‰.
- Bottom: Honey imports from China *after* setting a limit for carbon isotope ratios.
- Stable isotope analysis established as a useful screening tool for honey authentication.

White et al. 1998 *Journal of AOAC International*

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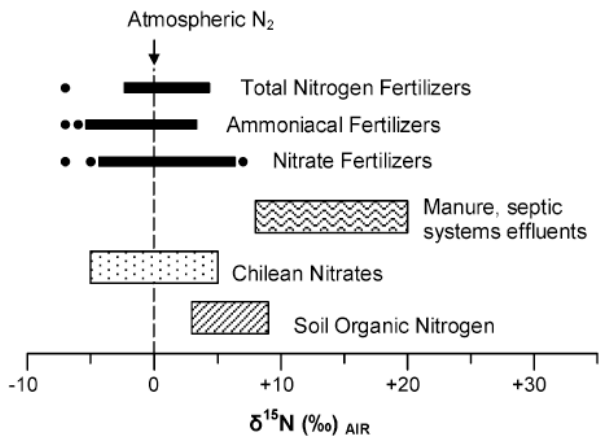
Nitrogen isotope ratios of fertilizers vary based on type & source



Vitòria et al. 2004 *Environ. Sci. Technol.*

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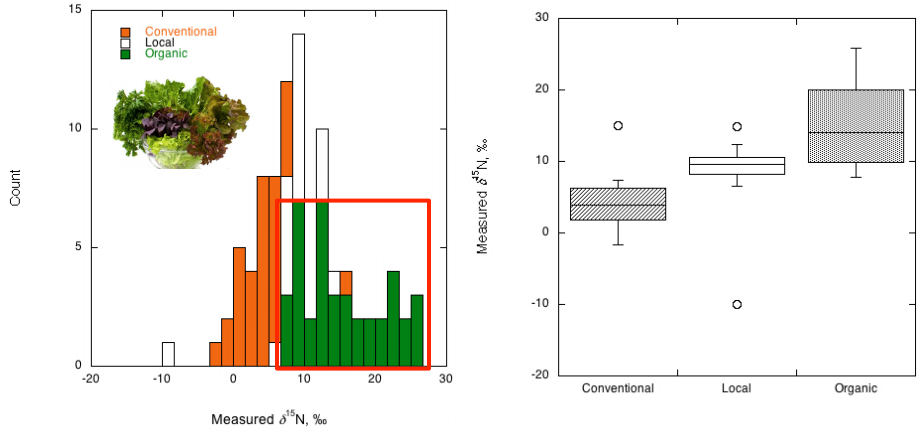
Nitrogen isotope ratios of organic & conventional greens differ



L. Chesson & J. Ruff, *unpublished data*

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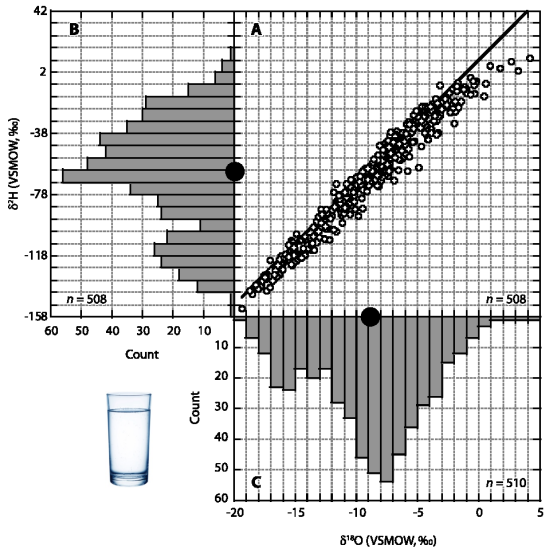
No. 3: Beverages
Tap waters generally fit the Global Meteoric Water Line (GMWL)



Bowen et al. 2007 *Water Resources Research*

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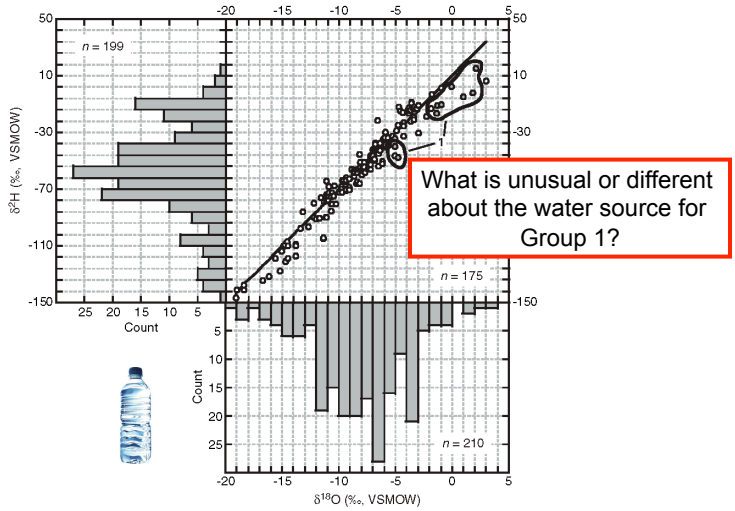
Most bottled waters fit the Global Meteoric Water Line (GMWL)



Bowen et al. 2005 *Rapid Commun. Mass Spectrom.*

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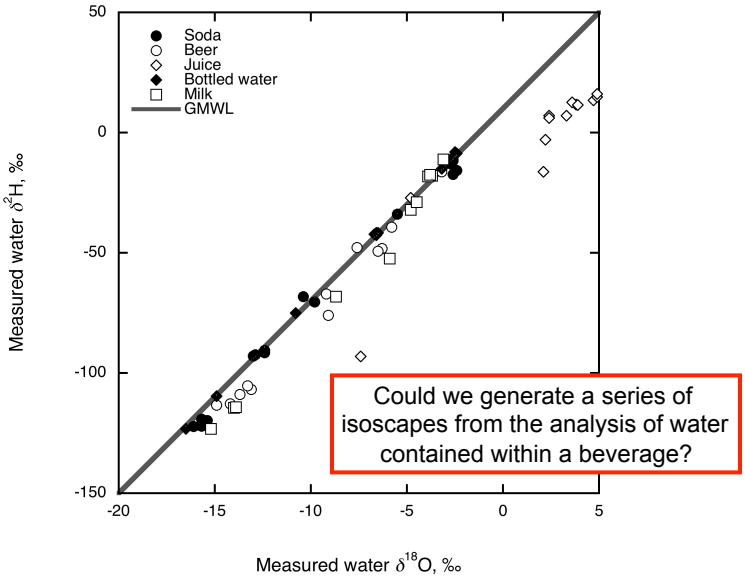
Several other beverage types also generally fit the GMWL



Could we generate a series of isoscapes from the analysis of water contained within a beverage?

Chesson et al. 2010 *Rapid Commun. Mass Spectrom.*

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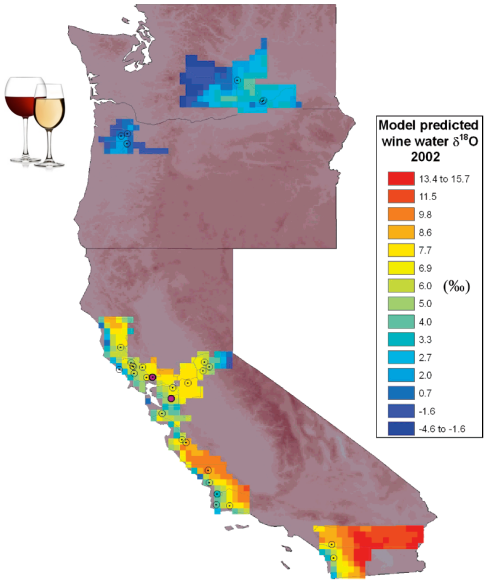
There is a preliminary wine isoscape for the West Coast, USA



West et al. 2007 *J. Agric. Food Chem.*

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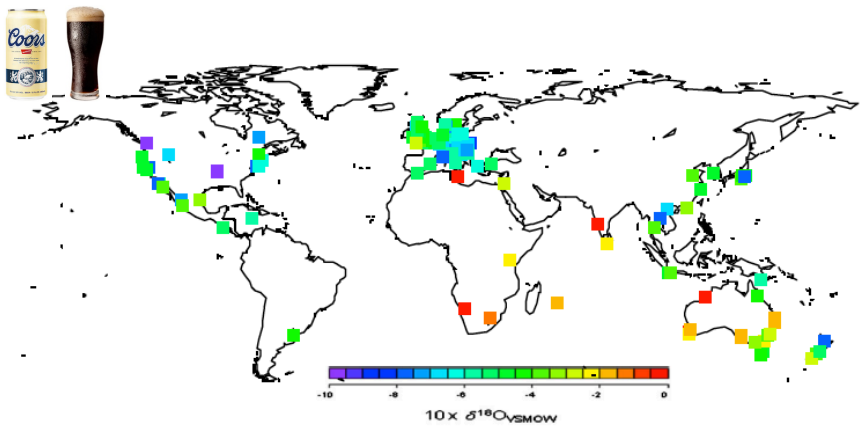
A global beer isoscape (an “alcoscape”?) was published in 2015



Carter et al. 2015 *Sci. Justice*

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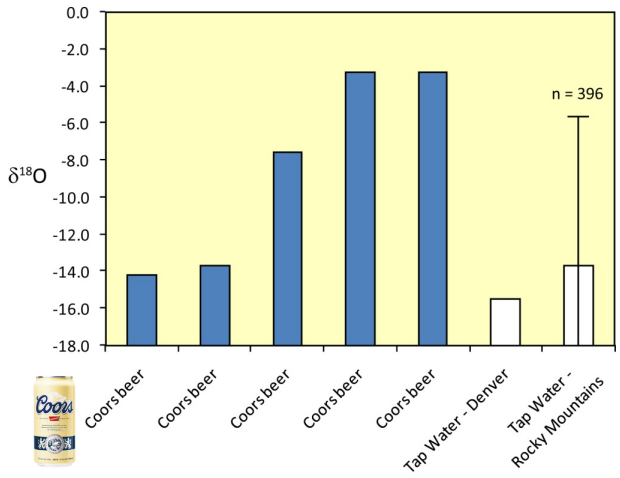
Oxygen isotope ratios reveal an odd feature of beer production



“…stubbornly brewed in the same Golden, Colorado brewery with 100% Rocky Mountain water and High Country barley since 1873…”
(www.millercoors.com, accessed 06/04/14)

L. Chesson, *unpublished data*

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Beverage H & O isotope ratios can answer questions of origin

- Is Evian bottled water really from Evian, France?
- Is Fiji bottled water really from the nation of Fiji?
- Where was your Coca-Cola bottled?
- Does your Napa Valley wine contain grapes grown in Napa Valley, California?

- **Related application and spoiler alert:** We can use the H & O isotope ratios of your hair to predict your fluid intake (and therefore, your potential location).

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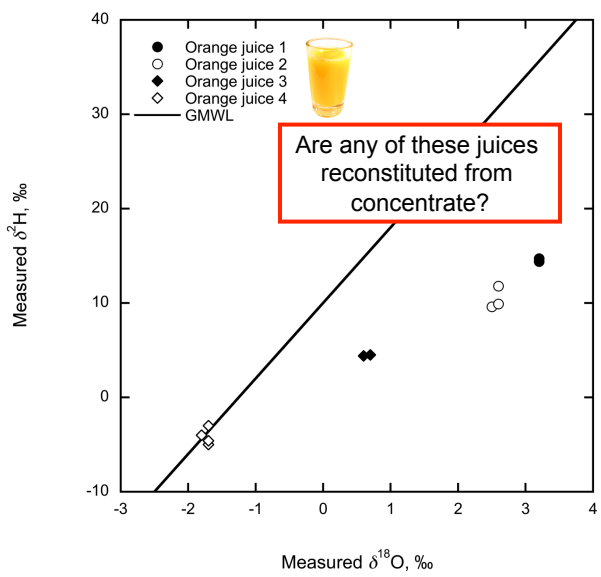
Beverage H & O isotope ratios can also reveal adulteration



L. Chesson, *unpublished data*

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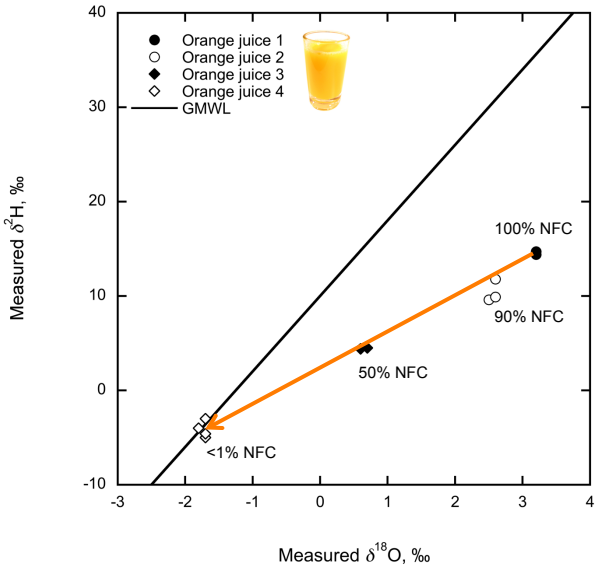
Beverage H & O isotope ratios can also reveal adulteration



L. Chesson, *unpublished data*

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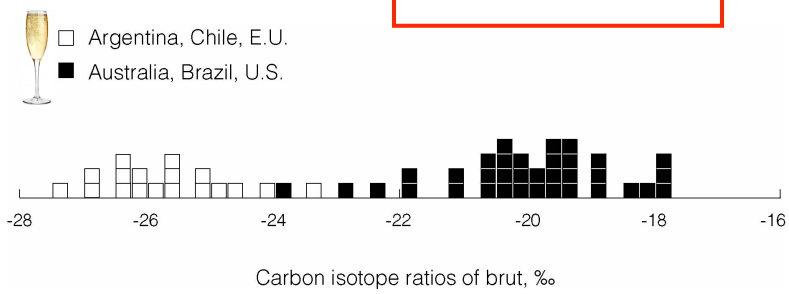
Carbon isotope ratios often used to detect beverage adulteration

"It is clear that there may be at times a 50% or more contribution of C_4 sugar to the overall isotopic signature of the wine."



Martinelli et al. 2003 *J. Agric. Food Chem.*

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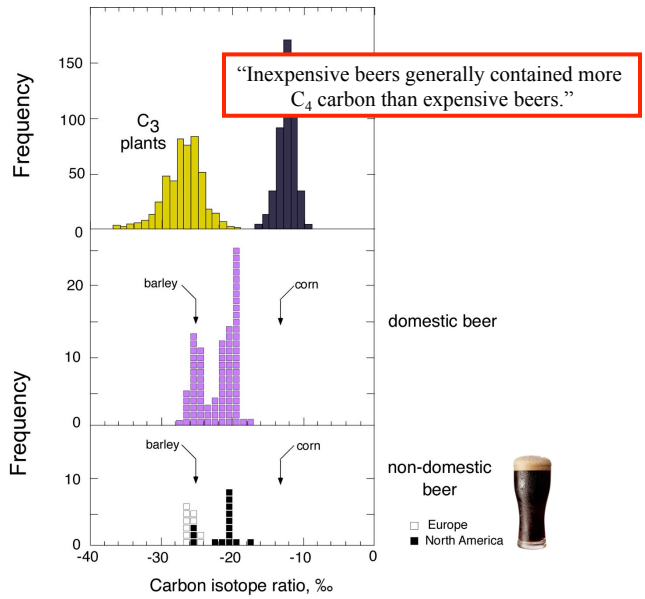
Carbon isotope ratios often used to detect beverage adulteration



Adapted from Brooks et al. 2002 *J. Agric. Food Chem.*

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If you want to know more…

- Camin, et al. (2017) Stable isotope techniques for verifying the declared geographical origin of food in legal cases. *Trends in Food Science & Technology*, 61:176-187.
- Zhao, et al. (2014) Recent developments in application of stable isotope analysis on agro-product authenticity and traceability. *Food Chemistry*, 145:300-305.
- Gonzalvez, et al. (2009) Trace-element composition and stable-isotope ratio for discrimination of foods with Protected Designation of Origin. *Trends in Analytical Chemistry*, 28:1295-1311.
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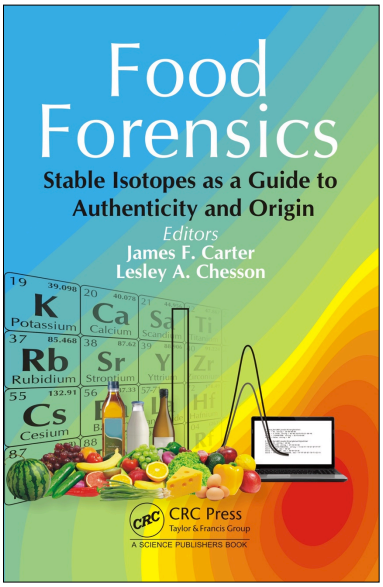

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