ENHANCING FOOD SAFETY AND QUALITY THROUGH ISOTOPIC TECHNIQUES FOR FOOD TRACEABILITY

A. Introduction

Producing safe and high quality food is a prerequisite to ensure consumer health and successful domestic and international trade, and is critical to the sustainable development of national agricultural resources. Systems to trace food or feed products through specified stages of production, processing and distribution play a key role in assuring food safety. Such traceability systems are typically based on a continuous “paper-trail” and effective labelling. However, analytical techniques that enable the provenance of food to be determined provide an independent means of verifying “paper” traceability systems and also help to prove product authenticity, to combat fraudulent practices and to control adulteration, which are important issues for economic, religious or cultural reasons.

Food traceability primarily focuses on food safety and quality, but also impacts on food security - the quantity and overall availability of food. Applying food traceability techniques can reduce food losses by minimizing recalls of food consignments if the production region can be determined scientifically.

Proof of provenance has become an important topic in the context of food safety, food quality and consumer protection in accordance with national legislation and international standards and guidelines. Provenance means to identify and ensure the origin of a commodity and thereby the region where it was produced. Recent incidents, such as the outbreak of food poisoning from Salmonella in contaminated peppers from Mexico, which occurred in the USA in 2008, have demonstrated the need for effective traceability systems and the deficiencies in current paper-based systems. The failure to trace the contaminated batch of peppers to their origin resulted in a wide-scale, costly and lengthy recall procedure involving many producers in Mexico and retail outlets in the USA.

FIG. II-1. Traceability of food covers various factors along the food chain. Beyond regulatory and organizational criteria, the verification of documentation is essential (picture courtesy of K. A. Donelly, Nofima).

Therefore, an independent and universally applicable analytical strategy to verify the declared country of origin of food can be an invaluable tool to enable regulatory authorities to trace contaminated foods back to their source. Isotopic and elemental fingerprinting provides a robust analytical tool to determine the origin of food. These techniques, when used in conjunction with food safety surveillance programmes, provide independent verification of food traceability systems, thereby helping to protect human health and facilitate international trade worldwide. The availability of verified traceability systems can also facilitate the targeted withdrawal and/or recall of contaminated products from the market if necessary. Such action can reduce the enormous economic impact of a 'blanket withdrawal/recall' and the subsequent damage to industry and consumer confidence. Furthermore, public awareness of the existence of tools and protocols to determine product origin, in a food safety context, can act as a deterrent to traders who knowingly re-route contaminated products to mislead importers, thus preventing the occurrence of incidents related to "third country" dumping of unsafe commodities.

The capability to certify food origin or authenticity is of significant economic importance to many stakeholders in developing countries. For example, some food products can be marketed using labels, e.g. Geographic Indication (GI) or organic produce, that are based on standards of identity or composition related to a very specific production area or production practices. This adds value to such products in terms of marketability and increased export value. Basmati rice from India and Pakistan, for example, is defined by its cultivar and also by its area of production. Genomic techniques can easily confirm the cultivar of Basmati rice, while isotopic and elemental fingerprinting is essential to determine its geographical origin. Isotopic parameters have also recently been added to the Protected Denomination of Origin (PDO) technical specification of Grana Padano Cheese (Italy) and other food commodities are undergoing similar characterisations.

In addition to their application to enhance food safety, these techniques can be applied to address religious or cultural issues. For example, whilst safe for human consumption, the animal or botanical source of a food may render it unfit for some consumers. For instance, gelatine derived from porcine sources and ethanol derived from wines or spirits are not compliant with Halal guidelines, nor are they in accordance with definitions given by Codex Alimentarius (1997).

B. Nuclear and nuclear-related techniques for food traceability

Nuclear techniques are uniquely tailored for the determination of food provenance. These methodologies, such as those listed in Table 1, may be used for traceability and authentication of food and have the potential to be applied in many developing countries, thereby enhancing their capacities to improve food safety and quality. Where appropriate, these techniques can also be complemented by conventional, non-nuclear approaches.

The following nuclear techniques can be used to measure the isotopic (e.g. hydrogen-3/ hydrogen-2/ hydrogen-1, carbon-13/ carbon-12, nitrogen-15/ nitrogen14, oxygen-18/ oxygen-16, sulphur-34/ sulphur-32, strontium-87/ strontium-86, lead-208/ lead-207/ lead-206) and elemental (e.g. macro, micro, and trace) composition of food:

TABLE 1. GLOSSARY OF TERMS FOR ANALYTICAL METHODS USED FOR FOOD TRACEABILITY AND THEIR FINGERPRINT CHARACTERISTICS

Method	**Abbreviation**	**Fingerprint**
Neutron activation analysis	NAA	Isotopic and elemental
Multicollector inductively coupled plasma – mass spectrometry	MC-ICP-MS	Isotopic and elemental
Nuclear magnetic resonance	NMR	Isotopic
Thermal ionization – mass spectrometry	TIMS	Isotopic
Isotope ratio mass spectrometry	IRMS	Isotopic
Cavity-ring-down spectroscopy	CRDS	Isotopic
Atomic absorption spectrometry	AAS	Elemental
Atomic emission spectrometry	AES	Elemental

In order to determine an isotopic fingerprint of a specific product, an extensive quality-assured database of authentic food samples is required. Appropriate modelling of the isotopic and elemental data, using multivariate statistics, is a prerequisite for identifying isotopic and elemental fingerprints. Only a few databases covering wide regional distribution exist so far. A considerable amount of additional data needs to be collected to provide universally applicable information that could make data comparable from diverse regions world wide.

The application of the cavity-ring-down spectroscopy (CRDS) technology to distinguish between C3 (sesame, soybean) and C4 (corn) plants through isotopic techniques is an example. Photosynthetic carbon isotope fractionation is related to carbon dioxide uptake and enzymatic processes. The C3 plants, named due to the number of carbons in an intermediate molecule in the relevant biochemical pathway, discriminate more heavily against carbon-13 than the C4 plants and therefore have more negative δ13C values (calculated from the ratio of carbon-13 to carbon-12 in the plant). Plotting the data from δ13C measurements therefore shows a dramatic difference between the oils from C3 plants (sesame, soybean) and that from corn, which is a C4 type plant. From the plot, it can be observed that even the two oils from C3 plants are clearly distinct. Accordingly, the adulteration of respective plant oils with materials of lesser quality and value can be determined.

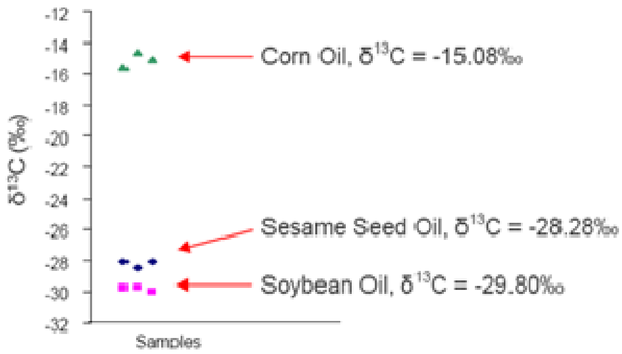

FIG. II-2. The graph displays δ13C (‰) values for different food commodities. Sesame and soybean are C3 plants that can be distinguished from C4 plants due to different isotope discrimination patterns. Adulteration with other ingredients can be detected by shifts in the δ13C values.

C. Supplementary and combined analytical techniques supporting food traceability

In addition to nuclear and nuclear-related approaches, various non-nuclear techniques can provide complimentary data to complete or confirm results obtained by isotopic and elemental fingerprinting. For instance, seafood is a highly perishable food item which is increasingly traded globally. Particular conditions and difficulties have to be taken into account compared to other food products and different analytical techniques are applicable for characterizing seafood. More than 500 species are traded in the European market alone. A large number of processed fish products have lost their morphological characteristics and for instance fraud, where low grade fillets are substituted for high-value fillets, can only be discovered by means of a combination of reliable analytical methods to determine the species and geographical origin.

The most appropriate technique is related to the specific condition of the seafood product, e.g. processed vs. unprocessed, or whether or not it is a closely related or a different species. Separation and characterisation of specific proteins through isoelectric focusing (IEF) of sarcoplasmic proteins (water soluble proteins) is the method of choice for the identification of fish species. DNA based methods using polymerase chain reaction (PCR) for nucleic acid amplification is the key technology for species identification, as there is no limitation when different processing treatments of fish and seafood are used.

The most important techniques for the determination of geographical origin are the methods using the variability of stable isotopes (hydrogen-2/ hydrogen-1, nitrogen-15/ nitrogen-14, carbon-13/ carbon-12, oxygen-18/ oxygen-16) in different biological tissues. It is well known that freshwater ecosystems are generally carbon-13- and nitrogen-15-depleted in comparison to marine ecosystems. Consequently, the analysis of these stable isotopes ratios can be used for distinguishing between freshwater and marine fish. Furthermore, different feedings often lead to significant fingerprints in terms of stable isotope ratios in the body so that stable isotope analysis can be also used to distinguish between different environments. Nuclear magnetic resonance, coupled with isotopic ratio mass spectrometry (NMR/IRMS) and site-specific natural isotope fractionation by nuclear resonance (SNIF-NMR), are the two main methods to determine the ratios of stable isotopes.

D. Isotopic traceability techniques for rapid response to emerging food safety risks

The food supply is vulnerable to a range of food hazards (microbiological, chemical, physical) that may arise at any stage of the food supply chain. In addition to well publicized food safety incidents such as aflatoxins in maize, dioxins in pork, melamine in dairy products, and Salmonella in peanuts, new hazards and risks are continually emerging. These may be related to unintentional contamination with, e.g. agrochemicals or bacteria, or intentional contamination (adulteration for economic fraud or with the intent to harm consumers). Other issues may also pose threats to food safety which are not yet well understood or characterized, for example the effects of climate change on food production, or emerging technologies such as the use of nanoparticles in food.

Isotopic measurement techniques can provide an effective means for the identification and tracking of food products, allowing a rapid first response to counter any threat by efficiently tracing and removing affected products from the market. Stable isotope and radio-labelling techniques can also provide a second-tier analytical portfolio to help to detect, identify and characterize the hazard. For example, radio-labelling offers a uniquely sensitive traceability method to investigate the fate of nanoparticles in foods. Nanoparticles are increasingly applied on a broad range of applications and may also play a vital role as food additives. However, the respective risk assessment and evaluation are just in their infancy. The development and application of these techniques would address vulnerabilities in the food supply chain due to emerging threats and help establish effective preventative systems and incident response strategies.

Specific future tasks would be to develop isotopic traceability methodologies and systems to facilitate the rapid tracking of contaminated products and their removal from the market. The development of related stable and radio-isotope techniques that can be applied to detect and characterize emerging food safety hazards and assess and control the risks associated with those hazards is also envisioned.

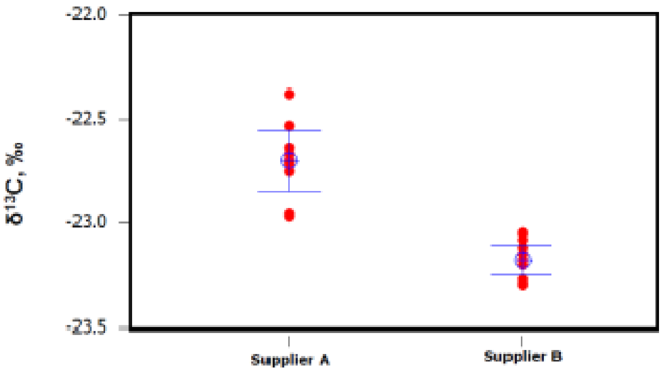

FIG. I1-3. Synthetic chemicals feedstock suppliers distinguished by δ13C ranges. Adulteration is detectable unequivocally by differences in δ13C values (graphics from Picarro Inc.).

E. Analytical and managerial challenges for food traceability

Multi-element and isotopic analyses have previously been applied to a range of foodstuffs to develop methods that will permit their geographical origin to be determined with varying degrees of certainty. A vast array of analytical techniques and parameters have been studied to verify the provenance of regional foods, such as aroma, sugar, phenolic and flavour compound profiling, by gas and liquid chromatography and 'fingerprinting' or chemical profiling by 1H NMR (using hydrogen-1 as target atom), near Infra-Red and Fluorescence spectroscopy. These techniques can be extremely powerful tools for food origin determination in their own right and NMR profiling is often reported to be used in conjunction with multi-element isotopic and trace element analysis.

Food authentication requires a database of genuine samples to which the 'suspect' test sample can be compared to establish its authenticity. In order to characterize markers for an authenticity parameter, such as geographical origin, there is a requirement for a large number of independent variables to be measured and statistically 'screened' in order to identify key tracers that differentiate the regions or countries of interest. Measuring elemental concentrations and isotopic variation in regional products is arguably the best analytical strategy for accurately verifying geographical origin.

Meat (beef and lamb), dairy products (milk, butter, cheese), beverages (tea, coffee, juice), cereal crops (rice, wheat) and wine have, to date, been the main commodities of interest investigated using the techniques mentioned above. Other commodities such as olive oils have been analysed for geographical classification using multi-element data together with sensory parameters, combined with multivariate statistics.

Further research activities have also been undertaken to identify the regional provenance of asparagus using strontium isotope ratio measurement by multicollector - inductively coupled plasma - mass spectrometry (MC-ICP-MS). Tracing to origin is also an important issue for protection of the market and trading interests for other commodities such as saffron.

The relative abundance of natural strontium (Sr) isotopes is related to local geological conditions and may therefore provide information on the origin of raw cheese products. Values of Sr isotope abundance ratios in terrestrial vegetation are linked with the Sr isotopic composition of the soil, which is influenced by bedrock, soil/water properties and atmospheric inputs. The typical strontium mass content of mobile strontium in soil and in solution ranges from 0.2 to 20 mg kg^{-1} (µg Sr leached per g of soil). For different types of geological samples the overall mass contents of Sr range from 1 up to 2000 mg kg^{-1} and from 0.01 to 7620 mg L^{-1} for hydrological samples (seawater, rivers, rain) and from 8 to 2500 mg kg^{-1} for biological samples (wood, roots). Biological processes, whether involved in plant or animal metabolism, do not significantly fractionate strontium isotopes. It has been found that geological properties (e.g. Sr isotope abundance ratios) are reflected directly in the cheeses when the cows are kept under a controlled dietary regime and are not fed with industrially produced feeds or feeds from geographically distant sources.

There is still considerable room for improvement in both sampling and analytical methodology. In particular, there is a need to ensure that:

- Procedures used by exporting countries are in harmony with those used by the competent authorities in importing countries as provided under applicable legal norms. For example at the European level under the Regulation (EC) 882/2004, on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules;
- Attributes of food authenticity are clearly identifiable, documented and/or measurable by all involved parties;
- The limitations inherent in analytical data, in particular concepts such as uncertainty and limits of quantification, are understood by all concerned, and;
- Mechanisms are developed to assist in the preparation of appropriate commercial specifications as well as making certain that sampling and analytical methods used ‘in house’ (usually rapid methods) are fit for purpose. This involves taking into account not only the analyte but also the food matrix in which it is analysed.

Also in the field of food authenticity, besides reliable but time consuming analytical methods, there is a need for the development of fast, simple, robust methods of proven efficacy and reliability.

F. Conclusion

A deeper understanding of how meteorological and geochemical signatures are transferred into food systems would allow the generation of isotopic and multi-element maps for foods from different geographical locations, which could be incorporated into traceability systems. Comparative databases constructed from these data can then be used as benchmarks in ongoing scientific developments of the future.

Food traceability is an emerging topic that is becoming increasingly relevant especially in terms of international trade. For the export and import of food, the development of traceability systems has been identified as a priority, especially in connection with food safety. Therefore, the implementation of food traceability mechanisms, including analytical methodologies for verification, is particularly relevant for developing countries who wish to increase extending their share in international food trade. International organizations, such as the IAEA, can play a role towards providing equal access to such technologies in the future as well as assisting developing countries to build the necessary capacities to use them.

REFERENCES

[II-1] Official Journal of the European Communities, REGULATION (EC) No 178/2002 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety,
http://eurlex.europa.eu/pri/en/oj/dat/2002/l_031/l_03120020201en00010024.pdf

[II-2] Factsheet Food Traceability, 2007, European Commission, Health & Consumer Protection Directorate-General, http://ec.europa.eu/food/food/foodlaw/traceability/factsheet_trace_2007_en.pdf

[II-3] CARCEA, M., et al., Food authenticity assessment: ensuring compliance with food legislation and traceability requirements. Quality Assurance and Safety of Crops & Foods ISSN 1757-8361.

[II-4] SCHRÖDER, U., Challenges in the Traceability of Seafood. J. Verbr. Lebensm. **3** (2008) 45-48.

[II-5] SUZUKI, Y., et al., Geographical origin of polished rice based on multiple element and stable isotope analyses. Food Chemistry **109** (2008) 470–475.

[II-6] LO FEUDO, G., et al., Investigating the Origin of Tomatoes and Triple Concentrated Tomato Pastes through Multielement Determination by Inductively Coupled Plasma Mass Spectrometry and Statistical Analysis. J. Agric. Food Chem. (2010) **58** (6) 3801–3807.

[II-7] SWOBODA, S., et al., Identification of Marchfeld asparagus using Sr isotope ratio measurements by MC-ICP-MS. Anal Bioanal Chem 390 (2008) 487–494.

[II-8] KELLY, S., et al., Tracing the geographical origin of food: The application of multi-element and multi-isotope analysis. Trends in Food Science & Technology **16** (2005) 555–56.

[II-9] ODDONE, M., et al., Authentication and Traceability Study of Hazelnuts from Piedmont, Italy. J. Agric. Food Chem. **57** (9) (2009) 3404-3408.

[II-10] MOLKENTIN, J., Authentication of Organic Milk Using 13C and the Linolenic Acid Content of Milk Fat. J. Agric. Food Chem., **57** (3) (2009) 785-790.

[II-11] AURSAND, M., et al., C NMR Pattern Recognition Techniques for the Classification of Atlantic Salmon (Salmo salar L.) According to Their Wild, Farmed, and Geographical Origin. J. Agric. Food Chem. **57** (9) (2009) 3444-3451.

[II-12] MAÇATELLI, M., et al., Verification of the geographical origin of European butters using PTR-MS. Journal of Food Composition and Analysis **22** (2009) 169-175.

[II-13] RASPOR, P., Bio-markers: traceability in food safety issues. Acta Biochimica Polonica **52** 3 (2005) 659–664.

[II-14] STANIMIROVA, I., et al., Tracing the geographical origin of honeys using the GCxGC-MS and pattern recognition techniques, Food Chemistry, **118** (2010) 171-176.

[II-15] CAMIN, F., et al., Multi-element (H,C,N,S) stable isotope characteristics of lamb meat from different European regions. Anal. Bioanal. Chem. **389** (2007) 309-320.

[II-16] PROHASKA, T., et al., Identification of Marchfeld asparagus using Sr isotope ratio measurements by MC-ICP-MS. Anal. Bioanal. Chem. **390** (2008) 487-494.

[II-17] FORTUNATO, G., et al., Application of strontium isotope abundance ratios measured by MC-ICP-MS for food authentication. J. Anal. At. Spectrom. **19** (2004) 227-234.

[II-18] ALONSO, G.L., et al., Worldwide market screening of saffron volatile composition. J. Sci. Food Agric. **89** (2009) 1950-1954.

[II-19] RHODES, et al., Emerging Techniques in Vegetable Oil Analysis Using Stable Isotope Ratio Mass Spectrometry. Grasas y Aceites, **34** 53, Fasc. **1** (2002) 34-44.