Appendix 1 – Background to stable isotope and multi-element analysis in food origin determinations

1. Natural abundance isotope ratios

A broad overview of the way in which natural abundance isotope ratios can be used for provenance determinations is shown in Table 1.1. Many natural phenomena, classed as physico-chemical effects, can lead to isotope fractionation (measurable changes in the ratio of the 'heavy' to 'light' isotope of a given element). For example, evaporation and condensation, crystallisation and melting, absorption and desorption, diffusion and thermodiffusion. As a first approximation natural abundance measurements will provide information on plant 'type' or diet (carbon and nitrogen isotope ratios), and geographical origin (hydrogen, oxygen, sulfur and strontium isotope ratios). Although it will be seen that diet effects on $^{13}C/^{12}C$ ratios can act as reliable geographical markers or proxies.

Table 1.1: Possible origin information available from different isotope ratios

Isotope ratio	***Fractionation***	***Information***
$^{2}H/^{1}H$	evaporation, condensation, precipitation	geographical, latitude, altitude
$^{13}C/^{12}C$	C_3 and C_4 plants	diet (geographical proxy)
$^{15}N/^{14}N$	trophic level, marine and terrestrial plants, agricultural practice	diet (geographical proxy)
$^{34}S/^{32}S$	agricultural practice	geological origin, distance to sea

The measurement of the stable isotope ratios of hydrogen and oxygen are applicable to the characterisation of geographical origin because they are strongly latitude dependent. Meteoric water that has passed through the meteorological cycle of evaporation, condensation and precipitation finally constitutes groundwater and this exhibits a systematic geographical isotope variation (Yuntseover and Gat, 1981). Decreasing temperatures cause a progressive heavy-isotope depletion of the precipitation when the water vapour from oceans in equatorial regions moves to higher latitudes and altitudes (Craig H, 1961). Evaporation of water from the oceans is a fractionating process that decreases the concentration of the heavy isotopomers of water ($^{1}H^{2}H^{16}O$, $^{1}H^{1}H^{18}O$) in the clouds compared to the sea. As the clouds move inland and gain altitude further evaporation, condensation and precipitation events occur decreasing the concentration of deuterium. Consequently, the ground water reflects this isotopic gradient from the coast to inland areas (Dansgaard W, 1964). The variation of oxygen-18 (^{18}O) in the hydrosphere follows an analogous pattern to that of deuterium (^{2}H). Previous studies have clearly shown that the oxygen-18 and deuterium content of water, consumed by animals, exhibits a strong correlation with the oxygen-18 and deuterium content of organic compounds present in animal products such as milk, butter and cheese (Manca G et al., 2001). This correlation is also found in animal tissues and has been exploited by ecologists to determine the migratory patterns of birds (Wassenaar and Hobson, 2001) and insects (Hobson *et al*., 1999).

Local agricultural practices and animal diet affect $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios respectively. C_3 plants use the Calvin photosynthetic pathway to assimilate CO_2. During this process the plants discriminate against ^{13}C and therefore possess relatively lower $^{13}C/^{12}C$ ratios than C_4 plants that utilise the more energy efficient Hatch-Slack pathway. Since C_3 plants predominate at higher latitudes and C_4 plants are more common in warmer climates at lower latitudes (such as the tropics), there is a gradient of decreasing $^{13}C/^{12}C$ in plant material from the equator to the poles, which can also be used as a proxy for geographical origin determination.

2. Delta Notation

Differences in isotope-effects mentioned in the previous section are usually of the order of a few percent. Consequently, changes in the isotopic ratio at natural abundance levels often occur around the third or fourth significant figure. Isotope ratio analysis therefore requires very precise measurement and this is achieved by measuring the ratio of the heavy and light stable isotopes in the test material and comparing it to a reference compound of nominal isotope ratio. The technique of differential analysis of sample and standard permits very small differences in the isotopic composition of test samples to be reliably and accurately determined. Thus, isotopic abundance of a sample relative to a reference is normally expressed by the differential equation (1):

$$\delta\ \mathrm{ref} = \left(\frac{\mathrm{Rsamp} - \mathrm{Rref}}{\mathrm{Rref}} \right) .1000 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Which can be simplified to (2):

$$\delta\ \text{ref} = \left(\frac{\text{Rsamp}}{\text{Rref}} - 1 \right).1000 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

'δ ref' is the isotope ratio of the sample expressed in delta units (‰, per mil), relative to the reference material. 'R_{samp}' and 'R_{ref}' are the absolute isotope ratios of the sample and reference material respectively. Multiplying by 1000 converts the value to parts per thousand (‰), or the more commonly used expression per mil (derived from the Latin mille meaning one thousand and used in an analogous way to per centum or per cent). In essence the use of equation (1) or (2) facilitates the comparison of isotope ratios especially at the natural abundance level when the differences being examined are relatively small.

3. Multi-element concentrations

Multi-element profiling consists of analysis of three distinguished groups of elements: macro elements [including aluminium (Al), calcium (Ca), magnesium (Mg), phosphorus (P), sodium (Na) and potassium (K)], micro elements [e.g. manganese (Mn), iron (Fe), cobalt (Co), Nickel (Ni), copper (Cu) and zinc (Zn)] and trace elements [e.g. **strontium** (Sr), molybdenum (Mo), cadmium (Cd), **lead** (Pb) and the Rare Earths].

The multi-element composition of animal tissues reflects, to some extent, that of the vegetation that they eat. The vegetation is the compositional reflection of the bio-available and mobilised nutrients, present in the underlying soils, from which they were cultivated.

Alkaline metals - especially rubidium (Rb) and caesium (Cs) - being easily mobilised in the soil and transported into plants, are good indicators of geographical identity. Trace element availability depends on several factors such as soil pH, humidity, porosity, clay and humic complex etc (Kim and Thornton, 1993). Consequently, the range of soils present and bioavailability mean that elemental composition may provide unique markers in food that characterise geographical origin. It is important to note that the chemical composition of the soil does not necessarily reflect that of the underlying lithology due to the deposition of glacial till (or 'drift' geology) during the 'ice-ages'. This is the case for most of Northern Europe which was covered by glacial till in lowland areas.

It is also important to note, even though multi-element profiles can provide additional discriminatory power to stable isotope fingerprints, these concentration profiles vary over time with agricultural practices and present only snapshots at the time of measurement. Hence, multi-element profiling was not applied to this project, but heavy stable isotope ratios (longterm indicators) of lead and strontium instead.

4. References

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