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1st IMEKOFOODS
Metrology Promoting Objective and Measurable Food Quality and Safety

October, 12th - 15th 2014
Rome (Italy)
ABSTRACT BOOK
International Conference
1st IMEKOFOODS
Metrology Promoting Objective and Measurable
Food Quality and Safety

October, 12th – 15th 2014
Rome (Italy)

Edited by
Giovanna Zappa
Claudia Zoani

2014 ENEA
Italian National Agency for New Technologies, Energy and Sustainable Economic Development

Lungotevere Thaon di Revel, 76
00196 Rome
1st IMEKOFOODS
Promoting Objective and Measurable Food Quality & Safety
12th-15th October 2014 Rome (Italy)

ORGANIZATION BODIES

Organizing MO

Organizing IMEKO TC
TC23 – Metrology in Food and Nutrition

Event Secretariat:

1st IMEKOFOODS National Organizing Committee
e-mail: imekofoods@enea.it
Tel. +39 06 3048 3665
FAX: +39 06 3048 6258

Organizing Secretariat - Symposia S.r.l.
e-mail: imekofoods@grupposymposia.it
Tel. +39 06 3972 5540
Fax: +39 06 3972 5541

Conference webpage: http://imekofoods.enea.it
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Conference Editors:

Giovanna Zappa – ENEA (giovanna.zappa@enea.it; +39 06 3048 3436)
Isabel Castanheira – INSA (isabel.castanheira@insa.min-saude.pt; +35 1217 508 153)
Ruth Charrondiere – FAO/INFOODS (ruth.charrondiere@fao.org; +39 06 570 56 134)
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### IMEKO Secretariat:

Dalszínház utca 10, 1st floor, Office room No.3  
H-1061 Budapest (6th district) - HUNGARY  
Secretary: Judit Faragó  
E-mail: imeko@t-online.hu  
Phone/Fax: +36 1 353 1562

### IMEKO TC23 webpage:


### IMEKO webpage:

Dear Participants,

it is a true pleasure for me to welcome you to Rome for the 1st IMEKOFOODS, a Conference conceived by IMEKO TC23 “Metrology in Food and Nutrition” and organized by ENEA (Italian National Agency for New Technologies, Energy and Sustainable Economic Development) with the significant contribution of leading research organizations represented in the SSC and IPC.

This first edition of IMEKOFOODS is focused on “Metrology promoting Food Quality and Safety”. The aim is to encourage the exchange of ideas, the scientific debate and the encounter among the different realities that revolve around the "world of measures" for food Quality and safety, promoting the harmonization and integration and addressing the "world of research" towards the emerging needs of the civil society and the productive sectors.

We have succeeded in organizing this important event in a few months thanks to the great interest, participation and cooperation of all of you. This is already a first success and a positive sign that shows us how much enthusiasm and desire to collaborate on the theme of Metrology for promoting food quality and safety there is among researchers, food industry, manufacturers of analytical instrumentation and software, institutions and standardization bodies.

We are honored to have such a high level of speakers with an extensive participation by many Countries and by so many leading research organizations.

The excellence and the high number of patronages and sponsorships are also a great honor for us. In particular the patronage and collaboration with EXPO 2015 constitutes a major and concrete possibility to give continuity to our discussion and scientific debate.

I wish you a successful, scientifically stimulating symposium and a pleasant stay in Rome.

Giovanna Zappa
1st IMEKOFOODS General Chair
KEYNOTE LECTURES

**KL01** - Standardized scientific tools for food safety and quality control to protect trade and European consumers
*Anklam E.*

**KL02** - Real-time PCR – a reality check
*Bustin S.A.*

**KL03** - Thinking, talking, and understanding the same thing in the same way: a difficult but necessary task for the 21st Century
*De Bièvre P.*

INVITED LECTURES

**IL01** - Currently available measurement systems for food control
*Ulberth F.*

**IL02** - Some Actual Challenges with Global Equivalence in the Analysis of Milk and Milk Products
*van den Bijgaart H.*

**IL03** - Improve precision in sensory evaluation technique by means of calibration and standard samples. The case of the IOC methods for the classification of the virgin olive oil and the table olives
*Giamo A.*

**IL04** - Peptide based e-nose for food quality and process control
*Compagnone D.*

**IL05** - Sample Preparation and Detection Methods for Natural or Engineered Nanoparticles in Food
*Larsen E. H., Loeschner K. and Correia M.*

SPECIAL LECTURE

**SL01** - COST Action FA1101 SaffronOmics (Omics technologies for crop improvement, traceability, determination of authenticity, adulteration and origin in saffron):
From goals to achievements
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Standardized scientific tools for food safety and quality control to protect trade and European consumers

Anklam E.

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Retieseweg 111, 2400 Geel (Belgium) - elke.anklam@ec.europa.eu

Safe food products are the pre-requisite for a healthy diet. Consumers expect that the foods they buy are not only safe but also genuine, i.e. are not subject to fraud and are of as high quality food as possible. This holds especially true for high priced products such as wine, honey, olive oil and labelling of food products needs to be truthful and not misleading.

It is important that food control authorities around the world have comparable tools at hand to provide appropriate surveillance to ensure food safety and the prevention of frauds in the food chain. The European Union has established an appropriate framework on issues related to food and feed safety and quality control. Control laboratories need to follow harmonized procedures and the results obtained need to be trustable, reproducible and of high quality. Laboratories need to follow internationally harmonized and recognized standard methods for analysis. Whenever possible, methods used should be internationally validated and standardized. European Reference Laboratories support National Reference Laboratories of the European Union to obtain high quality and harmonized results by the provision of reference methods, reference materials, proficiency testing schemes and training to laboratory staff.

This presentation will show the importance of standardized methods to keep the confidence of consumers in food they buy and of trade partners in food they import.
Real-time PCR – a reality check

Bustin S.A.
Anglia Ruskin University, Postgraduate Medical Institute, Bishop Hall Lane, Chelmsford, UK
stephen.bustin@anglia.ac.uk

Food-borne diseases caused by bacteria, viruses and parasites are a significant problem worldwide. At the same time, there is increasing concern with regards to quality aspects such as food authentication or the identification of allergens and genetically modified organisms. The speed, sensitivity, specificity and potential for high throughput and automation of the real-time polymerase chain reaction (qPCR) have made it the benchmark technology for the quantitative analysis of nucleic acids, resulting in its routine use in the agricultural and food industries.

Despite the popularity of qPCR technology, the complexity of its workflow is frequently overlooked and the potential for false positive or false negative results is often underestimated. A reliable qPCR assay depends on a multipart workflow that starts with sample acquisition and handling, template extraction and quality control, design, validation and optimisation of appropriate qPCR primer, probes and reagents, the inclusion of proper controls and ends with accurate interpretation of results.

This complexity can lead to inaccurate or wrong results. Of particular concern are the early steps in the workflow, such as target extraction and quality control, particularly when the target is RNA, as well as questions regarding the reliability and robustness of the qPCR assay itself.

Many qPCR assays are derived from published data. However, these are often contradictory because of frequent use of poorly designed assays, inappropriate data analysis and lack of transparency of reporting. In addition, choice of instrumentation and reagents significantly affects the reliability of qPCR results. The “minimum information for publication of real-time quantitative PCR experiments” (MIQE) guidelines describe the minimum information necessary for evaluating qPCR experiments and so aim to encourage better experimental practice, allowing more reliable and unequivocal interpretation of qPCR results. This presentation will identify the main issues associated with qPCR-based results and suggest appropriate workflows to minimise the problem of inaccurate data acquisition.
Thinking, talking, and understanding the same thing in the same way:
a difficult but necessary task for the 21st century

De Bièvre P.
Independent Consultant on Metrology in Chemistry (MiC)
Former Adviser (1998-2002) to the Director IRMM GEE (Belgium)

Founding Editor and Editor-in-Chief 1994-2011
“Accreditation and Quality Assurance”
Journal for Quality, Comparability and Reliability in Chemical Measurement

B-2460 KASTERLEE (Belgium)
paul.de.bievre@skynet.be

In June 2008, an International Vocabulary of Metrology (VIM) was published on the website of the Joint Committee for Guides in Metrology (JCGM), hosted by the Bureau International des Poids et Mesures in Paris-Sèvres. See http://www.bipm.org/vim

A version with “minor corrections” has been released in 2012 and the preparation of additional “Annotations” is in progress.

Now the terms covering these concepts can be validly translated in other languages because their meaning has been fixed. Especially chemical measurement results can now be described in a way which is commonly understood everywhere in the world by all who perform and use them. This is especially important when large amounts of money may be involved (the value of goods, and of food and drinks). The very import into big trading blocks such as the EU, is increasingly permitted or refused on the basis of the reliability of (chemical) measurement results. Similarly, a common understanding of comparability and equivalence of clinical measurement results is becoming very important for the many people travelling the world, either for business or for leisure. Hence, an IUPAC study was generated on “Metrological Traceability of Measurement Results in Chemistry”. It is available at http://iupac.org/publications/pac/83/10/1873

It is important to understand that a commonly understood Vocabulary is needed for countries using other languages than English. These countries should fully realize that they are at a considerable disadvantage over countries where English is spoken. A recipe for unfair trade.

“To prevent war, be very precise in your speaking”
[Kongfutze 551-479 B.C.]
INVITED LECTURES
Currently available measurement systems for food control

Ulberth F.

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM), Geel, Belgium

The free movement of safe and wholesome food is an essential aspect of the internal market and contributes significantly to the health and well-being of EU citizens and to their social and economic interests. Due to globalisation and the availability of new technological processes the European food and feed sector is becoming more and more complex. National legislation as well as international agreements refer to documentary standards (product as well as procedural standards) to enable international trade as well as to protect the health and interests of consumers. Mutual recognition of measurement data is extremely important to avoid duplication of analyses, thereby reducing costs and resources associated with testing. A diverse array of instruments such as validation and standardisation of methods, laboratory accreditation, participation in proficiency testing schemes, use of reference materials, training, etc., has been put in place in order to build trust in the competence and the measurement capabilities of testing laboratories. Legislation covering official food and feed control in the EU (Regulation (EC) No 882/2004) describes a tiered approach to methods of analysis eligible for official control. If available, standardised methods, preferably those issued by the European Committee for Standardisation (CEN), shall be used for official control purposes. Moreover, official control laboratories shall be ISO/IEC 17025 accredited. Use of validated methods and (certified) reference materials (CRMs), if available, are key-elements for ensuring analytical data quality. Many of the standardized methods available nowadays have been validated by a collaborative study organized and evaluated according to internationally agreed protocols. Methods that have been performance tested in a collaborative study are highly appreciated by users and are a prerequisite for formal standardisation by standardisation bodies such as ISO, CEN, AOAC and certain intergovernmental organisations such as IOC and OIV. The effectiveness of this integrated system depends to a large degree on the harmonised implementation of control, monitoring and enforcement mechanisms across the EU Member States. A network of EU and national reference laboratories was created that should contribute to a high quality and uniformity of analytical data produced by official food and feed control laboratories.

The European Commission’s JRC Institute for Reference Materials and Measurements (IRMM) promotes the harmonisation and standardisation of measurements relating to the study of food, the environment, health and biotechnology by offering an extended range of services (certified reference materials, method validation, proficiency testing schemes, etc) to the measurement community. Next to that the IRMM operates several EU reference laboratories, which also contribute to an effective dissemination of metrological traceability.
Some Actual Challenges with Global Equivalence in the Analysis of Milk and Milk Products

van den Bijgaart H

International Dairy Federation (IDF) - ISO/TC34/SC5 Milk and Milk Products
Qlip BV, Oostzeestraat 2a, Zutphen (The Netherlands), bijgaart@qlip.nl

Dairy is a vital part of the global food system, providing economic, nutritional and social benefits to a large proportion of the world’s population. Globally, around 150 million dairy households, equivalent to 750 million people, are engaged in the production of close to 800 million tonnes of milk. In terms of value, the trade of dairy trade equals over 50 billion Euro. The worldwide consumption of dairy products is expected to increase by 20% or more before 2021.

Objective measurements play an essential role for a swift functioning of the dairy chain:

- in animal production and in payment of raw milk on composition and quality to dairy farmers;
- in control and optimization of dairy processing;
- in monitoring and safeguarding food safety and food quality;
- to check compliance with regulatory limits;
- to check with agreed upon specifications in dairy trade;
- to fulfil consumer demand for information.

IDF is at the forefront in the development and sharing of scientific and technical knowledge, best practices and guidelines on nutritional and sustainability practices and policy, on standards in food composition and on animal health and welfare. In the field of methods of analysis and sampling, IDF closely cooperates with ISO TC34/SC5. The two partners maintain valuable working relationships with other standardization organizations, i.e. Codex Alimentarius, AOAC International, CEN and ICAR. The joint work working programme has culminated in over 170 joint international standards for the determination of a wide range of analytical parameters in milk and milk products.

The presentation will highlight some of the challenges contained in the actual working programme, a.o.:

- a better safeguarding in the global equivalence in milk somatic cell counting through the application of a reference system approach, where the present reference method is lacking precision;
- the exploitation of infrared milk spectra in combination with data from other sources to build new indicators for the efficiency of animal production.
Improve precision in sensory evaluation technique by means of calibration and standard samples. The case of the IOC methods for the classification of the virgin olive oil and the table olives

Giomo A.
Member of International Olive Council Commission of Experts on the Organoleptic Assessment of Olive Oil and IOC Statistics Expert

A test method must be: representative, reproducible and repeatable, it must also: to provide evidence of the adequacy of the properties measured and objective uncertainty associated with them (confirming the representativeness of the method), and placed under control of the influencing factors (confirmation the reproducibility and repeatability of the method).

The measures of the method for the organoleptic assessment of the virgin olive oil (COI/T.20/Doc. N.15/Rev. 6 November 2013) that must be put under control are:

- the result of the classification category (nominal scale) resulting from the calculation of the maximum perceived defect (MPD);
- and the measurement (continuous scale) of the MPD (continuous scale from 0 to 10 cm with one decimal place).

The monitoring of the dispersion of the results during panel regular activity is performed through the calculation of a computed index called CVr% (robust coefficient of variation), the limit value, which it has been superimposed, is 20%. There are other two indices for the panel homogeneity evaluation and for the judge performance evaluation:

- repeatability index;
- deviation index (COI / T.28 / Doc. No 1 September 2007).

The procedure to improve the sensory performance of the panel (COI/T.20/ Doc. No 14/Rev. 4 May 2013) is a continuous training of judges through standard defect samples and a periodic calibration (suggested) of the panel during the current activity with the residues of proficiency test samples.

For the future, it will be on evaluation the feasibility of obtaining the chemical odor standard for training and calibration of the panel as implemented by the method of sensory assessment of table olives (COI/OT/MO/Doc. No. 1).
Peptide based e-nose for food quality and process control

Compagnone D.
University of Teramo, Faculty of Biosciences and Technologies for Food Agriculture and Environment, Mosciano S.A., Italy, dcompagnone@unite.it

Food aromas and flavors are mainly dependent on the original volatile molecules composition and product matrix, buts are also strictly influenced by the production and modification occurring during food processing and packaging. Quality and process control appear then using a non-invasive approach as the detection of headspace volatiles using e-noses. A new generation of e-nose using short peptides immobilised on gold nanoparticles and deposited on quartz cristal microbalances (QCMs) has been developed.

An array of seven sensors with short peptides, different in length and amino-acid sequence, has been used to check the discriminating ability in different types of food samples. After optimization of the measurement procedure (using N\textsubscript{2} as carrier gas) the array was tested with samples from the confectionery (candies), chocolate, olive oil demonstrating an excellent discriminating ability. Classification of olive oil samples, detection of artificially prepared chocolate off-flavoured samples, as well as discrimination among natural and natural-identic flavours and colouring agents in candies was achieved.

A simple computational procedure, was then developed in order to predict the binding ability of the peptides for further development of the gas sensor array. The simulated affinity binding properties of 5 peptides versus 14 volatile compounds belonging to relevant chemical classes was evaluated. The same 14 volatile compounds were then analyzed with the gas sensors. Considering the entire dataset, virtual and experimental results matched in 70% of cases. Best results (up to 93%) were obtained with longest peptides. The molecular modeling approach was proved to be a convenient tool in predicting the behavior of sensors array for gas detection.

These results can open the use of “ad hoc” e-noses for any practical application for food quality and process control.
Sample Preparation and Detection Methods
For Natural or Engineered Nanoparticles in Food

Larsen E.H., Loeschner K. and Correia M.

Technical University of Denmark, National Food Institute, 19, Mørkhøj Bygade, Bldg. B, DK-2860 Søborg, Denmark; ehlar@food.dtu.dk

Accurate and precise characterization of metrics such as size, mass, shape etc. of nanoparticles (NPs) remains a challenging task. In order to determine quantitative metrics that are relevant in food monitoring or in risk assessment, an instrumental separation method like asymmetric field flow fractionation (AFFF, or AF4) coupled on-line to light scattering (LS), UV or fluorescence (FL) spectroscopies and ICP-MS have proven useful and powerful [1,2,3]. Furthermore, additional information obtained by an imaging method such as transmission electron microscopy (TEM) proved to be necessary for trouble shooting or independent confirmation of results obtained from AFFF-LS-ICP-MS. These methods are currently being studied in the EU FP7 project NanoDefine, which in support of the proposed European definition of nanoparticles.

Enzymatic degradation of chicken meat was used to release AgNPs prior to AFFF-ICP-MS analysis in the EU FP7 NanoLyse project. The fractograms showed a major nano-peak (about 80 % recovery of AgNPs spiked to the meat) plus new smaller peaks that eluted close to the void volume of the fractograms. In order to gain further insight into the sizes of the separated AgNPs, or their possible dissolved state, fractions of the AFFF eluate were collected and subjected to ICP-MS analysis in single particle (sp) mode. Moreover, the fractions were also subjected to imaging analysis by transmission electron microscopy. Both methods, which resulted in number-based size distributions of AgNPs in the collected fractions, demonstrated that the early eluting AFFF peaks contained some dissolved Ag-species and the later eluting peaks primarily contained AgNPs of increasing sizes.

In-situ derivatisation by sulfite was used to convert elemental Se deposited in rats’ tissues to the dissolved species selenosulfate-anion. This species, which was detected by anion exchange HPLC-ICPMS following dosage of of Se0NPs or Se(IV), showed that chemical analysis by a classical speciation technique such as HPLC-ICP-MS was useful also in studies of Se nanoparticles [4].

Finally, the possibility of using alkaline pre-treatment of rat spleens prior to sp-ICP-MS analysis of their content of gold nanoparticles (AuNPs) was tested and compared with enzymatic sample preparation [3]. The results showed that the same results, with respect to the obtained number-based size distribution for AuNPs, were obtained for the two preparation methods. In contrast, the alkaline method was by far superior for quantification of AuNPs and was comparable with that obtained by ICP-MS after digestion of the samples in aqua regia. The reason for this is however, not fully understood, and requires further study.

COST Action FA1101 SaffronOmics (Omics Technologies For Crop Improvement, Traceability, Determination Of Authenticity, Adulteration and Origin in Saffron): From Goals to Achievements


1) Aristotle University of Thessaloniki, School of Chemistry, Laboratory of Food Chemistry and Technology, GR-54124, Thessaloniki, (Greece) – tsimidou@chem.auth.gr
2) University of Castilla-La Mancha, Edificio ITQUIMA. Avda Camilo Jose Cela s/n, 13071, Ciudad Real (Spain) - marta.roldan@uclm.es
3) Austrian Institute of Technology GmbH – Konrad-Lorenz Strasse 24, 3430 Tulln, (Austria) – silvia.fluch@ait.ac.at
4) Agricultural University of Athens, Department of Science, Laboratory of Chemistry, 75 Iera Odos, 11855, Athens, (Greece) - mopol@aua.gr; ptara@aua.gr
5) Centro Agrario de Albaladejito, Carretera Toledo-Cuenca km 174, 16194, Cuenca (Spain) - omarsantana@gmail.com
6) Istituto per lo Studio delle Macromolecole (ISMAC), Lab. NMR, CNR, v. Bassini 15, 20133 Milan (Italy) - roberto.consonni@ismac.cnr.it

Saffron is the highest priced agricultural product and a good example of profitability, sustainability, cultural and social values, and high labour demand. The COST FA1101 Action addresses coordinated research on Saffron -OMICS for crop improvement, traceability, determination of authenticity, adulteration and origin to provide new insights that will lead a sound Saffron Bio-Economy. Research groups involved in this Action join experience in different plant sciences. According to the Memorandum of Understanding [http://www.cost.eu/domains_actions/fa/Actions/FA1101] the main goals of this COST network are summarized to the following: (i) Analysis of the Saffron genome by mapping (physical, large fragments) and sequencing (genome, ESTs, SNP polymorphisms, AFLPs, 454 cDNA sequencing).(ii) Analysis of the Saffron metabolome by two strategies: metabolic profile (precise quantification of specific metabolites of interest in Saffron) and metabolic fingerprinting (semi quantitative data acquired by LC-MS or 1H-NMR and (bio)markers revealed by multivariate statistical tools). (iii) Development of robust techniques to be used in traceability, determination of authenticity and origin, and adulteration detection, based on DNA fingerprinting and chemical fingerprinting. (iv) Dissemination of knowledge and know-how (students, researchers, Saffron growers and industry), dialogue with society. Progress in joint research has been achieved mainly through exchange visits of young scientists from laboratories of established experience in saffron quality and authenticity aspects (Spain, Italy, Greece) to laboratories of established experience in new analytical techniques (Greece, Spain, Italy, the Netherlands, New Zealand) and between laboratories involved in Plant Genomics (Spain, Italy, Germany). The major achievements of these Short Term Scientific Missions (STSMs) concerning authenticity and quality control of saffron using metabolomics are discussed. FT-IR, NMR, PTR-MS, LC-MS techniques have been applied successfully to address the abovementioned issues.

Acknowledgement: European Science Foundation (ESF) through the COST Action FA1101 (Saffron -OMICS: OMICS TECHNOLOGIES FOR CROP IMPROVEMENT, TRACEABILITY, DETERMINATION OF AUTHENTICITY, ADULTERATION AND ORIGIN IN SAFFRON. http://www.saffronomics.org) is acknowledged for funding STSMs.
Feasibility study for development of candidate reference material for chloramphenicol in milk powder: preparation and homogeneity testing

Rego E. 1,2), Guimarães E. 1), Rodrigues J. 1), Pereira Netto A. 2)

1) National Institute of Metrology, Quality and Technology (Inmetro), Chemical Metrology Division – Av. Nossa Senhora das Graças, 50, 25250-020, Duque de Caxias, RJ (Brazil) – ecrego@inmetro.gov.br; efguimaraes@inmetro.gov.br; imrodrigues@inmetro.gov.br

2) Federal Fluminense University (UFF), Department of Analytical Chemistry – Outeiro de São João Batista, s/n - 24020-150, Niterói, RJ (Brazil) – annibal@vm.uff.br

Chloramphenicol (CAP) is a broad-spectrum antibiotic isolated from Streptomyces venezuelae and belongs to the amphenicols drug family, which has been widely used in veterinary medicine for treatments of various infections. However, the uptake of chloramphenicol in humans can cause serious hemotoxic effects and consequently, its use was banned for treatment of food-producing animals in the EU [Decision 2002/657/EC] and several other countries, including Brazil. A minimum required performance limit (MRPL) for analytical methods of 0.3 μg kg \(^{-1}\) was fixed [Decision 2003/181/EC] for the detection of residues of CAP in different matrices, including meat and milk.

In order to achieve and safeguard reliable analytical results which are necessary to ensure effective consumer protection, the National Institute of Metrology, Quality and Technology (Inmetro) evaluated the feasibility of a candidate certified reference material production for CAP in milk with target concentration around the MRPL. In order to get incurred milk material, a cow was treated for one day with chloramphenicol succinate 20 mg/kg intramuscularly. Milk samples were collected before and after drug application. The milk was pasteurized and spray-dried. The incurred material was partly diluted with blank milk and the original fat content was reduced 50%. Fifty units with about 2 g of milk powder in amber glass bottles were produced. Half of bottles were deep-frozen and subsequently lyophilized resulting in two lots of twenty-five samples, with and without additional lyophilization. The samples were stored at -80°C. Nine samples of each lot were used for homogeneity studies. The samples were prepared in duplicate and analyzed by liquid chromatography with isotope dilution mass spectrometry (LC–IDMS). The water content was measured by Karl Fischer coulometric titration.

The inhomogeneity contribution for CAP in lots with and without additional lyophilization was 6.6 % and 13.8%, respectively. The study showed the use of spray dryer with additional lyophilization directly in bottles have the best results. The production of this type of CRM is feasible and INMETRO will proceed to the production of this candidate CRM, that can be applied as a tool for quality assurance.
Preparation of Matrix Reference Materials From Milk Powder and Infant Formula Certified for Vitamins A and E

Shehata A.B. 1), Rizk M.S. 2), Farag A.M. 2) and Tahoun I.F. 1)

1) National Institute of Standards, Tersa St, El-Matbah, Haram, P. O. Box: 136 Giza, Code 12211, Giza, Egypt
2) Department of Chemistry, Faculty of Science, Cairo University, Giza 12613, Egypt

Vitamins are important food constituents, which can be present in almost every foodstuff. Food quality and safety depends on food surveillance by reliable quantitative analysis enabled by appropriate quality control. Certified matrix reference materials (CRMs) are versatile tools to support quality assurance and control. However, in the case of vitamins, which are important in various foods, there is a lack of matrix reference materials. Two certified reference materials for the determination of all trans-retinol, retinyl palmitate, α and γ-tocopherol in milk powder and infant formula were developed by the Egyptian National Institute of Standards. This article presents the preparation, characterization, homogeneity and stability testing as well as statistical treatment of data and certified value assignment. The assignment of the certified values and their associated uncertainties were based on the widely used approach of combining data from independent and reliable analytical methods. Certification was carried out by four independent analytical methods in full compliance with ISO Guides 30-35. A very good agreement between the results obtained from all methods was found.
Development of innovative Reference Materials for the agrofood sector

Zappa G., Caprioli R., Gatti R., Zoani C.

Technical Unit for Sustainable Development and Innovation of Agro-Industrial System (UTAGRI)
Casaccia Research Centre - Via Anguillarese, 301- 00123 ROMA (Italy) - claudia.zoani@enea.it

By means of Italy and EU-funded Research Projects, ENEA has developed real plants for Reference Materials (RMs) preparation with some distinctive features and is carrying out R&D activities on the development of new RMs. With these plants, we have already prepared some monoelemental solutions with concentration values traceable to SI and many RMs of agrofood products, such as: lyophilized tomato paste, peeled tomatoes, strained tomatoes, champignon mushrooms, strawberry fruits, broccoli; lyophilized and partly skimmed milk, swine muscle, bovine muscle, bovine leaver, fish muscle and mussel tissue (in cooperation with EU RL-CEFAO); fluid and lyophilized honey; rectified concentrated grape must; fish feed (in cooperation with IZS AM); lake sediment. In addition, we are applying innovative approaches for the development of new kinds of RMs. In fact, besides reference solutions and matrix-RMs obtained following conventional procedures, also different kinds of RMs could be very useful in order to cover different phases of the measurement process and make RMs more representative, easier in use and less subject to alterations. Furthermore, RMs with many well-known different characteristics are more and more necessary, in order to set up multiparameter techniques for traceability and authenticity studies. Therefore, in order to fill the gaps and meet the needs of new RMs, we are studying the possibility to realize: Single Use-RMs; Double Phase-RMs; Multiparameter-RMs; Procedural-RMs.

Single Use-RMs (SU-RMs) are RMs supplied in a pre-weighted form to be directly submitted to the analytical procedure. They can be supplied in form of pellets of lyophilized agrofood matrices - very useful for avoiding segregations, humidity, contaminations and to facilitate powder weighing - or in form of capsules (e.g. encapsulated pre-weighted liquid honey), skipping the lyophilization step, with great advantages in terms of preparation costs and time and useful for avoiding the difficulties on weighing and homogeneous pick up of the test portion. Double Phase-RMs (DP-RMs) are RMs split, during the preparation procedure, into their two aqueous and anhydrous components and supplied in their two separated components (liquid and solid phases) to be re-combined before use. DP-RMs permit to have highly representative RMs and could represent a good solution when the analyte is volatile or degradable (e.g.: for food flavor analysis). Multiparameter-RMs are intended for use in multiparameter determinations, qualitative analyses and identity studies, through the definition of elemental, molecular and/or genetic markers or patterns for traceability of food products. Procedural-RMs are RMs representative of the sample at different analytical treatment stages (e.g.: RMs of extracts of volatile compound or secondary metabolites for defining the aromatic profile, RMs of extracts of mycotoxins). They could represent a solution when it is impossible to obtain RMs stable with respect to the parameters of interest and give advantages in term of ease of use and stability.
Moisture determination in food samples

Rolle F., Sega M., Verdoja A., Beltramino G., Fernicola V.

INRIM, Thermodynamic Division – Strada delle Cacce 91, Torino (Italy) - f.rolle@inrim.it

Moisture analysis of foodstuffs is significant for the safety and the sensory quality of processed food, as well as for defining the storage conditions and the shelf life of food. It is a determining factor for many physical and chemical properties of materials, which depend on their water content. In this sense, moisture analysis has also influence on the design of food process technology where the economic importance of moisture measurements is clear. Indeed, the products sold beyond international borders must meet the requirements of the receiving country. In addition many products, especially in food and agricultural fields, are sold on a dry matter basis [1].

The Istituto Nazionale di Ricerca Metrologica (INRIM) is developing the metrology infrastructure for providing traceability to moisture in material measurements. Such efforts are carried out within an European joint research project (EMRP SIB64 METefnet) with several other EU National Metrology Institutes. Part of this activity concerns the set up of metrologically-sound methods for the determination of water content in food samples, by using two indirect analytical methods: coulometric Karl Fischer titration (cKF) and Evolved Water Vapour analysis (EWV).

cKF is an electrochemical technique based on a selective reaction for water, using the iodine production in an electrochemical cell, which is stoichiometrically equivalent to the water present in the sample. EWV determines the amount of water in a sample by oven-heating it and thus detecting the evolved water vapour carried by a dry gas stream by means of a phosphorous pentoxide electrochemical sensor.

As a starting point, the optimisation of operating parameters of both instruments was carried out by using reference materials and was followed by preliminary studies with real matrices. Simple sugars (e.g. fructose) and milk powder (due to its high content of lactose) were chosen.

The choice of the matrices is due to the fact that determination of water in sugars can be useful for determining properties and biological origin of some foodstuffs (e.g. honey), while lactose is present in almost all dairy products and in infant formulas.

These techniques will be used in parallel for water determination in food samples and the results cross-compared. Particular attention is given to the establishment of metrological traceability and to uncertainty evaluation.

References:
Next generation MS based methods applied to multi-allergen screening in foods

Monaci L., Pilolli R., De Angelis E. and Pascale M.
Institute of Sciences of Food Production, National Research Council of Italy, Via G. Amendola 122/O, 70126 Bari, Italy (ISPA-CNR)

Sensitive and high throughput mass spectrometry based methods are strongly required as screening tool to assess the presence of chemical contaminants in foods. As far as food allergen monitoring is concerning, a challenging issue is represented by complex food matrices where the antibody-based kits commercially available might encounter objective limitations consequently to epitope masking phenomena or epitope modification occurring upon application of thermal treatments. The development of a liquid chromatography - tandem mass spectrometry (LC-MS/MS) method based on the extraction and simultaneous detection of soy, egg, milk and nuts allergens in a cookie chosen as reference food matrix, will be presented in this communication. Thanks to the innovative configuration and the versatility shown by the dual cell linear ion trap MS system implemented in this work, the most intense and reliable peptide markers were first identified by untargeted survey experiment, and subsequently utilized to design an ad hoc multi-target SRM method, basing on the most intense transitions recorded for each selected precursor peptide. Aiming at achieving a sensitive multi-allergen method, both the extraction procedure and the MS acquisition scheme were duly optimized and a method capable of tracing 5 different allergenic ingredients in cookie was finally developed. In addition, the effect of baking on the final peptide detection has been also taken in consideration and investigated.

The monitoring of a total of ten peptides, with two most sensitive peptide markers/protein selected, allowed to retrieve quantitative information about the presence of each allergenic protein in the food matrix under analysis reaching limits of detection comprised between 5 and 15 µg/g, depending on the specific allergen.

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Hidden allergen labelling and food metrology: a chance to solve the problem

Giuffrida M.G. 1), Lamberti C. 2), Cavallarin L. 3)

1) CNR-ISPA, Bioindustry Park S. Fumero, Via Ribes 5, 10010 Colleretto Giacosa (TO) (Italy) – gabriella.giuffrida@ispa.cnr.it
2) CNR-ISPA, Bioindustry Park S. Fumero, Via Ribes 5, 10010 Colleretto Giacosa (TO) (Italy) – cristina.lamberti@ispa.cnr.it
3) CNR-ISPA, Largo Braccini 2, 10095 Gruglisco (TO) (Italy) – laura.cavallarin@ispa.cnr.it

Food allergy is an increasing pathology. Since 2003 European Community is trying to protect food allergy sufferers by providing comprehensive ingredients listing information, to allow the characterization of food they need to avoid. Validated analytical methods for hidden allergen investigation in different food matrices are needed.

Mass Spectrometry-based methods are now approaching the field of current routine food allergen quantification, which is currently mainly based on immunochemistry techniques (ELISA). However, ELISA technique for allergen detection and quantification could be affected by cross-reactivity and/or by unpredictable effects caused by food processing. On the contrary, MS methods offer the possibility to confirm the molecular identity of the allergen and obtain a validated quantification. One step further for MS techniques is the possibility to achieve metrological traceability of the allergen throughout the absolute quantification. Up to now, these concepts are very poorly taken in account but it is clear that, for example, the requirement for certified reference materials developed for hidden allergen detection is now of a paramount importance. Certified reference materials could improve the development of a validated method. Actually, several papers have been published so far, proposing MS-based methods for the detection of allergens in food, but very few of them showed a validated approach. It is clear that a metrological approach in the field of food allergen quantification could facilitate the harmonization and standardization of the current analytical techniques.
Analytical determination of inorganic arsenic in food: from research to legislation

D’Amato M., Aureli F., Raggi A., Cubadda F.

Istituto Superiore di Sanità - National Health Institute, Department of Food Safety Safety and Veterinary Public Health – Viale Regina Elena 299, Rome (Italy) – francesco.cubadda@iss.it

Arsenic occurs in the environment in different inorganic and organic forms. Inorganic arsenic is more toxic as compared to organic arsenic and the need arises to discriminate the different arsenocompounds in food by speciation analysis in order to appropriately assess the risk of dietary arsenic exposure. Especially in seafood, most of the arsenic is present in organic forms that are less toxic or virtually non-toxic (arsenobetaine). Consequently, a risk assessment not taking into account the different species but considering total arsenic as being present exclusively as inorganic arsenic would lead to a considerable overestimation of the health risk related to dietary arsenic exposure.

Analytical methods for arsenic speciation and selective determination of inorganic arsenic have been developed in research laboratories in the last twenty years, mainly based on HPLC-ICP-MS. Following the ESFA and JECFA risk assessment of arsenic in food in 2009, the process leading to the introduction of legal limits for inorganic arsenic in foodstuffs has initiated at both the European and the international (Codex) level. In the EU, Regulation EC 1881/2006 will be revised to include maximum limits for inorganic arsenic in rice by the end of 2014.

The analytical issues related to the determination of inorganic arsenic in food will be discussed, along with latest developments in method standardization and the outcome of recent interlaboratory comparisons aimed at assessing the performance of European laboratories.
Development of a method for the determination of Cadmium (Cd) and Lead (Pb) in honey by Graphite Furnace Atomic Absorption Spectrometry


Istituto Superiore di Sanità, European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO), Department of Food Safety and Veterinary Public Health - Rome, Italy – andrea.colabucci@iss.it

One of the emerging issues regarding the analysis of metals in food is the determination of Cadmium (Cd) and Lead (Pb) in honey. Maximum Levels are not set for these element/matrix combinations in the relevant EU legislation (CR 1881/2006), but this kind of analysis is foreseen in most of the National Residue Control Plans that EU Member States have to perform according to Directive 96/23.

The lack of certified reference materials and the difficulties related to the sample preparation for such a sugary matrix led the EURL-CEFAO to plan the organization of a Proficiency Test on honey with the aim to test the ability of the National Reference Laboratories of its network.

In this framework, a method on direct determination of honey by GF-AAS after dissolution in an aqueous mixture of 2.5% HNO₃(v/v) and 12.5% H₂O₂(v/v) was in-house validated. The basic validation scheme foresaw the assessing of repeatability and of the limits of detection and quantification on a preliminary batch prepared with similar concentrations as those that would be proposed for the PT (about 20 and 100 µg/kg for Cd and Pb, respectively).

The results obtained under repeatability conditions were compared with those produced both by GF-AAS and ICP-MS after microwave digestion of the sample and no statistically significant differences were found.

The method was then fully validated according to the EURL procedure at the values of interest. Accuracy was estimated on the sample used for the 19th PT taking into account the assigned value obtained by consensus of the participants.

All this considered, the method proved his efficacy not only in terms of analytical capability, but also in terms of low costs and time saving. Moreover, compared to a microwave sample preparation, the environmental impact was also decreased being the amount of acid used in the preparation considerably reduced.
A new approach for non-destructive measurement of quality and maturity parameters of peach fruits

Matteoli S. 1), Remorini D. 2), Corsini G. 1), Massai R. 2), 3)

1) Department of Information Engineering, University of Pisa – Via G. Caruso 16, 56121 Pisa (Italy)
2) Department of Agriculture, Food and Environment, University of Pisa – Via del Borghetto, 80 - 56124 Pisa (Italy)
3) Interdepartmental Research Center for Nutraceuticals and Food for Health “Nutrafood”, University of Pisa

In order to strike a balance between ensuring the highest eating quality for the consumers and enabling marketing flexibility, most fruits are generally harvested when mature but not when fully ripe. Measures of quality and maturity stage are then performed just after harvest, at the packing facility or in the warehouse, to assess fruit shelf-life and sort fruit towards different distribution channels according to some organoleptic properties. Several indicators may serve this purpose, such as flesh firmness, soluble solid contents, and titratable acidity, which are generally measured with laboratory analyses (e.g. penetrometer, refractometer, pH-meter) entailing sample fruit destruction.

Non-destructive measurement techniques make use of remote/proximal sensors, e.g. high-resolution spectrometers, to retrieve fruit properties by suitable processing of the fruit reflectance spectra, thus avoiding fruit damage and waste and, in turn, allowing faster, repeated measures on each fruit of the batch. Reflectance indexes (e.g. band ratios) are typically extracted from some fruit spectra and correlated to destructive measures of specific fruit properties via linear regression. The regression coefficients are then used to infer the properties of the remaining fruits. Examining just a few spectral bands, the rich information content enclosed in the spectra is not fully exploited. Furthermore, the fruit samples used to learn the regression coefficients may originate from different rootstocks, which influence fruit quality and maturity evolution.

In this work, a new approach is adopted to fully exploit the spectra information content. Methods based on multivariate statistical models for the fruit spectra are employed to classify the fruits with respect to the rootstock. Then, the classification outcome is used to drive the linear regression procedure in a class-conditional fashion, both using the band ratios and the full spectra as regressors. Experimental results featuring peaches originating from four different rootstocks show that the proposed approach is promising for improving non-destructive measurement of fruit quality and maturity parameters.
Elemental composition and stable isotope techniques used to trace milk and dairy products

Ogrinc N, Lojen S., Horvat M.

Jožef Stefan Institute, Department of Environmental Sciences, Jamova 39, 1000 Ljubljana, Slovenia – nives.ogrinc@ijs.si, sonja.lojen@ijs.si, milena.horvat@ijs.si

Food authenticity and traceability of origin have been given high priority in the recent years. Due to their high nutrient content milk and dairy products represent an important part in the healthy balanced diet. Stable isotope measurements combined with multi-elemental analysis are used to characterize Slovenian milk according to geographical origin. These data present the basis for a database of authentic samples of milk in Slovenia. Slovenia is a small country regarding surface area, but pedologically and climatically diverse, and thus it offers an ideal area for studying the natural factors which govern the isotopic distribution in milk and its products. The obtained information should be used to increase the transparency of milk and dairy products supply chain and provide information related to authenticity.

This research represents a part of the “ISO-FOOD” ERA Chair for isotope techniques in food quality, safety and traceability. The research activities of the ERA Chair will focus on interdisciplinary topics combining the expertise in radiochemistry, stable isotope biogeochemistry, electron microscopy and microanalysis and combined analytical techniques (e.g. organic compound profiling, NMR chemical profiling), as well as multivariate statistics and data management, to develop universally available analytical strategies to verify the origin (provenance) of agricultural produces and foodstuffs, agricultural practices, food contamination, contamination sources and their safety. Nuclear and nuclear-related techniques for food traceability, which are tailored for fingerprinting of food provenance and authenticity, will be developed and combined with elemental markers and biomarkers.
Analytical Differentiation of the Geographical Origin of Milk Samples by their Fatty Acid Composition

Werteker M. 1), Huber S. 2), Motie A. 1), Rossmann B. 3), Schreiner M. 2)

1) Austrian Agency for Health and Food Safety, Institute for Feed and Animal Nutrition – Spargelfeldstraße 191, 1220 - Vienna (Austria) – manfred.werteker@ages.at
2) University of Natural Resources and Life Sciences, Institute of Food Science – Muthgasse 18, 1190 - Vienna (Austria) – matthias.schreiner@boku.ac.at
3) Austrian Agency for Health and Food Safety, Institute for Food Safety – Spargelfeldstraße 191, 1220 - Vienna (Austria) – birgit.rossmann@ages.at

Reliable analytical methods for the verification of authenticity are not only an important precondition for the protection of regional and traditional food production against fraudulent competitors, but also a defense of the consumers against faked products.

Aim of the present study was the development of a procedure for the analytical differentiation of cow milk samples according to their geographical origin. 30 samples of milk from Tyrol and 31 samples from Lower Austria were collected by the governmental food control. The milk fat of the samples was extracted according to a modified Roese-Gottlieb procedure and transmethylated by methanolic KOH. The fatty acid methyl esters (FAME) were extracted with n-hexane and analyzed by gas chromatography (GC). An HP-88 capillary column (100 m) and a temperature gradient from 60°C to 240°C were applied. The results were evaluated by principal component analysis (PCA, Unscrambler©, Version 10.2) using the standardized evaluation mode.

109 FAMEs could be separated by the applied analytical methods, whereof approximately one third could be identified by use of external standards. By PCA a clear distinction between the two groups of milk samples was possible which could be improved significantly by use of higher principal components up to PC5. By evaluation of correlation loadings an estimation of the contribution of distinct FAMEs to the characterization of samples was possible.

The high number of analyzed compounds provides a high potential of this method to make differentiations of products under various aspects as geographical origin, process conditions and others. In the presented study it was not possible to prove, if the differentiation of the samples was due to the different areas of origin, the breed of cattle or the method of feeding, a deficit, which could be resolved by validation of the method with an extended assortment of properly defined samples.
Validation of methods for H, C, N and S stable isotopes and elemental analysis of cheese: results of an international collaborative study

Camin F.1), Bertoldi D.1), Santato A.1), Bontempo L.1), Ziller L.1), Stroppa A.2), Larcher R.1)

1) Fondazione E. Mach (FEM), Via Mach 1, 38010 San Michele all’Adige (TN) ITALY – federica.camin@fmach.it
2) Consorzio Tutela Grana Padano, Via XXIV Giugno 8, 25010 San Martino Della Battaglia Desenzano del Garda (BS) ITALY

The results of an international collaborative study performed according to the IUPAC and ISO protocols, are here presented. Aim of the study was to determine the performance characteristics of methods for isotopic (H, C, N and S stable isotope ratios) and elemental (Li, Na, Mn, Fe, Cu, Se, Rb, Sr, Mo, Ba, Re, Bi, U) analyses of cheese, currently used for establishing the authenticity of PDO cheeses. The average standard deviations of repeatability (sr) and reproducibility (sR) and the accuracy of both methods resulted satisfactory, if compared to the results of the same methods applied to other food matrices. The validation data obtained here can be submitted to the standardisation agencies to obtain official recognition for the methods, which is fundamental when they are used in commercial disputes and legal debates.
Olive oils: looking for reference materials for an effective international harmonisation of control methods

Conte L.
Dept of Food Science, University of Udine – Via Sondrio 2/a 33100 Udine Italy lanfanco.conte@uniud.it

Olive oils are important products for European economy and nowadays several trials (someone successful and someone less) of production outside from Europe or Mediterranean basin had been attempted,

Its high value had been established on the basis of its peculiar composition, e.g. the presence of compounds named “biophenols” that recently had been accepted to be mentioned in the “health claim” of olive oil.

Such an high value make this oil subject of a number of frauds, performed both by mixing it to lower value oils and by selling as high brand oil (extra virgin) oils extracted by olives, but of lower quality.

A very structured net of chemical and sensory control had been established through a number if years and this surely helps in ensuring consumers about quality and purity control, however, on the side of method standardization and validation, some drawbacks still exists, depending on poor work carried out on the hand of reference standards.

In this lecture, some cases will be highlighted and some experimental proposed will be presented.

For what concerns chemical analysis, the quantitative evaluation of erythrodial and uvaol had been proposed several years ago and nowadays some new proposal will be developed, while on the side of polyphenols, the validated method maybe not adequately performing to satisfy request of Reg(EU)432/12 on health claims.

In this case, a complete change of approach is probably necessary, as standards are available for few compounds, only.
Measurement uncertainty in the determination of stigmastadienes in olive oil

Bešter E.1), Valenčič V.2), Bučar-Miklavčič M.3), Miklavčič Višnjevec A.4), Butinar B.5)

1) UP ZRS IZO – Zelena ulica 8 c, Izola (SLOVENIA) – Erika.Bester@zrs.upr.si, Vasilij.Valencic@zrs.upr.si, milena.miklavcic@guest.arnes.si, Ana.VisnjevecMiklavcic@zrs.upr.si, Bojan.Butinar@zrs.upr.si

The method for the determination of stigmastadienes is particularly suited for detecting the presence of refined oils in virgin olive oil, because refined oils contain stigmastadienes and virgin oils do not. Therefore, it is very important to be able to measure very low concentrations of stigmastadienes and in this respect to establish the measurement uncertainty properly. Measurement uncertainty obtained with the aid of the model equation is not appropriate in this case. Moreover, another approach suggested by EURACHEM / CITAC Guide CG 4, where the measurement uncertainty is assessed on the basis of collaborative trial results, was not suitable for the purpose in the concentration area up to 0.33 mg/kg, because the assessed measurement uncertainty was relatively high. The proper assessment of uncertainty in this concentration area is crucial, because the maximum permissible levels of stigmastadienes in virgin olive oil were set in this concentration area (Reg. UE 1348/2013). Based on these facts the measurement uncertainty was assessed according to the procedures set by VAM project 3.2.1. The main stages in the process were specification, identification of uncertainty sources, quantification of uncertainty components and the quantification of combined uncertainty. The determination of stigmastadienes is carried out in 6 stages: saponification in the presence of internal standard solution, liquid-liquid extraction of unsaponifiable matter, separation of steroidal hydrocarbon fraction by column chromatography on silica gel and analysis by capillary gas chromatography. Based on the identification and quantification of the significant contributors of uncertainty using the Plackett-Burman matrix and the quantification of precision and accuracy of the method, the relative expanded uncertainty of the method amount to 13 % in the measurement area up to 0.33 mg/kg. Hence, the method applied for the assessment of the uncertainty was appropriate for the scope of the established limit in the regulation.
Aromatic stability and degradation of Extra Virgin Olive Oil

Caciotta M.¹, Giarnetti S.¹, Leccese F.¹, Orioni B.¹, Oreggia M.², Rametta S.¹

¹ Roma Tre University, Science Department – Via della Vasca Navale n.84 00146, Rome (Italy) – maurizio.caciotta@uniroma3.it
² E.V.O. srl – Via Positano n.100 00134, Rome (Italy)

The Panel Test (PT) is performed by certified assayers and recognized by European Union. From a statistic point of view, it is a methodology sufficiently objective and repetitive so realizing a Metrology. The aim of this work is the study of extra virgin oil olive degradation and stability.

The method uses a cascade of two neural networks which inputs are an essential set of quality parameters coming from the Panel Tests made on extra virgin olive oils samples and gas chromatographic analysis on them. Nine extra virgin olive oil samples have been examined. The results of the PT are classified by an unsupervised neural network and related on a Kohonen Map. Then, they are correlated to Gas Chromatographic analysis of olive oil by the Multi Layer Perceptron with Back-Propagation (MLP-BP), a supervised neural network. The MLP-BP is able to classify a sample different from those used for the learning.

The olive oil degradation is analyzed by comparing the results on the same sample performed in different periods. Instead, the aromatic stability is observed by comparing different sample from the same producer.

The degradation has been observed by the change of the intensity of the signals: the reduction of aromas "fruity" and "green grass" is detected, typical flavors of freshness of the product, in in some oils the flavors "spicy" and "mint" appear.

As regards the aromatic stability, many products have maintained the bearing characteristics while the few variations are related to the disappearance of aromas of flowers and/or apple.
Novel and rapid molecular method for enumeration of bacterial pathogens contaminating food at low levels

Pasquali F., De Cesare A., Manfreda G.

University of Bologna, Department of Agricultural and Food Sciences – via del Florio 2, Ozzano dell’Emilia (Italy) – frederique.pasquali@unibo.it

At present food industry have to verify the compliance of their food products according to the EU Commission regulation 2073/2005 in which the number and size of samples, the point of collection of samples and the reference analytical method to be used for identification and/or enumeration of pathogens in different food matrixes are specified. The reference methods are ISO standard traditional cultural-based methods which are labor-intensive methods producing results in no less than 4-5 days. Moreover for pathogens contaminating specific foods at low levels, a zero tolerance approach is often applied and the absence of the pathogen in a defined number of samples is required (i.e. Salmonella in meat, egg and dairy products; *Listeria monocytogenes* in food products intended to be consumed by vulnerable people). However for quantitative risk assessment a rapid enumeration of those pathogens in food is recommended. Real-Time PCR is a novel and rapid molecular method for the identification of pathogens in food. In particular the application of a probe-based chemistry and the inclusion of an Internal Amplification Control make real-time PCR a reliable, accurate and specific method for rapid identification without the need of a confirmation step. However this method cannot be applied for the direct enumeration of low level pathogens in food due to the relatively high detection limit (approx. $10^3$-$10^4$ CFU/ml). This point might be overcome by the inclusion of a short pre-enrichment step prior to DNA extraction and PCR. During this step pathogen bacteria grow to their exponential phase and are therefore both detectable and quantifiable. An enrichment qPCR assay for the detection and enumeration of *Salmonella spp.* on table eggs will be described as example.
Next generation sequencing for quantitative measurements in food molecular microbiology

Puglisi E. 1), Bassi D. 2), Polka J. 1), Rebecchi A. 2), Cocconcelli P.S. 1), Morelli L. 3)

1)Istituto di Microbiologia, Facoltà di Scienze Agrarie, Alimentari ed Ambientali, Università Cattolica del Sacro Cuore - Via Emilia Parmense 84, 29122 Piacenza (Italy) – edoardo.puglisi@unicatt.it
2)Centro Ricerche Biotecnologiche, Università Cattolica del Sacro Cuore - Via Milano 24, 26100 Cremona (Italy)

The microbiological composition plays a pivotal role in the production, final composition and safety of fermented foods. Such composition can be assessed by means of culture-based and molecular methods, where the latter are necessary to analyze non-culturable species. PCR-DGGE has been for several years the most applied technique in molecular microbiology: advantages include the relatively low cost and ease of use and the possibility of processing samples altogether; the method is however only semi-quantitative and the resolution power is limited to a few dominant species.

Recent advancements in next generation sequencing technologies (NGS) are revolutionizing our comprehension and quantitative measurements of microbial communities. The initial approach is the same as for PCR-DGGE, i.e., the extraction of microbial DNA from food and the PCR amplification of 16s rRNA hypervariable regions. The PCR amplicons instead of being analyzed on a denaturing gel can be instead sequenced on NGS platforms such as Illumina, which provide multi-million reads of hundreds of base pairs.

Here we present the validation of this approach on two case studies, one dealing with typical Italian fermented dry-sausages (salami) from different producers and ripening stages, the second with Grana Padano cheese produced with and without the use of lysozyme. A total of 722,196 high-quality reads were obtained for the salami, 1,240,592 for the cheese case study. More than 95% of these sequences could be classified up to the species level, and the number of species identified was very high: in the case of salami, 33 different species of lactobacilli were quantified, whereas on the same samples PCR-DGGE identified much less. Comparisons of RT-PCR measurements also showed that NGS has the potential for a quantitative assessment of hundreds of species altogether: further studies will be useful in order to validate and standardize the method for metrological measurements in food science.
The range of phytobased on medicinal plants is large and constantly growth. For insurance quality, effectiveness and safety of these products is necessary to identify and authenticate plants. The quality of herbal drugs, obtainable both from wild or grown plants, is maintained by proper sampling, cultivation, harvesting, drying, fragmentation and conservation procedures. Events of sophistication, adulteration or deterioration in the herbal drugs, both fraudulent or incidental, are not rare. Recognition of medicinal plants is made traditionally by chemical methods or botanical methods. The type of botanical analysis traditionally carried out for the identification of plants in the species level, however, have limitations related to the fact that in some raw materials that have been treated, not shredded or altered is no longer possible to recognize; it is also not possible to obtain precise indications of such botanical area geographical production nor on the variety used as raw material. To overcome these problems, have been developed molecular methods based on genomics, which exploit the barcode DNA for the identification of medicinal plants. Molecular methods based on DNA, which exploit the PCR or its variants, allow to uniquely identify and reproducible the genus, species and variety of source of the raw material even when this has been processed (dried, juice, crushed, pulverized...) so also in finished or semi‐finished product. The present work describes the process for the development, optimization and validation of methods for the authentication of medicinal plants on different materials (leaves, roots or extracts) from the choice of the target genes to the interpretation and validation of the final results.
In field implementation of risk based metrics to promote food safety

De Cesare A., Bovo F., Pasquali F., Manfreda G.

University of Bologna, Department of Agricultural and Food Sciences – via del Florio 2, Ozzano dell’Emilia (Italy) – alessandra.decesare@unibo.it

In 2002, Regulation (EC) 178 of the European Parliament and of the Council states that, in order to achieve the general objective of a high level of protection of human health and life, food law shall be based on risk analysis. Commission Regulation No 2073/2005 on microbiological criteria for foodstuffs requires that food business operators ensure that foodstuffs comply with specific microbiological criteria, defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of micro-organisms, and/or on the quantity of their toxins/metabolites, per unit(s) of mass, volume, area or batch. The same Regulation describes a food safety criterion as a mean to define the acceptability of a product or a batch of foodstuff applicable to products placed on the market and a process hygiene criterion as a mean indicating the acceptable functioning of the production process. Both food safety criteria and process hygiene criteria are not based on risk analysis. On the contrary, the metrics formulated by the Codex Alimentarius Commission in 2004, named Food Safety Objective (FSO) and Performance Objective (PO), are risk based and fit the indications of Regulation 178/2002. In the context of food safety, an appropriate level of human protection (ALOP) is a governmental expression of its national public health goal for foodborne risks, presenting a level above which the risk is unacceptable. A FSO has been defined as the level of a hazard (in terms of concentration and/or frequency) that can be tolerated in the final product when it is consumed. Because, conceptually, an FSO should be derived from the ALOP, there is a need for additional milestones that ensure the appropriate frequency and/or concentration of a hazard at specific steps along the food chain. This need has been addressed with the PO representing the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain before consumption that provides or contributes to an FSO or ALOP, as applicable. The sources of uncertainty on the definition of a PO can be the inputs associated with the production-to-consumption process, the uncertainties in the dose-response model, and the assumed industry responses to efforts to control risk at the level of production and processing/slaughter. A possible reason for the PO targets not being use could be that there is a little guidance on how to establish them and how to implement them in practice linked to each other. In this context, the contribution of the BASELINE EU project has been to suggest a scientific and statistically based approach to derive POs for different food/biological hazard combinations and such approach will be presented at the conference.
Metrological traceability of PAHs measurements in food matrices

Rolle F. \textsuperscript{1)}, Perini S. \textsuperscript{1)}, Sega M. \textsuperscript{1)}

\textsuperscript{1)}Istituto Nazionale di Ricerca Metrologica - INRIM, Thermodynamics Division – Strada delle Cacce 91, Torino (Italy) – f.rolle@inrim.it

Food safety is nowadays among the hottest issues on which the concerns of various disciplines (e.g. basic science, economy, industrial and health sectors) are focused. For these reasons, it is necessary to have reliable methods and instrumentation to perform accurate and efficient quality controls, in order to prevent adverse effects on the consumer health. The contribution of metrology is fundamental, as it provides the means to obtain accurate and traceable measurement results, which can be compared even if determined in different conditions, places and times.

Many organic micropollutants have been classified as Persistent Organic Pollutants (POPs) by the United Nations Environment Programme and twelve classes of POPs have been considered until now. Among them, Polycyclic Aromatic Hydrocarbons (PAHs) play an important role due to their relevance under a toxicological point of view. Considering that food is the major way of PAHs intake, metrological traceability of these measurements in food matrices is a relevant issue due to the potential adverse effects that PAHs may have on human health.

At the Istituto Nazionale di Ricerca Metrologica (INRiM), an analytical procedure was developed for the determination of PAHs in infusion herbs, namely green tea (\textit{Camelia sinensis}), decaffeinated green tea and yerba mate (\textit{Ilex paraguariensis}). A metrological traceability chain was established for all the procedure steps, starting from the sample preparation to the quantification of analytes. The uncertainty evaluation of the results was also carried out, taking into account the significant sources.

Eight PAHs were chosen as analytes on the basis of their presence both in the US EPA list of priority PAHs in the environment and in the EU list of priority PAHs in food. The herbal samples were spiked with a proper Certified Reference Material, NIST SRM 1647e, extracted by Soxhlet extractor and quantified by gas-chromatography coupled with mass spectrometry (GC-MS) under metrologically traceable conditions. Due to the absence of a standard method for the determination of PAHs in the above cited matrices, a part of the work was also dedicated to the validation of the analytical procedure.
Measurement Uncertainty for Dioxins and PCBs by Isotope Dilution Mass Spectrometry

**Scortichini G.**¹, Iamiceli A.L.², Ceci R.¹, Diletti G.¹, Abballe A.², Iacovella N.²

¹Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise – Campo Boario 1, Teramo (Italy) – g.scortichini@izs.it
²Istituto Superiore di Sanità – Viale Regina Elena 299, Roma (Italy) – annalaura.iamiceli@iss.it

Whenever decisions are based on analytical results, it is generally acknowledged that their fitness for purpose cannot be correctly assessed without measurement uncertainty (MU). Estimation of MU is not only required by ISO/IEC 17025 for testing laboratories but it is also mandatory for compliance assessment of feed and food regarding the content of chemical contaminants. Dioxins (PCDD/Fs) and PCBs are persistent organic pollutants for which maximum limits (MLs) have been established in feed and food by European Union (EU) legislation. In this context, MU is used to assess if analytical results are compliant to MLs.

The Guide to the Expression of Uncertainty in Measurement (GUM), published by ISO, established general rules for evaluating MU. The concepts of the ISO guide were applied to the analytical chemistry by EURACHEM. The GUM component-by-component approach (i.e. bottom-up) is proposed for the calculation of MU associated to PCDD/Fs and dioxin-like PCBs (DL-PCBs) determined by isotope-dilution mass spectrometry.

A preliminary study was conducted in order to identify the most significant sources of uncertainty. The approach distinguished Type A components derived from the statistical distribution of the quantity values from series of measurements; e.g. within-laboratory reproducibility, calibration curve, and Type B components evaluated from probability density functions based on experience or other information; e.g. standard solution concentration, volume of internal standard added to the sample. Overall bias (best estimated by repeated analysis of relevant CRMs) and data obtained from proficiency testing were also included in the uncertainty estimation. Other factors subjected to daily variation, such as mass response linearity (variation of RRFs), calibration curve drift and analyte-specific detection limit in the actual sample, were taken into account for a comprehensive MU evaluation.

By definition, MU is associated with a measurand (e.g. a congener concentration). However, MLs are expressed in Toxic Equivalents (TEQ) units, therefore MU must be also expressed in TEQ for decision-making and compliance assessment. Among different approaches to calculate MU associated to TEQ values, the square root of the sum of squares (RSS) considering each relevant congener provided the best estimate based on a large quality control data set.
A metrological approach to evaluate epidemiological forecasting models

Sanna F.1), Bellagarda S.2), Roggero G.2), Merlone A.2)

1) IMAMOTER-CNR - Istituto per le Macchine Agricole e Movimento Terra – Consiglio Nazionale Ricerche, Strada delle Cacce, 73, 10135, Turin, Italy – f.sanna@ima.to.cnr.it
2) INRiM - Istituto Nazionale di Ricerca Metrologica, Strada delle Cacce, 91, 10135, Turin, Italy – s.bellagarda@inrim.it, g.roggero@inrim.it, a.merlone@inrim.it

The evolve of the vineyard diseases, such as Grapevine Downy Mildew (*Plasmopara viticola*), are strictly depending by temperature, humidity and rain. The pathology is currently controlled with the massive use of fungicides, which has considerable economic costs, negative effects on environment, human health and wine quality.

For a correct defense against pathogen attacks, it is necessary to know the incubation period to act promptly and reduce the use of chemicals. In order to identify high-risk and fungicide sprays periods, several forecasting models have been proposed. These are useful tools which may assist in agricultural management risks and require accurate knowledge of meteorological variables such as temperature, humidity and precipitation. To date, the models used have improved the quality of the output data, but none of them considered the quality of the input data in terms of evaluation of measurement uncertainty and traceability of the sensors of the weather stations.

In situ calibration of weather stations installed in agricultural sites is usually performed by comparison. This procedure was metrologically evaluated and showed relevant weak points. Standard sensors are not always made to operate in open air, it is not possible to cover the whole range for the quantities, thus it is not possible to evaluate linearity and uncertainties for several sensors over the whole range and the evaluation of the mutual influences between parameters is not achievable. A calibration procedure for automatic weather stations for agrometeorological scope is proposed.

The aims of this study, part of the European MeteoMet project, are the improvement of the meteorological observations in field by disseminating the calibration methods, the implementation of traceability in agrometeorological measurements, and the improvement of the forecasting models by inclusion of traceable data and uncertainty components in the input values in order to reduce the use of chemicals in viticulture.
Non-selective signals in food analysis

Leardi R., Bagnasco L., Casale M., Casolino C., Lanteri S.

University of Genoa, Department of Pharmacy – via Brigata Salerno 13, Genova (Italy) – riclea@difar.unige.it

The analysis of samples by using technologies producing non-selective signals (e.g., NIR, FT-IR, UV-vis), is more and more common and widespread. Such approaches are very convenient, since they are usually fast, cheap and non destructive. In many applications no sample pretreatment is required, the acquisition of the spectrum can be performed in about one minute and no reactants/solvents are required. As a consequence, the return on investment of the related technology is very high.

The “disadvantage” of these techniques is that, being the signal non specific, simple mathematical approaches (e.g., the Lambert-Beer law) cannot be applied. Instead, a multivariate calibration must be performed by using chemometrical tools, the most common of which is PLS (Partial Least Squares).

Besides food-related problems, there are several fields in which these techniques are successfully applied (e.g., petrochemical, pharmaceutical, clinical, environmental, textile, leather); furthermore, they are the basis of PAT (Process Analytical Technology) and are becoming more and more relevant for process monitoring.

In what concerns food analysis, they can be applied in several steps, from the evaluation of the quality and the conformity of raw materials to the assessment of the quality (and therefore the economic value) of the final product, to the monitoring of the shelf life of the product itself. Another interesting field of application is the verification of food-authenticity claims, this being extremely important in the case of foods labeled as protected designation of origin (PDO), protected geographical indication (PGI) and traditional speciality guaranteed (TSG).

In the present talk some real examples of application will be shown, on different foods and having different goals.
Nutrient intake assessment at the European level

Heraud F., Ioannidou S., Valsta L.M.
European Food Safety Authority, Risk Assessment and Scientific Assistance Department – Via Carlo Magno 1A, Parma (Italy) – fanny.heraud@efsae.europa.eu, sofia.ioannidou@efsae.europa.eu, liisa.valsta@efsae.europa.eu

The European Food Safety Authority (EFSA) is responsible to provide scientific advice on nutrient intake as the basis of actions in the field of nutrition at the European level. In this framework, EFSA developed an approach to assess nutrient intakes of the European population, considering variability of food composition and food consumption patterns between European countries.

Information on food consumption patterns in Europe are gathered in the EFSA Comprehensive European Food Consumption database. This database is regularly updated and now includes data from 28 surveys representing 17 European countries. Food composition information available in 14 countries have been compiled in order to create a food composition database for nutrient intake. This database covers over 100 nutrients. The EFSA standardized food classification and description system, called FoodEx2, is used to combine both information.

Nutrient intake is assessed at the individual level by combining for each food consumed, the average consumption level with the amount of nutrient present in that food, and summing over the diet. Statistics are then derived at the population level for each country, age group and gender. The nutrient composition is country specific. In absence of information at the country level, an average value estimated at the European level is considered. Assumptions are also made for foods consumed but not present in the composition database, by attributing the nutrient level of similar food.

This approach has already been implemented on ten minerals and vitamins considering twelve food consumption surveys from the Comprehensive Database. Overall, the average intakes estimated were -/+ 15 % different from published national estimates. Such variation is mainly attributed to the use of different food description systems and to different modeling approaches. It shows the importance of harmonizing the food composition and consumption data collection at the European level.
Antioxidant activity and characterization of antioxidants from Argentina Oca

Rego A.¹, André C.¹, Delgado I.¹, Samman N.², Castanheira I.¹

¹ National Institute of Health Doutor Ricardo Jorge, Department of Food and Nutrition, Av. Padre Cruz, 1649-016 Lisbon, Portugal - isabel.castanheira@insa.min-saude.pt
² Universidad Nacional Jujuy, Jujuy, Argentina

Oxalis tuberosa is the scientific name of Oca a tuber similar to potatoes. This plant was brought into cultivation in the central and southern Andes for its tubers, which are used as a root vegetable. Oca is important to local food security because of its role in crop rotations and its high nutritional content.

Sample extraction was with MeOH:H2O 80:20, ultrasonicated, centrifuged, decanted and repeated for re-extraction.

Total Phenolic Content (TPC) was determined by Folin-Ciocalteu method with absorbance measured at 765 nm. Calibration curve with gallic acid and samples were in incubated at 40 °C during 30 min.

DPPH• method was used to determine free radicals scavenging property of phenolic compounds. The mixtures were done under controlled conditions and kept in the dark for one and half hour. The absorbance of solutions was measured at 517 nm. Antioxidant activity was expressed as IC50.

Ultra Performance Liquid Chromatography with Photo Diode Array detector (UPLC-PDA) optimization was carried out using polyphenol chemical standards of high analytical grade. A gradient elution performed with water 0.1% formic acid and acetonitrile as mobile phase was developed to separate, identify and quantify existing polyphenols.

TPC is usually associated to antioxidant activity (Fig. 1). Major phenolic content traduces in lower IC50 and bigger antioxidant activity since IC50 gives extract concentration to inhibit 50% of test radicals.

Despite having Rosada the lowest TPC it contains a higher concentration of identified antioxidants compounds than Blanca and Overa. From these two we can conclude that some antioxidants compounds were not quantified either because they are below the limit of quantification or not match the 40 analytical standards we have available. For the same reason Morada should have more identified antioxidants than Amariilha (Fig. 2).
From Broccoli Seeds to In Vitro Assay of Biological Effects: a Pipeline for Nutritional Quality Evaluation

Baima S. 1), Natella F. 1), Maldini M. 1), Nardini M. 1), Giusti A. 2), Kajetan T. 3), Mattivi F. 2), Scaccini C. 1), Ferruzza S. 1), Ranaldi G. 1), Rossi C. 3), Murgia C. 3), Sambuy Y. 3), Morelli G. 1)

1) CRA-NUT, Food and Nutrition Research Centre – Via Ardeatina 546, 00178 Rome (Italy) – simona.baima@entecra.it
2) Department of Experimental Medicine - Section of Medical Physiopathology, Food Science and Endocrinology - University “Sapienza” of Rome – P.le A. Moro 5, 00185 Rome (Italy)
3) Food Quality and Nutrition Department, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all’Adige (Italy)

Assessment of nutritional value and scientific validation of health claims for (functional) foods is a very difficult and controversial task. In fact, the composition of plant derived foods is greatly influenced by both genetic and environmental factors. In addition, the interactions between various molecules within a complex food matrix must be taken into account. Finally, genotypic and experimental system variation can affect correct interpretation of the biological effects of food nutrients.

The aim of this work is to develop a pipeline for the evaluation of plant food nutritional quality through the production of a highly standardized food matrix, its compositional analysis and the assessment of its nutritional value.

Broccoli were chosen for this study because vegetables belonging to the Brassicaceae family (e.g. broccoli, cabbage and Brussels sprouts) are widely consumed in the world, and are considered natural functional foods for their high content of a number of secondary metabolites with a recognised beneficial role for human health. Young seedlings (sprouts), in particular, represent enriched sources of vitamins, minerals, and health promoting bioactive substances with a higher nutritional value than adult plants. After testing different light and temperature regimes, as well as some chemical and hormonal treatments, we established both standard and inductive growth conditions potentially increasing the nutritional value of broccoli sprouts. Compositional analysis and biological testing of broccoli sprouts extracts on human Caco-2 cells, an in vitro model of intestinal function, indicates that this is a very promising approach for the evaluation of the potential health promoting effect of nutritionally improved crops.
Harmonised European food composition data utilizing standards and best practice

Westenbrink S.\(^1\), Roe M.\(^2\), Pakkala H.\(^3\), Koroušić Seljak B.\(^4\), Finglas P.\(^2\)

\(^1\) National Institute for Public Health and the Environment - PO Box 1, 3720 BA Bilthoven, the Netherlands; susanne.westenbrink@rivm.nl

\(^2\) Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK; mark.roe@ifr.ac.uk; paul.finglas@ifr.ac.uk

\(^3\) National Institute for Health and Welfare, P.O. Box 30, FI-00271 Helsinki, Finland; heikki.pakkala@thl.fi

\(^4\) Jožef Stefan Institute, Jamova c. 39, SI-1000 Ljubljana, Slovenia; barbara.korousic@ijs.si

The standardisation of food composition data (FCD) presentation is important to facilitate data exchange between producers, compilers and users of these data. The European Food Information Resource (EuroFIR) has created a quality framework for European FCD compiler organisations to produce improved national datasets in the two EU funded projects (EuroFIR NoE, 2005-10 & EuroFIR Nexus, 2011-13). This was further enhanced by the European Food Data Standard (EN 16104:2012) covering the food data chain. The EuroFIR quality framework includes standardised food, component and value documentation, standard operating procedures and peer review of data production and management processes.

Food choices and composition of local foods differ between countries so harmonisation of food composition datasets relates to approaches applied to production and management of data, to presentation of data and to data structure rather than using the same data for each country.

The 28 European food composition databases were documented to conform to EuroFIR guidelines using the LanguaL faceted food description system and EuroFIR value documentation thesauri (including standardised description of units, matrix units, values, (analytical) methods, references and acquisition types). Documented datasets can be searched by using the EuroFIR FoodExplorer tool, and data can be downloaded and used for various applications. Standardised documentation allows for mapping to other databases that may use different systems and thesauri. Documented data was provided to users including the European Food Safety Authority, EU research projects and applications for SMEs.

Work is continuing under EuroFIR AISBL, a non-profit association to support the work of the FCD compilers and users. FCD compilers are encouraged to incorporate this approach in their national procedures and to continue to work on standardisation and harmonisation of data. Furthermore it is important that laboratory reports and scientific publications also present food composition information in a standardised way to avoid loss of information during data exchange and to assure data quality.
Correlation of amino acids profile with arsenic accumulation in rice grain (Oryza sativa L.) consumed in Portugal

Mota C. 1), Lopes J. 2), Coelho I. 1), Santos M. 1), Castanheira I. 1), Matos A.S. 2)

1) National Institute of Health Doutor Ricardo Jorge, Department of Food and Nutrition, Av. Padre Cruz, 1649-016 Lisbon, Portugal
2) UNIDEMI, Departamento de Engenharia Mecânica e Industrial, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal asvm@fct.unl.pt

Portugal is the largest consumer of rice in Europe, with a consume of 17 kg per capita per year. Therefore, its nutritional profile and characterization in terms of arsenic content is important for the public health. In this study the amino acid profile of two rice cultivars (Oryza sativa L.) produced in Portugal, both considering japonica and indica subspecies, was evaluated and compared with total arsenic. Amino acids determination was performed in an Ultra performance liquid chromatography (UPLC) system, while Arsenic speciation was conducted by high performance liquid chromatography coupled to inductively coupled plasma mass spectroscopy (HPLC-ICP-MS). The most abundant essential amino acids were aromatic acids with a 11.8% of Total Protein. Non-essential amino acids represent 65.5% of Total Protein, with glutamic acid as the most abundant (12.9 mg/g in Indica and 12.4 mg/g in Japonica). Considering the two cultivars, one on the upland and the other on the lowland, six of the seventeen amino acids analyzed revealed significant differences, whereas between subspecies no significant differences were found. Correlation analysis between amino acid profile and arsenic reveals significant correlation for two essential amino acids (phenylalanine and valine) and four non-essential amino acids (alanine, acids aspartic acid, cysteine and glutamic acid), with the smallest significant Spearman coefficient of -0.661 (p-value = 0.038). Two dendograms were constructed based on cluster analysis, where the first one grouped the 17 amino acids into three distinct clusters, with low, medium and high content. The second dendogram obtained for studying the origin and subspecies relationships among the 22 samples, allow us to identify three clusters, one completely differentiate by origin, but the other two with a mix between origins and subspecies. These results corroborates with those obtained with analysis of variance, revealing to be a good tool to monitor changes in rice protein profile.

Keywords
Oryza sativa, Japonica rice, Indica rice, amino acid profile, protein quality, arsenic, cluster analysis
**NUTRIRETE.lab – a network to collect and store Italian food composition data**

Salvini S.\(^1\), Gnagnarella P.\(^2\), Concina F.\(^3\), Marletta L.\(^3\), Camilli E.\(^3\), Parpinel M.\(^1\)

\(^1\) Dipartimento di Scienze Mediche e Biologiche, Università degli Studi di Udine, Udine (Italy) – simonetta.salvini58@gmail.com maria.parpinel@uniud.it

\(^2\) Divisione di Epidemiologia e Biostatistica, Istituto Europeo di Oncologia, Milano (Italy) – patrizia.gnagnarella@ieo.it

\(^3\) CRA- NUT Centro di Ricerca per gli Alimenti e la Nutrizione, Roma (Italy) – luisa.marletta@entecra.it

Food composition data are produced by public and private laboratories and by the food industry for purposes of research, food control and food labeling. Data are also needed to assess and monitor diet of humans (and other animals) for individual counseling, diet therapy, food catering, public health interventions: country specific food composition databases (FCDBs) are used for such purposes (1,2).

In a fast growing food market, FCDBs are limited in number of food and nutrients, they become obsolete very quickly and lack of food components of emerging interest. On the other hand, laboratories produce costly data for specific purposes, and these valuable data could be further exploited, if collected, routinely documented and selected in a systematic way.

With the purpose of making good use of existing and up-coming chemical information about food, a government funded project (3) includes a work package aimed at creating a network of laboratories producing food information data. The web site NUTRIRETE.lab (www.nutriretelab.it) was developed to stimulate Italian laboratories to join the network by sharing their data, to convey requests for specific data between users and laboratories and to facilitate dissemination of food composition information.

To store, document and harmonize the shared information, we created a database, in MS Access. Such instrument is structured and documented according to guidelines developed in the frame of the Network of Excellence EuroFIR (4). LanguaL and other classification systems are used for food description. Information about analytical methods, sample preparation and component quantification are coded according to the EuroFIR descriptors (thesauri), but links to other systems are also possible.

This database will serve as the main source of information to update the Italian FCDBs.

Activities are ongoing and will hopefully continue in the future, if appropriate ways to launch a sustainable project will be found.

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Olive Oil is the first food product for which the assessment of the organoleptic quality by means of the Panel Test (PT) was introduced at the level of European Union legislation as a criterion for the sensory evaluation in the Reg. EU 1348/2013 annex XII (revision of Reg. EEC 2568/91). The standard establishes that olive oil must be tasted according to the rigid standards and regulations of the Panel Test (PT) that is carried out by a group of at least 8 professional tasters with a chief, that, through the tasting, give a numerical subjective evaluation (from 0 to 5) to many questions about flavours, in a questionnaire. The ensemble of the scores, reached by each component, defines the organoleptic quality. The amount of these numbers suggests, for better readability, the use of an unsupervised Artificial Neural Network (ANN), which identifies the best two-dimensional representation of the samples that have stimulated the PT. Analysing the coefficients relating a flavour to output neurons of the Kohonen Map, it is possible to individuate the most heavier. If it happens that heavier coefficients inherent to different flavours, flights the same neuron, an internal correlation will be searched.
Honey Floral Classification by Biomimetic Sensors

Di Sanzo R.\(^1\), Carabetta S.\(^1\), Cefaly V.\(^1\), Russo Mt.\(^1,2\)

\(^1\) Dipartimento Agraria, Università Mediterranea di Reggio Calabria – Laboratorio Quasicatec, Feo di Vito, 89122 Reggio Calabria (RC) – mariateresa.russo@unirc.it
\(^2\) Fondazione Mediterranea Terina ONLUS, Area industriale, 88046 Lamezia Terme (CZ)

Several methods have been used for the determination of the floral and geographical origin of honey. They are mainly based on the analysis of its pollen content, sensory analysis, amino acids, volatile compounds, carbohydrates, phenolic compounds, organic acids, and marker presence. These methods are generally complex and time-consuming.

This work proposes a fast analysis based on combination of array sensing that can effectively discriminate the samples not only based on the compounds present in the sample but also mimic the way humans perceive flavours. The method is low reagent volumes consuming and it does not need previous samples processing.

E-nose has been designed for automated detection and recognition of odours. It doesn’t decompose the volatile fraction of matrix in its constitutive components but supplies a global evaluation of aroma miming the human olfactory system. The sensor signals was convert to data that can be analysed by an appropriate statistical software to determine that one sample is similar or different from another. The electronic tongue behaves similarly to non-volatile compounds in a liquid.

The present work is focused on classifying honey samples of four different botanical origins (\textit{Hedysarum coronarium} L., \textit{Robinia pseudoacacia} L., \textit{Citrus} spp. and \textit{Castanea sativa} Mill.), using an electronic nose (e-nose) and electronic tongue measurements. The data collected with the e-nose and the e-tongue were analyzed by statistical analysis (principal component analysis and discriminant function analysis) to find an analytical alternative for classification of honey samples with respect to pollen type, which requires long time and skilled workers. The initial data obtained shown that the e-nose and the e-tongue was able to separately discriminate monofloral honey samples, with a discrimination index of 100% and 86% respectively. It was found that the e-nose and the e-tongue have efficiently tools for botanical classification of honey samples.
Sensory analysis, organoleptic properties and polyphenols content of Slovenian olive oils

Bučar-Miklavčič M., Valenčič V., Hladnik T., Bešter E., Miklavčič Višnjevec A., Butinar B.

1) UP ZRS IZO; LABS d.o.o. – Zelena ulica 8 c, Izola (SLOVENIA) – milena.miklavcic@guest.arnes.si, Vasilij.Valencic@zrs.upr.si, tejahladnik@gmail.com, Erika.Bester@zrs.upr.si, Ana.VisnjevecMiklavcic@zrs.upr.si, Bojan.Butinar@zrs.upr.si

The “Istrska belica” variety is the most widely spread olive variety in the Slovenian olive orchards. The quality olive oil crop from the “Istrska belica” variety is distinguished by its rich aroma, reminding of healthy, fresh, optimal ripe olive fruits, and of freshly mown grass combined. High content of polyphenols give the oil its characteristic bitter taste and pungent tactile sensation. During three-year project sensory analyses were carried out both on fresh oils and stored oils produced in Slovenia. Therefore, the first year samples were analysed three times and the second year were analysed twice. In addition, polyphenols levels were determined in all collected samples. Organoleptic properties such as fruitiness, bitterness, pungency and other specific characteristics of selected samples were assessed based on method defined in the Annex XII to Regulation EEC No 2568/91, 640/2008. The polyphenolic minor polar compounds in olive oils, such as the natural and oxidised derivates of oleuropein and ligstrose, lignans, flavonoids and phenolic acids were extracted by means of 60% (w/w) methanol solution and quantified by high-performance ternary gradient liquid chromatography (HPLC). The levels of total polyphenols, oleuropein and ligstrose derivates and tyrosol and hydroxytyrosol determined in fresh olive oils varied from 145 to 966 mg/kg (median=417 mg/kg), 83 to 584 mg/kg (median=251 mg/kg) and 2 to 97 mg/kg (median=9 mg/kg), respectively. While the levels of oleuropein and ligstrose derivates significantly decreased, the end step compounds tyrosol and hydroxytyrosol increased after two years. Likewise, the bitterness and pungency of the same samples decreased after two years as well. The total content of polyphenols and organoleptic properties of olive oils can vary greatly on the year basis. The highest levels of total assigned polyphenols, bitterness and pungency in the selected samples were found in fresh olive oils from crop year 2012. This could be due to the extreme weather conditions, such as draught in the crop year 2012. In conclusion, the levels of specific polyphenolic compounds and the organoleptic properties of high quality Slovenian oils are closely related.
Naturally occurring isotopes of strontium (Sr) have been proved to be good tools for tracing the geographical origin of foods. Notwithstanding, traceability and authenticity purposes often require high precision (around 0.002% percent relative standard deviation) and accurate measurements. These requirements can be often met by using Multiple-collector inductively coupled plasma mass spectrometry (MC-ICP-MS), which, despite a slightly lower precision with respect to other instruments such as thermal ionisation mass spectrometric (TIMS), allows processing an higher number of samples increasing the productivity of the analysis especially when the aim is to develop reliable geographical origin models based on “statistical approaches”. Unfortunately, there are various parameters that can influence the precision and the accuracy of the analytical determination of Sr isotope abundance ratio, namely $^{87}\text{Sr}/^{86}\text{Sr}$, by MCICP-MS. The main factors may be the isobaric interferences of $^{87}\text{Rb}$ and $^{86}\text{Kr}$; the former comes from sample and the latter from argon gas used to operate the plasma. In this work, a deeper investigation of the influence of these interferences and the procedures to overcome their negative effects are presented. Firstly, the $^{87}\text{Rb}$ influence on the $^{87}\text{Sr}/^{86}\text{Sr}$ was investigated by spiking a SRM987 (Strontium Carbonate Isotopic Standard) solutions (concentration varying from 50 to 400 $\mu$g kg$^{-1}$), with different amounts of an isotopically certified SRM984 (Rubidium Chloride) solutions with final concentrations of Rb ranging from 0 to 200 $\mu$g kg$^{-1}$. Furthermore, to improve accuracy of measured data, Chemometrics approaches (Experimental Design techniques and Principal component analysis) were used in order to optimize the chemical separation method needed to minimize the isobaric interference Rb/Sr. Moreover, a detailed discussion about (i) the mathematical correction procedures (exponential law, bracketing procedure, blank corrections etc.) and (ii) the robustness of the obtained results in terms of reproducibility and repeatability (by analyzing results coming from more than two years of measurements) will be presented as well.
Coffee aroma is strongly affected by the bean variety and their geographical origin. Determination of geographic origin of coffee is highly demanded for product traceability, authentication and marketing. In this study, the aromatic profiles of six roasted *C. arabica* coffees (Brazil, Ethiopia, Guatemala, Costa Rica, Colombia, India) were analyzed by Proton-Transfer-Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS) to characterize aromatic profiles of coffee powders and brews.

Commercially available medium roasted coffees were brewed by steam pressure coffee extraction in a stove-top coffee maker known as “moka” in Italy. The headspace measurements of coffee powder and brews were performed by a commercial PTR-ToF-MS 8000 instrument in switchable reagent ion mode and \( \text{H}_3\text{O}^+ \), \( \text{NO}^+ \) and \( \text{O}_2^+ \) were used as ionization agents. Multivariate data analysis techniques were applied in order to visualize data and classify the coffees according to their origin.

The results showed that the volatile compositions of coffees were highly influenced by the geographic origin of the coffee beans. Significant differences were found among volatile concentrations of coffee powders and brews. Multivariate data analysis techniques allowed separation of coffees according to origin both for powder and brew in 3 ionization modes. Tentative identification of mass peaks aided the characterization of aroma fractions useful for aroma fingerprints and origin discrimination.
Wine provenience determination using FT-Raman spectroscopy

Mandrile L.\textsuperscript{1)}, Zeppa G.\textsuperscript{2)}, Rossi A.M.\textsuperscript{1)}

\textsuperscript{1)} Istituto Nazionale di Ricerca Metrologica (INRIM), Strada delle Cacce 91, Torino. Italy
\textsuperscript{2)} DISAFA Università degli Studi di Torino. Italy

European consumers require not only high-quality and safe products but also certification and reassurance on a product’s origin. In order to preserve quality food products coming from particular geographical areas and to protect consumers against imitations and false information the European Commission defined several Council Regulations\textsuperscript{1}. Reliable metrological methods are needed to facilitate surveillance programs and prevent fraud or adulteration in the global market.

Literature data demonstrate that FT-Raman spectroscopy is a powerful technique for detecting selectively the trace components in foodstuffs\textsuperscript{1}. In this work we demonstrate the possibility to obtain results in determination of wine provenience by Raman spectroscopy.

In order to promote the future application of Raman Spectroscopy in this field, 306 samples of different wines such as Nebbiolo, Dolcetto e Barbera produced in the Piedmont region in different years have been analyzed. These wines are particularly relevant Italian products in the context of the global market. In this work wine samples have been directly analyzed using 1064nm excited Fourier transform Raman Spectroscopy. The acquired spectra were subjected to discriminant analysis classification analysis, performed with TQ Analyst\textsuperscript{™} software. First, the method permits to recognize different types of wine and to classify them. What is more we are trying with success to distinguish sample of the same type of wine from the three different production areas. Miss-classified samples are below the 10\%, emphasizing the goodness of this analysis.

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The strontium isotopic systematics applied to Glera musts: a tracer for the authenticity of the Prosecco wine

Petrini R. 1), Sansone L. 2), Slejko F.F. 3), Buccianti A. 4), Marcuzzo P. 2), Tomasi D. 2)

1) Dipartimento di Scienze della Terra, Università di Pisa, via S. Maria 53, 56126 Pisa, Italy
2) CRA-VIT, viale XXVIII Aprile 26, 31015 Conegliano, Italy - riccardo.petrini@unipi.it
3) Dipartimento di Matematica e Geoscienze, Università di Trieste, via Weiss 8, 34127 Trieste, Italy
4) Dipartimento di Scienze della Terra, Università di Firenze, via La Pira 4, 50121 Firenze, Italy

The traceability of foods has become a priority among consumers, driven by the increasing demand for food quality and safety. In the traceability system, the discrimination of the geographical provenance of food products is essential to verify the claims of origin often declared on labels, and to prevent unsafe products from reaching the consumers. The geographical origin assessment of wine is of particular interest, being one of the most important factors that determine its commercial value. Under European laws, wine production, designation and distribution, as well as the methods for analysis, are defined by various regulations and Member States directives.

In the present study, Glera vineyards from the Prosecco wine district in northern Italy have been characterized in terms of the \(^{87}\text{Sr}/^{86}\text{Sr}\) isotope-ratio of musts from the 2010, 2011 and 2012 vintages, coupled with the isotopic analysis of Sr in the labile fraction of the soils of provenance. For a single vineyard, detailed Sr isotopic analyses were carried out in sequentially extracted soil fractions at three different depths, and in the grape components (skin, seeds, must and stem), in order to verify the lack of Sr isotopic fractionation within the plant.

The results show that the ammonium acetate extracts from soils from the Prosecco vineyards, intended as representative of the labile fraction, are characterized by a large Sr isotopic variability. Musts from the different vineyards are also characterized by variable \(^{87}\text{Sr}/^{86}\text{Sr}\) ratio, which remains reproducible in the different harvests. For each vineyard, the Sr-isotope ratio in must and that of the labile fraction in the corresponding soil are correlated within experimental uncertainty, indicating that the isotopic composition in must can be forecast on the basis of that of soil. These observations confirm that the Sr-isotope systematics may be a potential tool in discovering fraud in wine trade.
Characterisation of Italian honeys through the application of mineral elements and stable isotopes analyses

Bontempo L., Camin F., Ziller L., Perini M., Nicolini G., Larcher R.
Fondazione E. Mach (FEM), Via Mach 1, 38010 San Michele all’Adige (TN) ITALY – luana.bontempo@fmach.it

According to Council Directive 2001/110/EC and the following amendments (in particular Directive2014/63/EU) the country or countries of origin where the honey is harvested and the botanical variety have to be declared on the label. Conventional honey analysis (chemical composition, physical characteristics, organoleptic parameters and pollen analysis) are not always applicable and effective to determine the geographic origin and variety of a honey. In the last years stable isotope analysis of the bio-elements and mineral composition have gained increasing importance in the determination of the geographic origin of different foodstuffs. In this research 265 honeys of different botanical origin (polyfloral, citrus, rhododendron, eucalyptus, acacia, chestnut, honeydew) produced along Italy in different years, were analyzed for stable isotope ratios (by Isotope Ratio Mass Spectrometry) and mineral elements (by Inductively Coupled Plasma Optical Emission Spectroscopy) content, in order to verify the relationship of these parameters with honey geographic origin and botanical variety. The characteristic ranges of variability in SIRs and mineral content in genuine Italian honey samples are here presented as well as their relationships and compliance with the limits indicated by the AOAC.
Detection of very low levels food-borne bacteria by culture on a Fluidic-free microsystem

Livache T. 1), Roupioz Y. 1), Calemuk R. 1), Mercey T. 2)

1) 1CREAB, UMR SPRAM 5819 (CEA, CNRS, UJF) INAC, CEA Grenoble, 38054 Grenoble Cedex 9 France
thierry.livache@cea.fr

2) Prestodiag, Villejuif France. Prestodiag.com

There is a growing need for the development of new devices enabling the fast detection of pathogenic bacteria in samples. This need exists in various fields ranging from clinical, environmental, or agri-food sectors. One common challenge across these fields is how to detect a very low number of contaminating bacteria in complex samples such as human fluids, soil or food aliquots.

Microbial culture continues to be the most common protocol for bacterial detection and identification in medicine and agronomics. Using this process may take days to identify a specific pathogen for most bacterial strains. Surface Plasmon Resonance (SPR) detection is an emerging alternative technology that can be used for the detection of bacteria using protein microarrays although typical limits of detection are in the range of $10^3$–$10^6$ cfu/mL, which is not compatible with most Food Safety regulation requirements.

We proposed to combine concomitant “on-chip” microbial culture with sensitive SPR detection of bacteria thus allowing rapid specific detection of bacteria pathogens – including Salmonella, Streptococcus pneumoniae and Escherichia coli O157:H7 – cultured on a protein microarray. This Culture–Capture–Measure (CCM) approach significantly decreases both the number of processing steps and the overall assay time for bacterial detection. Signal analysis of SPR responses allowed the fast and quantitative assessment of bacterial concentrations initially present in the sample as low as a few bacteria per milliliter. Altogether, our results show how simple, easy-to-operate, fluidic-less and lo-tec microarrays can be used with unprocessed samples and yield – in a single assay – both qualitative and quantitative information regarding bacterial contamination.

![Setup for the detection of the bacterial growth](image)

![Salmonella detection on SPR imaging chip, initial concentration 3cfu/mL](image)

Organic Bioelectronics: a Powerful Tool for Food Control

Magliulo M., Manoli K., Dumitru L.M., Mulla M.Y., Seshadri P., Torsi L.
Dipartimento di Chimica, Università degli Studi di Bari A. Moro – Bari, Italy – maria.magliulo@uniba.it

Organic field effect transistors (OFETs) have attracted increasing attention in the past few years due to their wide variety of potential applications. They have already proved to be useful as devices to explore phenomena governing organic electronics, and have also demonstrated their applicability as sensors for environmental monitoring, food industry and medical diagnostics [1,2]. In addition, OFETs can be fabricated on flexible or transparent substrates by different methods suitable for mass production. Their versatile design along with the possibility to tune the chemical/physical properties of the materials involved, can improve their sensitivity and selectivity. Bio-receptors sensitive to a particular analyte, either in gas or liquid phase, can be incorporated in the OFETs, as well. Consequently, OFETs based transducers are promising candidates for the development of flexible chemical/bio-sensors on plastic or paper as disposable ‘Intelligent Tags’. For example, such tags can detect quantitatively and qualitatively, the volatile compounds released from the food products, as they gradually start to degrade during their shelf life.

With this in view, on-line sensing of n-butanol odours was realized using a bottom gate top contact configuration OFETs. The devices were fabricated in both rigid and flexible substrates, using different pairs of organic semiconductors and insulating layers. The response of each sensor depended on the nature of the OS and dielctric. A comparison was made in terms of response time, sensitivity and selectivity towards the analyte. Regarding sensing in liquid phase, an electrolyte gated FET (EGOFET) was investigated as sensor for differentiation of carvone enantiomers, using functionalized gate electrodes with odorant binding proteins (OBPs). Calibration curves for drain current change upon interaction of carvone enantiomers with OBP functionalized gate electrode were obtained.

References
Rapid electrochemical screening methods for food safety and quality

Moscone D.

University of Rome "Tor Vergata" Chemical Sciences and Technologies Department - Via della Ricerca Scientifica, 00133 Rome, Italy - e-mail: danila.moscone@uniroma2.it

The potential impact of foods on human health is receiving increasing attention by public opinion, scientists and legislators. The interest of consumers, as well as of producers, for food safety and quality testing has increased over the last years. Thus, it is mandatory to have efficient and sensitive analytical methods to detect food contamination. Food control analyses require robust, sensitive, and selective detection methods. The most commonly used methods such as chromatography and mass spectrometry require expensive instrumentations and skilled technicians. In this presentation the development of fast, simple and cheap electrochemical screening methods is presented. In particular, the screening methods will concern the detection of bacteria such as Salmonella sp, toxins such as Palitoxin, viruses such as Picornavirus, the Hepatitis A virus (HAV), and chemical compounds such polyphenols and pesticides.

Screen printed electrodes (SPEs) and/or electrochemical arrays based on a 8 screen-printed electrode strip connected to a cost effective and portable apparatus, have been adopted in the methods that will be illustrated, as electrochemical transducers or assembled as immunosensors.

In order to obtain higher sensitivity, in some case SPEs have been modified with nanostructured materials such as Carbon Black or Gold nanoparticles, while in the some case of immunosensors, the arrays have been coupled with Magnetic Beads (MB), where the immunological chain is made to happen.

Experiments illustrating the optimization and analytical characterization of the developed methods and their application in real samples to evaluate matrix effect and recovery will be discussed.
Multiplexed chemiluminescence-based biosensor for quantification of aflatoxin B1 and type B-fumonisins in maize flour

Mirasoli M.1, Zangheri M.1, Anfossi L.2, Calabria D.1, Di Nardo F.2, Giovannoli C.2, Baggiani C.2, Roda A.1

1) University of Bologna, Department of Chemistry – Via Selmi 2, Bologna (Italy)- mara.mirasoli@unibo.it; martina.zangheri2@unibo.it; donato.calabria2@unibo.it; aldo.roda@unibo.it

2) University of Turin, Department of Chemistry – Via P. Giuria 5, Turin (Italy)- laura.anfossi@unito.it; fabio.dinardo@unito.it; cristina.giovannoli@unito.it; claudio.baggiani@unito.it

Aflatoxins and fumonisins are known for their acute toxic, immunosuppressive, mutagenic and carcinogenic effects. Several instrumental analytical methods are available for detecting these toxins in foodstuff, but they require complex sample preparation and dedicated laboratory equipment.

Biosensors are promising analytical tools for rapid on-site detection of analytes in complex matrices. We recently described a biosensor for type-B fumonisins detection in maize samples based on a chemiluminescence Lateral Flow ImmunoAssay (CL-LFIA) coupled with a portable ultrasensitive CCD-based “contact” imaging device, reaching a limit of detection of 25 µg kg\(^{-1}\) in maize flour samples [1].

In this work, a multiplex CL-LFIA is presented, in which two competitive immunoassays are simultaneously performed on the same strip for detecting type-B fumonisins and aflatoxin B1. The assay involved a simple extraction of the analytes from maize flour samples followed by their detection by a multiplex competitive immunoassay with CL detection employing ready-to-use analytical cartridges. The use of CL detection allowed accurate and objective analytes quantification, enabling to detect simultaneously type B-Fumonisins and aflatoxin B1 down to 6 µg kg\(^{-1}\) and 1.5 µg kg\(^{-1}\), respectively, thus fulfilling the standard imposed by the legislation of European Union. In order to verify the performances of the method, analysis of naturally contaminated maize samples was performed, showing a good agreement with validated confirmatory HPLC-UV and commercial ELISA kit. The proposed CL-LFIA protocol is suitable for identifying, within the regulatory limits, samples that require further confirmatory analysis, therefore reducing the overall number of samples subjected to analysis by reference chromatographic assays and thus costs. This allows performing frequent analyses monitoring the entire production chain (e.g., on field, at harvest, during storage and transportation) according with the HACCP procedures.

Accurate Glutamate Monitoring in Foodstuffs by a Sensitive and Interference-Free Amperometric Biosensor

Centonze D., Mentana A., Nardiello D., Palermo C., Quinto M.

Università degli Studi di Foggia, Dipartimento di Scienze Agrarie, degli Alimenti e dell’Ambiente - Via Napoli, 25, I-71100, Foggia (Italy) – diego.centonze@unifg.it

L-Glutamate (Glu) is a well-known flavour enhancer that is present in several foodstuffs either as an additive or a natural compound. Glu monitoring is an important issue since the excessive intake of this flavour enhancer can cause allergic and neurotoxic effects. Glu is currently determined by chromatographic [1] or capillary electrophoretic [2] methods, which require extensive sample pre-treatment and expensive equipments. A suitable alternative is represented by amperometric biosensors, low cost devices that could provide specific, rapid and repetitive analyses of complex matrices. In the last decade a number of biosensors for glutamate detection have been proposed [3-7], but the above mentioned requirements have not been completely met. In order to face these problems a proper selection of the electrode material and the use of permselective films are required [8].

This work describes the development and optimization of an amperometric biosensor for glutamate monitoring in foodstuffs. The biosensor is based on glutamate oxidase (GLOD) immobilized by a gel of bovine serum albumine and glutharaldehyde onto a platinum electrode modified with a permselective overoxidized polypyrrole film. Different experimental conditions have been tested for the enzyme immobilization, and the optimized biosensor, integrated in a flow injection system, has been characterized in terms of linearity, LOD, LOQ, repeatability and stability of response. The excellent anti-interference characteristics towards the main interferents present in real food matrices have allowed the application of the biosensor in the accurate monitoring of Glu in different kind of foodstuffs.

Affinity sensing for food control

Scarano S., Mariani S., Minunni M.

Dip. di Chimica “Ugo Schiff”, Università di Firenze, Italy - e-mail: maria.minunni@unifi.it

Affinity sensors are based on the specific recognition between an immobilized receptor on a transducer surface and its ligand i.e. target analyte. From the recognition an affinity complex is formed at the sensor surface and its formation is revealed by the transducer, which generates an analytical measurable signal. The bioreceptor can be an antibody, a nucleic acid probe, or a biomimetic one such as an aptamer or a molecular imprinted polymer (MIP). Direct label free and real time analysis can be achieved by optical, as in the case of Surface Plasmon Resonance (SPR), and piezoelectric sensing.

Some studies related, in particular, to the detection of nucleic acids in different matrices will be presented. We will report about the analysis of genetically modified organisms markers in tobacco plants and in soybean in industrial food chain, and also of highly repeated sequences directly in non-amplified genomic DNA in meat in by biosensor-based approaches.

Behind this advancement in optical sensing i.e SPR imaging will be described coupled both DNA and protein analysis in complex matrices to allow real time, label free and multi-analyte detection.
Sample Preparation and Detection Methods for the Analytical Determination of Nanoparticles in Food

Aureli F., D’Amato M., Raggi A., Cubadda F.

Istituto Superiore di Sanità - National Health Institute, Department of Food Safety Safety and Veterinary Public Health – Viale Regina Elena 299, Rome (Italy) – federica.aureli@iss.it

Nanoparticles are particles with all three external dimensions in the size range 1-100 nm and can be present in food naturally, incidentally or as constituents of a manufactured nanomaterial. Nanomaterials exhibit new properties compared to coarser materials with the same composition and in recent years the food industry have been exploring the potential of nanotechnology for novel applications. Three main categories of applications exist, namely nanomaterials for (i) agricultural production; (ii) food processing (e.g. nano-sized ingredients); (iii) products that come into contact with food (e.g. packaging materials). Apart from projected use, some manufactured food ingredients have been shown to be already containing a nano-sized fraction, e.g. the food additives E551 (SiO₂) and E171 (TiO₂).

Despite many potential benefits, application of nanotechnology to the food sector has provoked concern and debate from the perspective of safety. A major issue is that there is insufficient knowledge on how altered physicochemical properties may influence their toxicological properties. Therefore, there has been an increasing interest in the latest years in the development of methods for the analytical determination of nanoparticles in food. Furthermore, with the aim to provide food information to consumers, Regulation EU 1169/2011 establishes that as of December 2014 ingredients present in the form of engineered nanomaterials have to be indicated in food labels and analytical methods are required for this purpose.

Sample preparation and detection methods for the analytical determination of inorganic nanoparticles in food will be reviewed, with focus on state-of-the-art mass spectrometric techniques such as single particle ICP-MS and asymmetric flow field flow fractionation combined on-line with optical detectors for size determination (MALS, DLS, UV) and elemental detection by ICP-MS. Using these tools our laboratory participated in the first three international interlaboratory studies on the determination of Ag particles in suspensions and real samples.
New Analytical Services for nano ingredients

Grandi S.¹, Mustarelli P.¹, Strada L.¹, Salvini A.², Nulli A.³

¹ University of Pavia, Dep. of Chemistry and INSTM, V.le Taramelli 12, 27100 Pavia, Italy; grandi@unipv.it
² University of Pavia, Laboratory of Applied Nuclear Energy (LENA) Via Aselli, 41 27100 Pavia, Italy asalvini@unipv.it
³ Freelancer, Via Beatrice d’Este 18, 27100 Vigevano (PV) Italy

Nanomaterials have recently become part of products already on the market, from antibacterial containers to self-cleaning concrete to cosmetics and many others. According to the most recent data, every week are marketed between 3 and 4 nanotechnological products. The Project on Emerging Nanotechnologies, carried out a census of existing products: from 2006 to 2013 products have grown from 212 to 609. According to the LUXresearch, by 2014 about 15% of the total production in the world will consist of goods that incorporate nanomaterials. For this reason on 11th July 2013, according to the European Regulation 1223/2009, nanoparticles in cosmetic products must appear in the list of ingredients in order to enable the consumer to know whether the product contains nanomaterials. In addition, on 13th December 2014, will be active the European Regulation no. 1169/2011, for foods which contains nanoingredients. At the same time the presence of some toxic nanomaterials as contaminants should be faced and solved and the first step to perform this is to find them in the products. In order to meet these needs our laboratory is intended to be an interface between private companies and institutions that are still studying standard material and methods for nano-ingrediants. The aim of our constituting Spin-Off of University of Pavia (Italy) N.A.M. (Nano Analysis and Materials) is to serve the needs of the market becoming one of the most important laboratory specialized in advanced separation, characterization and speciation of nanoparticles, proteins and polymers exploiting all the potentialities of FFF (Field Flow Fractionation) technique due to a full analytical and semi-preparative FFF platform. In order to complete, validate and support FFF analyses we can associate many others classical characterization techniques as: DLS, ZP, FTIR, ICP-MS, solid state NMR, SEM, TEM, BET, XRD; and not classical characterization techniques as: γ spectroscopy and radioactivity measurements. These particular analyses, in our University, are possible in presence of the following uncommon facility: a TRIGA Mark II nuclear reactor.
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Certified Reference Material ERM-AC626 “Arsenobetaine in water”
– a new calibrant from IRMM

Koleva B., Held A., Hemons H.

European Commission, Joint Research Centre, Institute for Reference Materials and Measurements (IRMM) – Retieseweg 111, Geel, B-2440, Belgium – Boryana.KOLEVA@ec.europa.eu

The Institute for Reference Materials and Measurements offers more than 170 different certified reference materials (CRMs) related to the analysis of food and feeding stuff, which are matrix and pure materials as well as synthetic mixtures. The certified parameters vary from proximates and conventional properties through element content to GMO content and microbiological properties. The main goal during the material production and certification is the high quality of the products. This is also the reason for the implementation and maintenance of a quality system according to ISO Guide 34 and ISO/IEC 17025.

ERM-AC626 “Arsenobetaine in water” is a successor of the CRM BCR-626. BCR-626 was widely used by laboratories dealing with food analysis as a calibrant and quality control sample. The production process of the new material has been improved following the modern concepts of CRM production, e.g. usage of amber glass ampoules instead of vials and preparation of the solution by substitution weighing. The metrological traceability of the certified value is guaranteed by using different approaches for quantification and confirmation. Special care was taken also during the production in order to certify the material with the smallest achievable uncertainty. The results from the short-term stability, long-term stability and homogeneity studies together with the confirmation analyses proved the quality of ERMAC626. The certified reference material is intended to be used as a calibrant and quality control sample.

This presentation provides an overview on the different stages during the production of ERM-AC626. Special attention is paid to the characterisation of the arsenobetaine raw material as well as to the uncertainty estimation. The critical points during the production are also discussed.
Feasibility study for development of reference material in cereal matrix for infant feeding: ash content, water content and protein content

Scarlato R. C., Miranda N. G. M., Costa R. S., Rego E. C. P., Caixeiro J. M. R.

INMETRO, Dquim – Avenida Nossa Senhora das Graças, 50, Duque de Caxias, RJ (Brazil) – rcscarlato@inmetro.gov.br, nmiranda@inmetro.gov.br, rsdcosta@inmetro.gov.br, ecrego@inmetro.gov.br, jmrodrigues@inmetro.gov.br

This study evaluated the feasibility for production of a candidate certified reference material whose matrix is a cereal for infant feeding from a mix of corn, wheat and rice, sold in Brazil. The cereal (9 kg) was purchased in the retail trade. It was ground, homogenized and manually filling by weighing on an analytical balance. Thirty two units with about 250 g of cereal in amber glass bottles were produced. They were listed sequentially according to the filling and identified with labels.

Three parameters of chemical composition were evaluated in respect to degree of heterogeneity and stability: water content by Karl Fischer Coulometric Titration, Kjeldahl for total proteins and ashes by calcination, the latter two being calculated on dry basis, due to the hygroscopicity of the matrix.

For the homogeneity study 05 bottles were analyzed. The contribution from the inhomogeneity in the content of water, ashes and proteins in the samples kept at the reference temperature were, respectively, 0.97, 1.53 and 2.34 (%). These degrees were considered acceptable for this type of matrix.

The temperatures evaluated were 4, 22 and 50 °C, relative to a reference temperature of -20 °C. All parameters were measured in duplicate in each bottle. The duration of study was 63 days.

The candidate CRM was stable for 63 days at temperatures of 4 °C and 22 °C and stable for 19 days at 50 °C for the water content parameter. For ashes and protein, both on dry basis, the material was stable for 63 days at 4, 22 and 50 °C.

This study demonstrated that the production of this type of CRM is feasible. So, INMETRO will proceed to the production of bath for this CRM, that will can be applied as a tool for quality assurance and traceability of measurements.
Feasibility study for the development of a Strained Tomatoes-Reference Material to be certified for contaminants from contact materials

Zappa G. 1), Caprioli R. 1), Dimiziani L. 2), Gatti R. 1), Zoani C. 1)

Casaccia Research Centre - Via Anguillarese, 301- 00123 ROMA (Italy) - rosanna.gatti@enea.it
2) Bruker Daltonics S.r.l. - Via Cluentina 26/R, Macerata (Italy)

The safe use of food contact materials in food packaging is ensured at EU and National level by many regulations (e.g.: Reg. 1953/2004; Reg. UE 10/2011; Dir. 2002/72/EC; Dir. 97/48/EC); in fact, the strong interactions between these materials and beverages can cause the migration of some contaminants from packaging into food.

Bisphenol A (BPA) is employed to manufacture plastics and resins, such as in polycarbonate (used to make food containers, such as returnable beverage bottles, baby bottles, tableware and storage containers), and epoxy resins (used to make protective coatings and linings for food and beverage cans and vats). Concerns have arisen over BPA since it has been found to migrate in small amounts into foods and beverages stored in containers and some studies indicated that high levels of the chemical could be carcinogenic. Also metals and alloys are used as food contact materials, mainly in processing equipment, containers and household utensils but also in foils for wrapping foodstuffs. These food contact materials can give rise to migration of metal ions into the foodstuffs and therefore could either endanger human health if the total content of the metals exceeds the sanitary recommended exposure limits, if any, or bring about an unacceptable change in the composition of the foodstuffs or a deterioration in their organoleptic characteristics.

In this work we report about a feasibility study - conducted according to EC recommendations - for the development of a Strained Tomato-Reference Material to be employed for evaluating the release of contaminants from food contact materials. In particular, BPA and some trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Sn) were considered. With this purpose, a test batch of commercial strained tomatoes was prepared. Two RMs were prepared, the first one with the contaminants at natural level (TQ), the second one spiked for As, Pb, Hg and BPA. The preparation procedure - especially with respect to the spiking - was set up in order to ensure the homogeneity of the RM. The RM was prepared both in lyophilized form, and - as already suggested for other agrofood RMs - in pellet form to be employed as “Single Use-RM”, with the aim to make easier the material handling, as well as to improve the performances of the RM itself. Homogeneity studies and stability studies under thermal and luminous stress are underway.
TXRF analysis of certified reference materials

Dalipi R.1), Borgese L.1), Zappa G.2), Zoani C.2), Depero L.E.1)

1) University of Brescia, Department of Mechanical and Industrial Engineering – Via Branze 38, Brescia (Italy)
Technical Unit for Sustainable Development and Innovation of Agro-Industrial System (UTAGRI)
Casaccia Research Centre - Via Anguillarese, 301-00123 ROMA (Italy)

Food is one of the main sources of essential major and trace elements in human nutrition. However, also potentially toxic elements (e.g., As, Cd, Cr, Ni, Pb) can contaminate food chain from the environment or during processing and storage.

Analysis of elements in samples of food is a complicated process, mainly dependent on the property of the sample matrix and nowadays fast and sensitive analytical techniques are desirable as a result of the increasing demand on multielemental information.

Total reflection X-ray fluorescence (TXRF) is a powerful analytical technique with respect to multielemental analysis capability, simplicity of quantification, detection limits and relatively short analysis time. Due to its benefits TXRF is gaining acceptance in the field of food safety and traceability.

In this work, TXRF was employed in the determination of major and trace elements in different Reference Materials (RMs) prepared by ENEA: strawberries, mushroom and peeled tomatoes. Accuracy and precision of the applied analytical method were evaluated. Two different sample preparation procedures for TXRF analysis were considered: sample digestion and direct analysis of suspended sample. The multielemental quantification of the CRMs samples was carried out by adding gallium as internal standard (IS).
Optimization for the accreditation of official control laboratories. Flexible scope accreditation

López Mª T., Berenguer J.

National Center for Food. Spanish Agency for Consumers Affairs, Food safety and Nutrition
– e-mail address: mlopeze@msssi.es

In accordance with Regulation (EC) 882/2004, the laboratories involved in official control should be accredited according to the standard ISO/IEC 17025.

To meet the requirements of the control and comply with their assigned functions, can require extensive accreditation, that they have the own difficulty to obtaining and presented, as added problem, the complexity of its maintenance in time.

To optimize resources, the method of accreditation by the system called “flexible scope” is a strategy of accreditation that can be very useful because does not require an assessment / audit by the accreditation body prior to the incorporation of new matrix/analyte to the scope of accreditation previously accredited as a category as a category of trials.

However, whether to apply a particular test procedure is not anticipated the need to include new analytes in the scope of accreditation the option to certify by closed scope whose validation includes all the possible range of matrices is a good option.
Freeze dried fish as Proficiency Test material: a procedure to obtain the planned concentration of endogenous Mercury

Ciprotti M.\textsuperscript{1)}, Giordano R.\textsuperscript{1)}, Sepe A.\textsuperscript{2)}, Sorbo A.\textsuperscript{3)}, Zoani C.\textsuperscript{3)}, Colabucci A.\textsuperscript{1)} and Ciaralli L.\textsuperscript{3)}

\textsuperscript{1)}Istituto Superiore di Sanità, European Union Reference Laboratory for Chemical Elements in Food of Animal Origin (EURL-CEFAO), Department of Food Safety and Veterinary Public Health – Rome, Italy – maria.ciprotti@iss.it
\textsuperscript{2)}Istituto Superiore di Sanità, Department of Food Safety and Veterinary Public Health – Rome, Italy
\textsuperscript{3)} ENEA - Italian National Agency for New Technologies, Energy and Sustainable Economic Development; Technical Unit for Sustainable Development and Innovation of Agro-Industrial System (UTAGRI)

Casaccia Research Centre - Via Anguillarese, 301- 00123 ROMA (Italy)

Proficiency Tests (PTs) are organized by the EURL-CEFAO to harmonize the quality of the results produced by the EU National Reference Laboratories belonging to its network.

With this aim, ad-hoc reference materials are in-house prepared at concentrations of interest, set according whether to Maximum Levels reported in EU legislation (CR 1881/2006) or routinely analyzed element/matrix combinations.

This work describes the preparation of a freeze dried fish sample at the desired concentration by mixing two fishes having unlike content of the element. In fact, as for Hg in fish, since the organic forms of the element are predominant (mainly as Methylmercury), a study on the feasibility to mix different fish species was performed to achieve the planned element concentration avoiding the spiking of inorganic standard solutions.

Therefore, after exploring the natural content of Hg in some species, a preliminary lot was prepared mixing a sea fish (swordfish, 0.39 mg/kg) and a fresh water fish (pangasius, <0.002 mg/kg). This preliminary lot was prepared to evaluate if a proper homogenization could be achieved, notwithstanding the different tissue structure. To this end, an appropriate amount of water was added to facilitate the amalgamation; swordfish, pangasius and their mix were then lyophilized.

The analyses carried out by Flow Injection Mercury System showed the achievement of the target values for the preliminary lot; moreover congruity of data was also achieved comparing the Hg content in fresh and freeze dried samples considering the lyophilisation yields. All this considered the EURL-CEFAO was able to adjust the ratio swordfish/pangasius (~1/2) for the preparation of the 150 test items to be distributed for the 13\textsuperscript{th} PT 2\textsuperscript{nd} Round.

The sufficient homogeneity test and the outcome of the exercise showed the fitness of the procedure and the capability of the EURL to obtain the planned concentrations.
Analytical Quality Assurance and Measurement Uncertainty in the Assessment of Bioaccessibility and Speciation of Se in Foods

Aureli F., D’Amato M., Raggi A, Cubadda F.

Istituto Superiore di Sanità - National Health Institute, Department of Food Safety Safety and Veterinary Public Health
– Viale Regina Elena 299, Rome (Italy) – francesco.cubadda@iss.it

Selenium is an essential micronutrient for humans and animals, involved as selenocysteine (SeCys) in functioning at the catalytic center of several selenoproteins (e.g., glutathione peroxidases, thioredoxin reductase, and iodothyronine-deiodinases). It enters the food-chain through plants, due to their ability to transform inorganic Se into a variety of organo-Se species, which has important implications for human nutrition and health.

Absorption in humans is not homeostatically regulated and is not believed to be affected by nutritional status. Selenomethionine (SeMet) is actively absorbed through the same enzyme transport system as does methionine (Met). In supplementation studies, organic forms of Se (wheat Se, SeMet and high Se-yeast) were found to be more bioavailable than selenate – Se(VI) – and selenite – Se(IV). This difference is due to the ability of SeMet from digested organic Se sources to be incorporated in place of Met into tissue proteins where it can act as a Se store and become available upon turnover of tissue proteins.

Foods that contain high proportions of SeMet are good bioavailable sources of selenium. There is good evidence that the increased selenium status attained after supplementation with organic forms of selenium is retained for a longer period after supplementation has ceased than is the case with inorganic selenium. The key step for selenium bioavailability is liberation of SeMet and other absorbable selenium species from the food matrix (particularly proteins) during gastro-intestinal digestion, i.e., selenium bioaccessibility.

The key factors affecting measurement uncertainty in the determination of selenium bioaccessibility after in vitro enzymolysis simulating human gastrointestinal digestion and in selenium speciation by either HPLC-ICP-MS or molecular MS are reviewed. Analytical quality assurance issues in these types of measurements are discussed based on the experience of our laboratory in studies on selenium bioaccessibility and speciation in food.
Validation of a gas chromatography method for fatty acids determination: application to bakery products

Albuquerque T.G.1,2, Beatriz Oliveira M.2, Sanches-Silva A.1,6, Costa H.S.1,2

1) National Institute of Health Dr. Ricardo Jorge, I.P., Department of Food and Nutrition – Av. Padre Cruz, 1649-016 Lisbon (Portugal) – tania.albuquerque@insa.min-saude.pt;
2) REQUIMTE/Faculdade de Farmácia da Universidade do Porto – Jorge Viterbo Ferreira n.º 228, 4050-313 Oporto (Portugal);
6) Centro de Estudos de Ciência Animal (CECA), Universidade do Porto – R. D. Manuel II, Apartado 55142, 4051-401 Oporto (Portugal)

Gas chromatography (GC) coupled with mass spectrometer (MS) or flame ionization detector (FID) is the most widely used technique for determination of fatty acids (FA), including trans fatty acids (TFA). TFA are associated with an undesirable effect on serum lipid profiles, and thus may increase the risk of cardiovascular disease, being considered in this respect, worse than saturated fat. The aim of this study was to optimize and validate a GC method for the quantification of FA in foods. Moreover, the developed method was applied for the FA quantification in bakery products. FA methyl esters were separated in SP-2560 column (100 m x 0.25 mm i.d., 0.25 μm). GC method was validated regarding linearity, limits of detection and quantification, recovery, accuracy and precision. FA profile of different types of bakery products (filled and unfilled sweet biscuits, sandwich biscuits, brioche with filling, filled croissants, salty snacks, wafers and crackers) was determined. According to the obtained results, the validated method presented good linearity for the following concentrations: palmitic acid (27 – 2730 μg/mL); palmitoleic acid (35 – 202 μg/mL); stearic acid (27 – 2730 μg/mL); oleic acid (45 – 4547 μg/mL); linoleic acid (45 – 4549 μg/mL); linolenic acid (53 – 300 μg/mL); elaidic acid (7 – 135 μg/mL); and linoelaidic acid (6 – 102 μg/mL). Determination coefficients (r2) were always equal or greater than 0.9989, indicating suitability for quantification. The most abundant saturated FA found in the analysed bakery products was palmitic acid. In summary, the validated method is suitable for routine analysis of FA. Moreover, FA profile of foodstuffs could be used for several purposes, namely for labelling of foods and quality control. Acknowledgements:

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Validation of an analytical method to determine coumaphos in honey samples by gC/MS/MS

Nardelli V., Berardi G., Calitri A., Casamassima F., Gesualdo G., Mambelli P.

1) Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Via Manfredonia 20, 71121 Foggia, Italy

e-mail: v.nardelli@izsf.it

The aim of the present work was to validate an efficient analytical method based on gas chromatography/tandem mass spectrometry (GC/MS/MS) for detection and quantification of coumaphos in honey samples. PCB 209 was used as internal standard (IS). This method was based on a preliminary extraction with ethyl acetate, followed by a clean-up by Florisil. GC/MS/MS analyses were carried out in the Selected Reaction Monitoring mode and the confirmation was made by establishing the presence of two significant transitions MS/MS for coumaphos, corresponding to m/z 109 and m/z 226 achieved by the fragmentation of parent ion at m/z 362. Coumaphos, used by the bee-keepers, is a common acaricide and is regulated by Maximum Residue Level (Reg. UE 2010/37). Validation parameters such as linearity, specificity, precision, recovery, LOD and LOQ, reporting limit and ruggedness were determined, resulting in compliance with Decision 2002/657/EC and SANCO Document N°12571/2013. Good linearity ($R^2 \geq 0.99$) was observed in the whole range of explored concentrations (5,0-150,0 ng/ml). LOD and LOQ were 0,12 ng/g and 0,37 ng/g respectively. Precision was evaluated by injecting six replicates spiked at three levels i.e. 10,0-50,0-100,0 ng/g in matrix and the calculated mean recovery (94%) was in compliance with the European Regulations. Specificity was verified by absence of significant interference in the maximum tolerance range ($\pm 0,2$ min) in relation to coumaphos retention time compared with those of spiked samples. Ruggedness was estimated for minor changes by means of the Youden Test. The measurement uncertainty was calculated by metrological approach, taking into account the following contributions: repeatability at level of interest, recovery, reference material, instrumental calibration, weighing. GC/MS/MS analysis demonstrated high sensitivity and specificity for detection and quantification of coumaphos at low level i.e. 5 ng/g (Reporting Limit). This analytical method was successfully applied to the routinely analysis of honey samples.
Milk hidden allergen quantification in bakery products by LC-MS/MS validated method

Lamberti C. 1), Acquadro E. 2), Corpillo D. 2), Decastelli L. 3), Garino C. 4), Arlorio M. 4), Ricciardi C. 5), Giuffrida M.G. 3), and Cavallarin L. 1)

1) ISPA-CNR, largo Braccini 2 10095, Grugliasco, Turin, Italy
2) ABLE BioSciences, Bioindustry Park S. Fumero, Via Ribes 5, 10010 Colleretto Giacosa, Turin, Italy
3) Centro Regionale Allergie e Intolleranze Alimentari, SS Controllo Alimenti, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d’Aosta, Via Bologna 148, 10154, Turin, Italy
4) Dipartimento di Scienze del farmaco & DFB Center, Università del Piemonte Orientale “A. Avogadro”, Largo Donegani 2, 28100, Novara, Italy
5) Politecnico di Torino, DISAT - Applied Science and Technology Dep., Corso Duca degli Abruzzi 24, 10129, Turin, Italy

A validated method based on LC-MS/MS and minimising sample preparation was set up to detect milk traces in bakery products. Two specific peptides from bovine α-s1 casein were selected: the one showing the most intense transition was chosen for quantification (FFVAPFPEVFGK), while the second for confirmatory purposes (YLGYLEQLLR). Incurred material were prepared “in house” at eight levels of milk contamination: 1, 2, 5, 10, 15, 25, 75 and 150 mg of milk/kg. Remarkable results were obtained for LOD (1.3 mg milk/kg cookies), LOQ (4 mg milk/kg cookies), intra-day repeatability (5-20% range) and inter-day repeatability (never exceeded 12%). In terms of recovery, values ranging from 20% to 26% were consistent with the method of calculation and with the type of processed food (bakery products).

Method applicability was then evaluated by testing commercial cookies with not exhaustive labelling. All samples tested were proven not to contain milk. These samples can be labelled as “milk-free”, giving an opportunity both to consumers (especially milk allergy sufferers) and to producers to safeguard their interests, both in terms of health and legal requirements.

The procedure proposed could be applied to the management of allergen risk, mainly as method of confirmation of both negative and positive outcomes obtained by the application of the ELISA method for milk allergen detection.
Determination of Polycyclic Aromatic Hydrocarbons in Water by Microextraction with Packed Sorbent and Gas Chromatography-Mass Spectrometry Analyses: A Comparison between “Draw-Eject” and “Discard” Methods under Equilibrium Conditions

Quinto M.1), Spadaccino G.1), Nardiello D.1), Palermo C.1), Amodio P.2), Centonze D.1)

1) Università degli Studi di Foggia, Dipartimento di Scienze Agrarie, degli Alimenti e dell’Ambiente, via Napoli 25, I-71100 Foggia (Italy) – maurizio.quinto@unifg.it
2) Università di Bari, Dipartimento di Matematica, via Orabona 4, I-70125, Bari (Italy) – amodio@dm.uniba.it

In this work, two different extraction procedures (namely, “draw-eject” and “discard”) for the analysis of different polycyclic aromatic hydrocarbons (PAHs) in water real samples by microextraction with packed sorbent (MEPS), have been compared in terms of sensitivity, reliability and time of analysis. The relevant partition equilibriums and extraction rates have been calculated by multivariate regression from data obtained after MEPS gas chromatography-mass spectrometry (MEPS-GC-MS) analysis of 16 PAHs. Partitioning parameters for a priori prediction of solute sorption equilibrium, recoveries, pre-concentration effects in aqueous and solvent systems have been calculated and compared for the two extraction procedures. Finally, real samples from sea, agricultural irrigation wells, streams and tap water were analysed. Detection (S/N ≥ 3) and quantification (S/N ≥ 10) limits were calculated for the extraction processes. Under the experimental conditions used, for the “draw-eject” procedure, these values ranged from 0.5 to 2 ng L⁻¹ and from 1.6 to 6.2 ng L⁻¹, while for the “discard” procedure ranged from 0.2 to 0.8 ng L⁻¹ and from 0.8 to 2.0 ng L⁻¹, respectively.
Honey origin determination by combining Raman Spectroscopy and Elemental Profiles

Menelao M. 1), Zappa G. 1), Mignani A.G. 4)

Technical Unit for Sustainable Development and Innovation of Agro-Industrial System (UTAGRI)
Casaccia Research Centre - Via Anguillarese, 301- 00123 ROMA (Italy)
2) Vrije Universiteit Brussel, Brussels Photonics Team, Pleinlaan, 2 – 1050 Brussel – Belgium
3) Institute for Agriculture & Fisheries Research (ILVO), Technology & Food Science Unit, Brusselsesteenweg, 370 –
9090 Melle, Belgium
4) CNR-Istituto di Fisica Applicata “Nello Carrara”, Via Madonna del Piano, 10 – 50019 Sesto Fiorentino (FI), Italy –
l.ciaccheri@ifac.cnr.it

In general, honey composition is closely associated with its botanical origin and the geographical area in
which is originated, because soil and climate characteristics determine melliferous floral. Chemical analysis
must be then combined with chemometric analysis for basic pattern recognition and honey classification
according to chemical composition (molecular and/or elemental profiles).

Optical spectroscopy is currently emerging as a modern and “green” analytical technique for intact food
analyses, thanks to the non-destructive nature of light measurements which enable rapid checks without
making use of reagents or chemical treatments, thus avoiding the problem of waste disposal. Optical
spectra can be considered to be a fingerprint from which to extract multiple information regarding adulteration and contamination. In particular, Raman spectra show sharp bands that identify the molecular
composition, and can immediately lead to the detection of multiple components and their quantification,
provided that a calibration is available. A straightforward multicomponent analysis from optical spectra can
be achieved by using multivariate chemometric techniques.

In this work we report about a study focused on the honey origin determination by combining Raman Spectroscopy and Elemental Profiles. In fact, also the contents of trace elements (including Rare Earth Elements - REE) could give an indication on honey geographical origin, other than on environmental pollution. In particular, 18 honey samples of different well know origin (botanical and/or geographical) were submitted to chemical characterization by applying Raman spectroscopy and elemental analysis. The instrument for Raman spectroscopy used in this experiment provides laser excitation at \( \lambda=1064 \) nm; this long excitation wavelength (which is not the most popular one for Raman experiments) makes it possible to avoid fluorescence effects that are common in sweeteners and could overcome the weak Raman signal. Elemental analysis has been performed applying both Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) and Inductively Coupled Plasma–Mass Spectroscopy (ICP-MS).

Results obtained by all the different techniques (Raman spectroscopy, ICP-AES and ICP-MS) were then
submitted to chemometric analysis.
Dispersive Raman spectroscopy at 1064 nm for rapid screening of deoxynivalenol in wheat bran: preliminary results

Ciaccheri L. 1), Mignani A.G. 1), Mencaglia A.A. 1), De Girolamo A. 2), Lippolis V. 2), Pascale M. 2)

1) Institute of Applied Physics “Nello Carrara”, National Research Council of Italy (IFAC-CNR) Via Madonna del Piano, 10 – 50019 Sesto Fiorentino (FI), Italy. E-mail: l.ciaccheri@ifac.cnr.it

2) Institute of Sciences of Food Production, National Research Council of Italy (ISPA-CNR) via G. Amendola, 122/O - 70126, Bari, Italy. E-mail: annalisa.degirolamo@ispa.cnr.it

Deoxynivalenol (DON) is a Fusarium toxin which frequently occurs in grains. Because of the toxic effects induced by DON, many regulations worldwide have established safety levels in food and feed. For instance, the EC maximum limit for DON in unprocessed wheat bran has been set at 750 μg/kg. New devices are envisaged for the rapid detection of DON in grain stocks in order to verify the compliance with EU regulation and to perform a quick assessment of contamination without using chemicals and bench analytical instruments. Optical spectroscopy is currently emerging as a modern and “green” analytical technique for intact food analyses, thanks to the non-destructive nature of light measurements which enable rapid checks without making use of reagents or chemical treatments, thus avoiding the problem of waste disposal.

The objective of this study was to assess the use of Raman spectroscopy, excited at 1064 nm by using a dispersive detection scheme, for rapid screening of DON in wheat bran. Twelve wheat bran samples contaminated with DON in the range ≤100-1600 μg/kg were considered. Four replica measurements were carried out for each sample, thus taking into account unavoidable inhomogeneity of contamination. Raman spectra were processed using Standard Normal Variate (SNV) and Orthogonal Signal Correction (OSC) for compensation of scattering influence, and removal of DON-independent effects. Then, Partial Least Square regression was applied as a predictive model for DON quantification. A coefficient of determination R²=0.72 was obtained, together with a root means square error of calibration RMSEC=313 μg/kg, thus indicating that Raman spectroscopy has good potential as a rapid tool for DON detection.

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Fluorescence Polarisation Immunoassays for rapid determination of T-2 and HT-2 toxins and ochratoxin A in cereals and derived products

Lippolis V. 1), Porricelli A.C.R. 1), Valenzano S. 1), Suman M. 2), Pascale M. 1)

1) Institute of Sciences of Food Production (ISPA), National Research Council of Italy (CNR), Via G. Amendola 122/O, Bari (Italy) e-mail: vincenzo.lippolis@ispa.cnr.it
2) Barilla SpA, Food Research Labs, Via Mantova 166, Parma (Italy)

Mycotoxins are toxic secondary metabolites produced by filamentous fungi under a wide range of climatic conditions on agricultural commodities, mainly cereals, in the field as well as during storage. To protect consumers from the risk of exposure to these toxins the European Commission has set recommended levels or maximum permitted levels for mycotoxins of major concern in a wide range of commodities. The development and validation of rapid, sensitive and reliable methods for mycotoxin determination in cereals and derived products is highly demanded.

Fluorescence polarisation immunoassay (FPIA) is a rapid homogenous assay that measures competition between a fluorescently labelled antigen (tracer) and unlabelled antigen in solution for binding a specific antibody. The FP signal is inversely related to the antigen content that competes with the tracer, and it increases when the binding of specific antibody to the tracer increases. Unlike most immunoassays (e.g., ELISA), the main advantage of this format is that additional manipulation steps, as multiple washing steps or separation of free from antibody-bound analyte, are not necessary.

We have recently developed FPIAs for the determination of ochratoxin A (OTA) in wheat and T-2 and HT-2 toxins in wheat, barley, oats and oatflakes. In-house validation of these assays has been performed on each tested matrix using either artificially and naturally contaminated samples, and reference materials. These FPIAs are rapid, easy-to-use, readily automated and suitable for high-throughput screening as well as for the quantitative determination of mycotoxins in foodstuffs.

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Deoxynivalenol (DON) is a mycotoxin mainly produced by several Fusarium species occurring in cereals and derived products. Rapid, robust and inexpensive methods using Fourier-Transform-Near Infrared (FT-NIR) spectroscopy have been recently developed at ISPA-CNR to predict DON levels in durum wheat. Linear Discriminant Analysis (LDA) models were developed based on different cut-off limits (i.e. 1000, 1200 and 1400 µg/kg DON) that were set at levels lower than the EC maximum limit for DON in unprocessed durum wheat (i.e. 1750 µg/kg). The overall classification rates of models were 89-91% with false compliant values of 3-7%. Model using a cut-off of 1400 µg/kg fulfilled the requirement of the European official guidelines for screening methods. Partial Least-Squares (PLS) regression analysis was also used to determine DON content in wheat samples in the range of <50-6000 µg/kg (as determined by a reference HPLC method). The model displayed good regression quality with a root mean square error (RMSE) of prediction of 868 µg/kg.

The feasibility of using FT-NIR spectroscopy was also investigated to rapidly predict DON in durum wheat bran at levels up to 1600 µg/kg by both LDA and PLS analysis. The LDA model used a cut-off value of 400 µg/kg that was lower than the EC maximum limit for DON in bran (i.e. 750 µg/kg) and displayed a classification rate of 80% with 5% of false compliant samples. Good performance results were also obtained by applying the PLS statistical model, confirming a good fit between HPLC and FT-NIR data in the tested range with an RMSE of cross-validation of 191 µg/kg.

These findings confirmed the suitability of FT-NIR to rapidly screen a large number of wheat samples for DON contamination and to verify the compliance with EU regulation.

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Combination of sugar analysis and stable isotope ratio mass spectrometry to detect the use of non-grape sugars in must of balsamic vinegar

Perini M., Malacarne M., Nardin T., Simoni M., Camin F., Larcher R.
Fondazione E. Mach (FEM), Via Mach 1, 38010 San Michele all’Adige (TN) ITALY – matteo.perini@fmach.it

The ‘aceto balsamico di Modena IGP’ (ABM) is a PGI (Protected Geographical Indication) vinegar obtained from cooked and/or concentrated grape must (at least 20% of the volume), with the addition of at least 10% of wine vinegar and a maximum 2% of caramel for color stability (EU Reg. 583/2009). This product could be counterfeited not only in its acetic fraction (e.g. by addition of acetic acids obtained from petroleum derivatives) but also in the must fraction by adding exogenous sugars mix (e.g. from beet or cane). In the case of grape must, fraudulent addition of cane and beet sugars has been detected since 1991 by analyzing the isotopic ratios of hydrogen (D/H) and carbon (13C/12C) in ethanol. High Performance Liquid Chromatography with Pulse Amperometric Detection (HPLC-PAD) represents an efficient method for the analytical profiling of minor sugars.

The aim of this work was to investigate whether the HPLC-PAD technique for sugar dosage (sucrose and maltose) is useful as low-cost and time-saving screening of the authenticity of ABM. Different samples of ABMs added with growing percentage of beet, cane and maltose syrup were set up and analysed. A specific experiment to evaluate possible degradation of the added sugar during the shelf time was conducted. Moreover 20 samples of commercial samples of ABMs were taken into account to have a picture of the market.

The results showed that maltose unlike sucrose is stable along the time and for maltose a concentration higher than 0.5 g/kg indicates a presence of non-grape sugars in must.
Chocolate authentication using Mass-Spectrometry techniques combined with chemometric tools

Acierno V. 1,2), Simeone F.C. 1), Alewijn M. 1), van Ruth S.M. 1,2)

1) RIKILT – Institute of Food Safety, Wageningen UR, P.O. Box 230, 6700 AE Wageningen, the Netherlands.
2) Food Quality and Design Group, Wageningen University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands.

Variability of ingredients and processing make it extremely difficult to trace the origin of chocolate and to ascertain its authenticity. The properties of chocolate, indeed, differ considerably from those of the raw cocoa beans; identifying chemical links between the starting cocoa beans and the finished product would expedite fraud control.

For chocolate authentication, we are developing methodologies based on rapid Mass spectrometry (MS) techniques to identify, in the finished products, chemical fingerprints of geographical origin and processing. In this poster, we used chemometrics to analyze the compositions, as obtained by MS, of the volatile and non-volatile components of 93 samples of dark chocolates available on the Dutch market. We used Proton Transfer Reaction–MS to analyze the volatile organic component, and Flow Infusion Electrospray–MS to investigate the non-volatile component of the chocolates. Chemometric analysis of MS data revealed that, for these samples, characteristic marks (i.e., fingerprints) of the cocoa beans remain in the finished chocolate, and made it possible to characterize and differentiate the chocolates, and their ingredients, according to their geographical origins, brands, and types of cocoa.

Beyond authentication of chocolate, the combination of MS techniques with chemometric analysis emerges as a convenient approach to identify, in finished products, chemical fingerprints of geographical origin, and of processing; screening of these fingerprints will ease the authentication of the finished products and empower fraud control.
Pure and mixed Arabica and Robusta roasted coffee analysis using FT-Raman spectroscopy: proof of principle

Mandrile L., Rossi A.M.

Istituto Nazionale di Ricerca Metrologica (INRIM), Strada delle Cacce 91, Torino. Italy

The main goal of chemical food analysis is usually to link food products with its distinctive chemical nutritional and organoleptic features. In order to facilitate surveillance programs and prevent fraud or adulteration in the global market the development of simple and rapid analytical methods which don’t need long and difficult sample pretreatment is urgently needed.

Coffea arabica L. (Arabica) and Coffea canephora Pierre (Robusta) are the two coffee varieties of commercial interest. They differ in a range of agronomic, genetic, and chemical properties. Therefore, it is crucial to have accurate methods to determine the Robusta-to-Arabica-ratio in blends due to the their significant price difference. Nowadays, chromatographic techniques are well-established methods to distinguish the two species but also spectrographic methods such as Fourier transform infrared spectroscopy are able to discriminate Arabica and Robusta beans.¹ Raman spectroscopy has recently demonstrated the possibility to obtain reliable results on soxhlet extracted oil from Robusta and Arabica beans.² In this work we try to demonstrate the possibility of distinguish pure Arabica and mixed Arabica-Robusta roasted coffee by Raman spectroscopy after an easy and fast water extraction.

A rapid and simple extracting method has been optimized the aqueous filtrate is directly analyzed with 1064nm excited FT-Raman Spectroscopy. The acquired spectra were subjected to discriminant analysis classification with the aim of separating the two variety of coffee and their blends. The final goal of this work is to provide a model with high predictive capability able to determine the Robusta and Arabica fraction in unknown blends.

Beside we applied FT-Raman and FT-IR spectroscopy to follow the roasting process of ground coffee at different time and temperature observing a gradual increase of the oxidised species (C=O 1730cm⁻¹ FT-IR peak) and studying the volatile compound decrease (CGA 1630cm⁻¹ FT-Raman peak).

The potential of the performed technique to provide analytical information on coffee samples on the basis of the mixture composition and the roasting method will be presented and the main advantages of using Raman Spectroscopy on ground and water extracted coffee will be addressed.


Two New Different Enzyme Devices for Ethanol Determination in Alcoholic Beverages. Comparison, Correlation and Statistical Data.

Tomassetti M., Angeloni R., Merola G., Campanella L.

“Sapienza” University of Rome, Department of Chemistry – P.Le A. Moro 5, 00185, Rome (Italy)

mauro.tomassetti@uniroma1.it

New different enzyme amperometric devices for the determination of ethanol in alcoholic beverages were developed by immobilizing alcohol oxidase or catalase in κ-Carrageenan gel layer overlapping an amperometric gaseous diffusion Clark type oxygen electrode. The variation of the oxygen concentration in the aqueous solution due to the enzymatic reactions, was measured at a constant applied potential. First of all the response of two biosensors toward standard solutions of methanol, ethanol, n-propanol, n-butanol, ethylenglycol and glycerol was recorded, compared and discussed. In the characterization studies of the biosensors several parameters such as pH, operational stability, response time, analysis time, measure and calibration repeatability, between-days and between-electrodes calibration reproducibility, linearity, sensitivity and substrate specificity were studied. Finally by using the developed biosensors, the ethanol concentrations of several wine and beer samples were determined and the results, obtained with the two enzyme electrodes, were compared. Precision and accuracy showed by the two methods were similar and the recoveries were greater than 90% for both biosensors. A detailed investigation was carried out concerning the correlation between data found measuring ethanol content of several commercial beverages, both using two devices and among these experimental results and nominal values given by producer firms. Lastly a statistical evaluation of variance by F-test was also performed. The response of the catalase biosensor was not influenced by the presence of the methanol. Therefore the biosensor method using catalase enzyme seemed to be the most suitable, between the two enzyme devices, for a selective and less expensive determination of ethanol in alcoholic beverages.
Impedance Spectrometer for Food Quality Control

Krejčí I., Musil M., Břoušek A.
College of Polytechnics Jihlava, Department of Electrical Engineering and Computer Science, Tolstého 16, Jihlava, Czech Republic – e-mail: krejci19@vspj.cz

The electrical impedance spectroscopy is widely used in material science, because this quantity and its frequency spectrum are important features of any material. This method became popular in measurement of biological and biochemical objects, including foods, especially in the specification of material ingredients and their concentration. This application requires quite different approach to the construction of impedance spectrometers, because they are usually designed for measurements in laboratory conditions [1], [2], while food materials are processed under rough climatic conditions (low temperature, high air humidity, etc.). Besides, portability and measurement automation are the accented features of the instrument. To reach these strict requirements, the new impedance spectrometer, taking advantage of modern battery powered techniques, capable of the impedance measuring within the impedance range from 10 W to 1 MW in the frequency range from 10 Hz to 1 MHz has been built. Specific impedance measurement conditions of biological objects [3], the state of the art, the instrument construction, used measurement method and achieved results are discussed.

Keywords: Electrical impedance, electrical impedance spectroscopy, signal processing.

References:
Vitamin C content in aromatic herbs:  
a contribution to food composition databases

Costa D.1), Reis A.R.1), Albuquerque T.G.1),2), Costa H.S.1),2), Castilho M.C.3),4), Ramos F.3),4), Machado A.V.5), Sanches-Silva A.1),6)

1) National Institute of Health Dr. Ricardo Jorge, I.P., Department of Food and Nutrition – Av. Padre Cruz, 1649-016 Lisbon (Portugal) – tania.albuquerque@insa.min-saude.pt;
2) REQUIMTE/Faculdade de Farmácia da Universidade do Porto – Jorge Viterbo Ferreira n.º 228, 4050-313 Oporto (Portugal);
3) CEF - Center for Pharmaceutical Studies, Health Sciences Campus, Pharmacy Faculty, University of Coimbra, Coimbra, Portugal;
4) CNC – Center for Neuroscience and Cell Biology, Pharmacy Faculty, University of Coimbra, Coimbra, Portugal;
5) IPC – Institute for Polymers and Composites/I3N, Department of Polymer Engineering, University of Minho, Guimarães, Portugal;
6) Centro de Estudos de Ciência Animal (CECA), Universidade do Porto – R. D. Manuel II, Apartado 55142, 4051-401 Oporto (Portugal)

There is an increasing interest in healthy lifestyles and food with potential health benefits. Therefore, specific spices and herbs are being used to replace sugars and salt as well as artificial additives.

Food Composition Databases (FCDB) provide information on important food components, however there is still lack of information regarding many aromatic herbs. The aim of this study was to determine vitamin C by high-performance liquid chromatography (HPLC) with diode array detection (DAD) in a vast range of aromatic herbs, including parsley (Petroselinum crispum Mill.), sage (Salvia officinalis L.), thyme (Thymus vulgaris), rosemary (Rosmarinus officinalis L.), oregano (Origanum majorana L.) and tarragon (Artemisia dracunculus L.).

The analytical method used was previously fully validated. The analytical column used was a SynergiTM Hydro-RP (150 x 4.6 mm I.D., 4.0 μm particle size) protected with a SecurityGuard Cartridge AQ C18. The mobile phase consisted of 20 mM NH4H2PO4, pH 3.5, and containing 0.015% of m-H3PO4 (w/v). The detection was monitored at 245 nm. Total vitamin C content per 100 g of edible portion ranged between 15.5 mg for tarragon and 149.2 mg for parsley. Parsley presented the highest content in ascorbic and dehydroascorbic acids (74.8 and 74.4 mg per 100 g edible portion, respectively). The global market of aromatic herbs is expanding, thus the obtained analytical results are an important source of reliable data to be included in the Portuguese FCDB.

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Recipe calculation of eleven Iranian stews (Khoresh)

Ghazizadeh M.1, Behnammoradi M.2

1) 1909-1082, Seymour St. Vancouver, BC, Canada - mitra1956@yahoo.com
2) 1909-1082, Seymour St. Vancouver, BC, Canada - moradi1330@yahoo.com

Material and Methods: The composition of 100 g edible portion of 10 Iranian stews (Khoresh) have been calculated, based on the composition of edible part of raw ingredients. Khoresh consists of pieces of red meat or chicken, fried with chopped onion in oil, some green herbs or vegetables which are first sautéed and then added to the meat; other ingredients may consist of legumes, fruits and nuts. The recipes, especially the proportion of the main ingredients have been adopted from the Honare Ashpazi cookbook (1). Cooking yield factors (YFs) have been measured by dividing the weight of edible part of cooked food to raw ingredients, and has been applied in recipe level. The data for nutrient content of ingredients has been extracted from the online Canadian Nutrient File, USDA nutrient database, Food Composition Tables of Iran, and Food Composition Tables for the Near East, FAO (2, 3, 4,). Retention factors (RFs) have been extracted from the USDA Table of Nutrient Retention Factors (5) and BFE (6), which have been applied at ingredient level. The whole procedure including preparation, cooking, calculating YFs and analyzing nutrient contents of each dish carried out in duplicate and results have been reported as mean values.

Results: The Proximate/Energy component of 100 g edible part of dishes is: 63.26 - 79.44 g moisture, 0.98 – 2.07 g mineral, 6.08 – 11.07 g protein, 5.77 – 16.25 g total fat, 4.44 – 17.01 g carbohydrate, 1.24 – 2.55 g dietary fiber, and 60.69 – 215 Kcal energy. The dishes also contain 164.48 – 331.41 mg sodium, 0.69 – 1.88 g saturated fat, 0.05 – 0.5 g trans-fat, and 13.22 – 36.41 mg cholesterol. Cooking yield factors of dishes are between 0.80 and 1.38.

Significance: These findings will be useful for professionals interested in knowing the composition of Iranian meals, and also for individuals who are curious about their daily diet.

Keywords: Recipe calculation, Iranian stew, Khoresh

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Proteomic Strategies for the Identification of Proteins in Durum Wheat

Palermo C., Mentana A., Nardiello D., Quinto M., Centonze D.

Università degli Studi di Foggia, Dipartimento di Scienze Agrarie, degli Alimenti e dell'Ambiente, Via Napoli, 25, I-71100, Foggia (Italy) - carmen.palermo@unifg.it

Although the fulfillment of genome sequences of several green plants has not yet been completed, there are many researches focused on the determination of the functional network of proteins by proteome analysis. In the last decade, a wide identification of proteins by MS was performed in rice, Arabidopsis, maize, barrel medic (Medicago truncatula) [1, 2]. The relatively small genome size of rice (420 Mb) and its importance as a food grain have made rice a primary target for genome sequencing and proteomic studies. On the contrary, proteomic studies in wheat are in progress and the database of wheat is incomplete also owing to the large genome size (16,000 Mb). In a recent work, to broaden the knowledge of the durum wheat gluten proteome, three cultivars were compared in two different growing seasons by a proteomic approach [3].

Most of the wheat proteins are very difficult to identify because they have homologous sequences to other proteins. For that reasons, new proteomic approaches have to be developed for the study of these wheat proteins.

In this work, two different enzymatic digestion methods and bioinformatics approaches coupled with nanoLC and electrospray ionization ion trap mass spectrometry is described for the identification of metabolic and gluten proteins of durum wheat cultivars, in order to increase the final number of identified proteins.

The use of MS techniques in combination with the Swissprot, NCBI and EST Viridiplantae databases allowed us to identify wheat proteins minimizing the risk of false-positive identifications.

Effects of apple de-hydration by innovative technologies on the microstructure and olfactory quality

Cammarota G. 1), Laurienzo P. 2), Fasulo G. 1), Di Stasio M. 1), Volpe M.G. 1)

1)Istituto di Scienze dell’Alimentazione, CNR, Via Roma, 64, 83100 Avellino, Italy. mgvolpe@isa.cnr.it
2)Istituto di Chimica e Tecnologia dei Polimeri, CNR, Via Campi Flegrei 34, 80078 Pozzuoli, Naples, Italy.

The effects of innovative technologies of apple de-hydration based on the application of biopolymer films on the flavour content and on microstructure have been investigated. The microstructure is analysed by means of Environmental Scanning Electronic Microscopy (ESEM), while the olfactory quality of differently preserved apple were analysed using an electronic nose equipped with Metal Oxide Semiconductors (MOS) sensors.

Our approach were based on the use of films made of blends of biodegradable and biocompatible polymers belonging to well know families of natural polysaccharides, already approved for use in agro-food industry. Different compositions of the two components of blends are been utilized in order to verify the differences in the final morphologies and olfactory quality.

The results have demonstrated that the utilized films made of polysaccharides are able to selectively control the loss of water from fruits by adsorbing it at slow rate, without losing volatiles and flavours. Moreover some compositions of the polymeric blends preserve the olfactory quality better than other. In fact the analysis of electronic nose data shows that the apple slices packed in blends composed by Agar as major component, better preserve olfactory properties.

The texture of dried apple slices, packed in polysaccharides films, has been investigated by ESEM analysis and compared to freeze dried slices. We found that whereas freeze drying leads to cell structure breakage, the slices dried by packaging in our films preserve a packed structure, with few regular cavities, with no evidence of cell wall collapse.

Re-hydration tests showed that the re-hydration rate of slices dried in polysaccharides films is very low respect to the freeze dried slices; moreover, after re-hydration, the texture closely resembles that of the fresh fruit.

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Sensory characteristics affected by storage of Escamoles *Liometopum apiculatum* M.

**Melo-Ruiz V.**, Sandoval-Trujillo H., Sánchez-Herrera K., Diaz-Garcia R., Calvo-Carrillo C.

1. Universidad Autónoma Metropolitana, Xochimilco, Departamento de Sistemas Biológicos – Calz. del Hueso 1100. Col. Villa Quietud, Coyoacán, C.P. 04960, México, D. F., (Mexico) – *vmelo@correo.xoc.uam.mx*

2. Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, – Vasco de Quiroga 15, Colonia Sección XVI, Tlalpan, C.P.14000, México D.F., MEXICO.

Escamoles a common food stuff in rural communities and a gourmet dish at high class restaurants in urban cities are seasonal available, and production is limited to four month a year, however customers demand is all year round. Insects are high in polyunsaturated fatty acids PUFAs, therefore, changes during storage such as rancidity, lipid oxidation, modification of sensory properties and α-tocopherol and protein loss may happen. The aim of this study was to investigate quality status of escamoles ant eggs *Liometopum apiculatum* M refrigerated at -4°C and frozen at -30°C for up to 12 months. Samples analysis included: lipid hydrolysis, free fatty acids formation and oxidation loss of antioxidant α-tocopherol, protein changes and sensory characteristics modification. Escamoles packed in polyethylene bags vacuum sealed and refrigerated at -4°C and frozen at -30°C for 1, 3, 6, 9 and 12 months. Data obtained were: Samples refrigerated, after the third month, lost water and sensory characteristics were slightly unpleasant, no rancidity and fatty acids, lipids proteins and α-tocopherol decreases flavor was unpleasant. Samples frozen maintain sensory characteristics up to 12 months but fatty acids, proteins and α-tocopherol present modifications. The use of low temperature -30°C inhibited rancidity, leading to good quality escamoles.

**Key words:** Storage, Escamoles, Insects, Sensory, Nutrition
UMAMI taste and Escamoles ant eggs edible insect palatability.

Melo-Ruiz V., Murata Ch., Quirino-Barreda T., Sánchez-Herrera K., Diaz-García R.

Universidad Autónoma Metropolitana, Xochimilco, Departamento de Sistemas Biológicos – Calz. del Hueso 1100. Col. Villa Quietud. Coyoacán, C.P. 04960, México, D. F., (Mexico) – vmelo@correo.xoc.uam.mx

It is well known among nutrition experts that nutritional status is strong determinant of food preferences. This can be established on the basis of the association between the sensory characteristics of a food and the physiological consequences of ingestion. Palatability promotes the selection, intake, absorption and digestion of foods. All five senses are involved in determining food palatability, with taste playing the major role, Umami is a characteristic taste imparted by glutamate amino acid present in many foods such as Escamoles ant eggs edible insects, glutamate plays an important role in taste, palatability and acceptability of insects by people. Umami taste discovered in Japan coined the term “UMAMI” to identify it. The umami taste has characteristics that enhance and differentiates it from other tastes, including a taste enhancing pleasurable sensation and is dependent of nutritional status. The umami taste does not have specific characteristics by itself. The aim of this study is to analyze the protein and amino acids content, focus in glutamic acid to determine the contribution of glutamic acid in sensory characteristics of escamoles. Escamoles collected at Mexico State content 51.63% of proteins and 9.2 mg/16g N of glutamic acid. Escamoles sensory characteristics are enhanced by glutamic content. The glutamic acid has other important functions in human’s metabolism, but it is not the topic to consider in this study.

Key words: Umami, Taste, Escamoles, Insects, Palatability.
Taste Analysis on Conventionally, Organically and Naturally Grown Cabbage

Yoshida K., Funabashi M.
Sony Computer Science Laboratories, Inc.
Takanawa Muse Bldg., 3-14-13 Higashigotanda, Shinagawa-ku, Tokyo (Japan)
kaoru@csl.sony.co.jp, masa_funabashi@csl.sony.co.jp

Today agricultural products through various farming methods are commercially available. Though the diversity on their compositions has been recently reported, the objective difference on their tastes remains to be clear. We conducted taste analysis on conventionally (C), organically (O) and naturally (N) grown cabbage that were available in Japan. For measuring tastes, a smart taste-sensing system was used, which consists of eight different chemical/biochemical sensors, each highly selective for Na⁺, K⁺, Cl⁻, H⁺, sucrose, glucose, glutamate or caffeine, and a human knowledge-base that maps the signals from the sensors to the scores of five different tastes (saltiness, sourness, sweetness, umami and bitterness) through a learning process with human senses [Citterio and Suzuki 2008]. After grinding up individual cabbage in H₂O, their supernatants were subjected to the taste measurement. Out of five different tastes, bitterness was the one that most significantly differentiated the samples, especially, N-cabbages from the others. Among the N-cabbages, one was exceedingly bitter, far more than the others. The bitterest one happened to grow under shadow, while the others in the sun. On sweetness and sourness, N-cabbages were low, while C-cabbages were high and O-cabbages were in between. On saltiness and umami, there was little difference among all samples. We also studied lasting effects of tastes, by first soaking sensors in the sample and then placing them out in the air, and there found that bitterness was the most lasting taste. In summary, in the case of cabbage, the growth condition may influence most significantly on the taste of bitterness, followed by sweetness and sourness. Many bitter phytochemicals are known to play defensive roles for plants and have physiological and pathological effects on human health. Accumulation of objective information on tastes, in addition to compositions, of agricultural products is important for systematic assessment of food quality and safety.
Head Space – SPME – GC/MS of virgin olive oils as a possible tool to improve sensory evaluation: critical evaluation of analytical data applying peak deconvolution software

Moret E., Conte L.

Dept of Food Science, University of Udine – Via Sondrio 2/a 33100 Udine Italy moret.ERICA@spes.uniud.it, lanfanco.conte@uniud.it

Sensory evaluation (“panel test”) of olive oils was established as official analytical method for olive oils both in the International Olive Oil trade standard and in the EU legislation [Reg(CEE)2568/91, Reg(CE) 796/2002]. As time passed and this method was wider and wider applied, a number of drawbacks were highlighted, mainly depending on disagreement between different panels, probably depending on lack of reference standards.

Even before panel test was adopted as official methods, several attempts were carried out trying to correlate sensory characteristics to presence and concentration of selected volatile analites.

In this poster, preliminary results of a three year project are reported, dealing with GC/MS analysis of volatiles of virgin olive oils that already underwent to panel test.

The used instrument was a double column Agilent capillary gas chromatography model 7890B coupled to an Agilent model 5977A single quadrupole mass spectrometry; this instruments was assisted by a Combi PAL autosampler suitable to perform pre incubation of vials, SPME sampling and injection.

The GC-MS apparatus was slightly modified in order to make two columns of different polarity (DB-5MS and VF-WAX) always connected to the spectrometer ionization camera.

Using such a configuration of the HS-SPME-GC-MS, each sample was analysed by using both column and linear retention index was calculated for each analyte in order to make identification more reliable.

Even if the elution on two different polarity column can help in identification, however if overlap occurs, this can affect the reliability of peak measurement; because of this, Agilent software make available a “Find by Chromatographyc Deconvolution “ algorithm, suitable to highlight the presence of different compounds co-eluted with principal peak of an analyte. The reliability of peak measurement is a critical point for this kind of studies, because often the concentration of selected analytes is correlated to sensory scores.
Contribution to a descriptive sensory analysis lexicon for the evaluation of commercial balsamic vinegars

Lalou S., Papadopoulou M., Hatzidimitriou E., Tsimidou M. Z.
Aristotle University of Thessaloniki, Chemistry Department, Thessaloniki (Greece) tsimidou@chem.auth.gr

Traditional Balsamic Vinegar of Modena and Reggio Emilia (TBVM and TBVRE) and Balsamic Vinegar of Modena (BVM) are highly esteemed and priced products worldwide. Recently, new industrial products are permitted by law to be traded as Balsamic Vinegars (BV). Limited is the knowledge for the composition and sensory profile of the latter. Sensory evaluation methods are important tools in food quality control. In particular, sensory descriptive analysis (DA) is recommended for the assessment of new products with complex sensory and texture profiles. In a recent publication, a lexicon with 20 attributes was used for the DA of TBVMs and TBVREs. Our work is a contribution toward a DA lexicon for industrial BVs, PGI or not. A panel of 8 assessors was trained in DA of balsamic vinegars. From a predefined list containing 46 terms, a final one consisting of 15 taste and aroma descriptors was produced according to ISO 11035:1994 multistep procedure. Six of them (caramel, raisin, wood, sweetness, bitterness and acidity) were common with those in the lexicon for TBVs. Pungent, aftertaste, tannic, sundried tomato, red fruits, ethyl acetate, quince, coffee, tapenade, were also found by the panelists in descending order of importance. Next, the sensory characteristics of 11 commercial BVs from Greek and Italian market were evaluated using this 15-term lexicon. The obtained data revealed a more homogenous sensory profile for the PGIs. The greater variability in the sensory profiles of the Greek industrial balsamic vinegars indicates the effect of different starting materials to the characteristics of the end product as well as the need for improvement and standardization of processing practices. These vinegars were characterized—though to a different extent—by less desirable attributes such as acidity, “pungent” and “tannic” in contrast to PGIs, to which attributes such as sweetness, raisin, red fruits and caramel prevailed.

References:
Minerals and trace elements contents in fruit juice: a contribution for Portuguese total diet studies

Coelho M. 1), Nascimento A. C. 1), Gueifão S. 1), Sardinha D. 2), Castanheira I. 1)

1) National Institute of Health Doutor Ricardo Jorge, Department of Food and Nutrition, Av. Padre Cruz, 1649-016 Lisbon, Portugal - mariana.coelho@insa.min-saude.pt
2) Instituto Superior de Ciências da Saúde Egas Moniz, Monte de Caparica, Portugal

Total diet studies complement traditional monitoring and surveillance by providing a scientific basis for population dietary exposure to contaminants, with potential impact on public health. This should be achieved by using quality assurance procedures and specifically by applying analytical methods where performance criteria have been established in compliance with metrological requirements.

The aim of this study is to determine the amounts of minerals and trace elements in 24 types of fruit juices, obtained from various kinds of fruits, available in the Portuguese market and representative of consumption by Portuguese population.

Inductively coupled plasma optical emission spectrometry (ICP-OES) technique was employed for determination of the elements Sodium, Potassium, Calcium, Phosphorus, Magnesium, Manganese, Iron, Copper, Zinc, and ICP-MS for the trace elements, Chromium, Nickel, Molybdenum, Strontium, Tin, Cobalt, Selenium, Arsenium, Cadmium and Lead.

In orange juice high Potassium and Phosphorus content were observed. High concentration of Sodium 6,9 % was determined in apple juice and Arsenic was found below limit of quantification for majority of samples under analysis. All the results have been achieved in agreement with rigorous metrological procedures as previous defined.

This information will be used for dietary exposure assessment, which combines food consumption data with data on the concentration of chemicals in food. The resulting dietary exposure estimate may then be compared with the relevant health based guidance value for the food chemical of concern, if available, as part of the risk characterization. Metrological procedures reveal a crucial tool to guarantee fiability of measurement results used in risk assessment associated with the consumption of fruit juice.
Issues for Harmonization in Dietary Exposure Assessment: Focus on Total Diet Studies

D’Amato M., Aureli F., Raggi A., Cubadda F.

Istituto Superiore di Sanità - National Health Institute, Department of Food Safety Safety and Veterinary Public Health – Viale Regina Elena 299, Rome (Italy) – francesco.cubadda@iss.it

Dietary exposure assessment is quantitative evaluation of the intake of chemical substances (including nutrients) via food at large, i.e. including beverages, drinking-water and food supplements. It encompasses different types of surveys, from Duplicate Diets Studies (for estimating dietary intakes at the individual level) to Total Diet Studies (TDSs). The latter represent the gold standard for calculating population dietary exposure and assessing potential impact on public health, and have been the subject of a joint guidance by EFSA, FAO and WHO in 2011.

A TDS consists of selecting, collecting and analysing commonly consumed food purchased at retail level, processing the food as for consumption, pooling the prepared food items into representative food groups, homogenising the pooled samples, and analysing them for harmful and beneficial chemical substances. TDSs are designed to cover the whole diet and to measure the amount of each chemical substance ingested by the population living in a country, ideally using average and high-level consumption data for final exposure calculations. In Italy, ISS is coordinating the 2012-14 TDS that will assess the exposure of the Italian population to both toxic and essential trace elements and a number of food contaminants.

Even though the key aspects of a TDS are well defined, there are many methodological differences in the way this type of study is performed at the national level that limit the possibility to compare exposure of different populations across Europe and worldwide. The European project TDS-Exposure was launched in February 2012 with the aim of creating an EU-wide network of TDS-Centers using common tools like databases and modelling software. With 26 participants from 19 countries, and strong links with European and international organisations like WHO, FAO or EFSA, TDS-Exposure aims at harmonizing the TDS methodology and ensuring data collected in the future can be compared across countries.
Analytical Quality Assurance in the Determination of Urinary Iodine

Aureli F., D’Amato M., Raggi A., Cubadda F.

Istituto Superiore di Sanità - National Health Institute, Department of Food Safety Safety and Veterinary Public Health – Viale Regina Elena 299, Rome (Italy) – francesco.cubadda@iss.it

Iodine is present in the body in minute amounts, mainly in the thyroid gland. It is an essential element, whose role is in the synthesis of thyroid hormones. Dietary iodine deficiency (ID) is associated with a large range of abnormalities reflecting thyroid dysfunction, grouped under the heading of “iodine deficiency disorders” (IDD). Goitre and cretinism are the most visible manifestations of ID; others include hypothyroidism, decreased fertility rate, increased perinatal death and infant mortality. Irreversible mental retardation is the most serious disorder induced by ID. A deficit in iodine resulting in thyroid failure during the critical period of brain development, i.e. from fetal life up to the third month after birth, will result in irreversible alterations in brain function.

It is estimated that nearly 2 billion people have inadequate iodine nutrition, with about 400 millions in Europe. Correction of ID, when carried out at the right time, reduces or eliminates all related consequences on human health. The technology to prevent ID – salt iodization – is easy to implement and affordable even by governments with limited health budgets. The recommended indicator for assessing the extent of ID within a population is median urinary iodine (UI). According to generally accepted criteria, ID is a public health problem in populations where the median UI concentration is <100 μg/l.

Accurate determination of UI is essential in IDD control programmes. Since 2008 our laboratory has participated to the Ensuring the Quality of Iodine Procedures (EQUIP) program, established by the US CDC to help laboratories worldwide assess the accuracy of their UI analyses, with a 100% success rate. Our experience in AQA of UI determination by ICP-MS is discussed and application to studies on populations with IDD and on the interaction between iodine status and exposure to endocrine disrupters with thyreostatic effects is presented.
Molecular methods applied for detection of primary and secondary microflora in raw materials for Grana Padano cheese-making process

Federici S., Ferrari S., Miragoli F., Rebecchi A., Morelli L., Callegari M.L.
Centro Ricerche Biotecnologiche, Università Cattolica del Sacro Cuore, Via Milano 24, 26100 Cremona, Italy

Introduction: Natural Whey Starters (NWS) are the most common cultures currently used to produce different varieties of cheeses; NWS together with raw milk microbiota are responsible for most cheese characteristics.

Methods: Twenty-eight Grana wheels produced in two different factories were characterized for their quality. The natural whey, row milk, ripened cheese and skimmed milk were investigated by qPCR and DGGE-PCR in order to establish a correlation between microbial composition and quality of the final product. Several sets of primers were tested on different genes in order to correctly quantify microbial populations of primary and secondary microflora. All qPCR was based on SYBRGreen chemistry followed by melting curves analyses.

Results: The analyses performed by DGGE-PCR highlighted an heterogeneous microbial pattern in Grana cheese, with common bands representing the Natural Whey Starters, and the secondary microflora coming from raw milk contamination. qPCR gave an enumeration starting from raw materials to the ripened cheese (18 months), revealing that strains belonging to Lactobacillus casei group (L. casei, L. rhamnosus and L. paracasei) were dominant in some productions, whereas in some specific periods of the year, the L. fermentum was the most abundant species in the secondary microflora.

Conclusions: The results show that the Real-Time PCR is a suitable and reliable method to characterize bacteria involved in the cheese-making process, that could be associated with the DGGE-PCR technique for a correct monitoring concerning bacterial population during the cheese-making and ripening.
The measure of the evaporation rate to evaluate the different behaviour of wine glasses during tasting

Venturi F., D’Agata M., Sanmartin C., Taglieri I., Andrich G., Zinnai A.
University of Pisa – Department of Agriculture Food and Environment (DAFE) – Via del Borghetto 80, 56124 Pisa (Italy) – francesca.venturi@unipi.it

In sensorial characterization of wine, the glass represent an essential tool which makes possible the interaction between wine and taster’s senses.

Since the choice of the best combination of "glass type" and "wine tasted" appears to be an important factor for the definition of the wine sensorial profile, an experimental research was developed to investigate the impact of glass shape on the sensory perception of the attributes of two different wines: a rosé wine and a full bodied red wine aged in oak barrels.

With the aim to better understand how the differences related to the glass type can influence the consumer perception of the wine, the same product was assessed in each glass, at the same moment, for three times (t= 0, 40', 120') during every tasting session.

The evolution of wine in each glass was characterized also by a chemical and physical point of view, in order to verify if the different characteristics of the glasses utilized influenced the chemical parameters analysed, so to justify the changes in the sensorial perception expressed by the panel components. The wine evaporation rate was measured by determining the losses of weight of the wine inside each glass at different tasting times. The time evolution of evaporation processes was represented by some straight lines passing through the origin of the axes. The slope of these lines could be used to express the rates of the loss of smell intensity of the wine tasted as a function of the glass used.

Thanks to the general overlapping between the experimental results related to the evolution of both sensorial, chemical-physical characteristics of the wines tasted and the development of evaporation processes, it was possible to identify which glass seemed to show the best influence on the sensory perceptions of the product.
The measurements of the kinetic constants used to describe the evolution of alcoholic fermentation in model solutions

Zinnai A., Sanmartin C., D’Agata M., Taglieri I., Andrich G., Venturi F.

University of Pisa – Department of Agriculture Food and Environment (DAFE) – Via del Borghetto 80, 56124 Pisa (Italy) – angela.zinnai@unipi.it

Over the last twenty years, grapes having great concentrations of phenols and aromatic compounds but also very high sugar contents were processed with the aim to obtain high quality wines. As a consequence, the vinification of these grape musts was more difficult to carry on because of the risk of slowing or stuck of fermentation.

With the aim of describing the sugars bioconversion during alcoholic fermentation, the time evolution of different initial concentrations of D-glucose and D-fructose, dissolved in a model solution simulating a must (citrate buffer at pH = 3.4 inoculated by two yeast strains: S. cerevisiae (strain C) and S. bayanus (strain B), were investigated in presence or not of ethanol in the initial reaction medium.

The concentrations of both the hexoses used and the products of the sugars conversions, as well as the number of viable cells of yeasts, were determined as a function of the alcoholic fermentation time and the kinetics constants used to describe the development of microbial population ($k_o$) and the hexoses metabolism ($k_H$) were measured.

The good degree of repetitiveness shown by constants in the same experimental conditions seem to confirm the reliability of these parameters to describe the kinetic evolution of alcoholic fermentation.

On the basis of the information collected using this kinetic approach, it would be possible to develop technical data sheets, specific for each yeast strain, useful to identify the strain more suitable to the different working conditions characterizing several biochemical processes (ex: wine making, sake making, brewing processes and bioethanol production).

Moreover these kinetic constants could be adopted as bio-markers in selection and breeding of wine yeast strains having a lower tendency for sluggish fructose fermentation.
Optimization of microbial fermentation of *Irvingia Gabonensis* seeds in “Itugha” production

Ekpe Onot O, Igile Godwin O, Eyong Ubana E, Eteng Mbeh U.

University of Calabar—Department of Biochemistry. College of Medical Sciences, Calabar, Nigeria.

Email: rooseh01@yahoo.com, giotech2000@yahoo.com, eubana@yahoo.com, mbeheten@yahoo.com

Optimization is meant to develop the design and process of fermenting *irvingia gabonensis* seeds, in the production of a product itugha considered more nutritious than the raw material from which it is produced (Ekpe, O.O and Igile, G.O. 2013). Itugha’s quality is measured by taste, aroma and flavour. This study assesses the optimal use of microbes, pH and temperature during the fermentation process.

Fermentation, has been implicated in the traditional production of itugha from fresh *irvingia gabonensis* seeds (Ekpe, O. O. 2009; Ekpe O.O. 2007). This production process was carried out under controlled environment by measuring pH, conditioned temperatures, % titratable acid and organic acids. Early stage fermentation is caused by Bacillus spp, pH 6-7, 30 °C and at 1.8% acidity of extract; the intermediary stage, Micrococcus spp, and Streptococcus, pH 5.6, 35-38 °C and 4.4% acidity of extract, late stage, principally Candida tropicallis DMB321, pH 4.5-5.1, 70 °C and 5.4% acidity of extract. Citric acid 2.4% DM, Glycolic acid 1.22% DM and oxalic acid 2.98% were quantified. For the Sensory analysis, 9-point Hedonic Scale (Ngoddy and Onuoha 1985) was used. Overall acceptability for Like Extremely=7.5; Like very much=8.8; Like moderately=9.00. Ranking (Larmond 1977) was 72. Population t-test analysis, t-value was 21.18 and F-value 12.25. Progress of flavor development showed, no flavour in the early stage, alcoholic aroma by the 3rd day (intermediate stage) and stringent spicy aroma by 6th day which became prominent after application of heat (final stage).
New genetic approaches for automated feed authentication

Braglia L. 1), Gavazzi F. 1), Giani S. 1), Mastromauro F. 1), Morello L. 1), Breviario D. 1), Imperi E. 2), Grosso V. 2)

1) Istituto Biologia e biotecnologia Agraria, CNR, Via Bassini 15, 20133 Milano, Italy - breviario@ibba.cnr.it
2) Labor srl, Tecnopolo Tiburtino, Via G. Peroni 386,00131 Roma

Animal feed are at the base of the human food chain and their composition is fundamental for animal health and growth, as well as for the safety, nutritional and organoleptic properties of the derived products. For this reason, EU law demands that the composition of compound feed is declared in the label and the quantitative amount of each ingredient is recorded by the producer to be made available on request. In addition, the production of specific PDO products such as Parmesan cheese must follow strict disciplinary rules with regard to the animal diet, including the assurance that prohibited species are absent.

The authenticity of feed composition can be determined by genetic approaches through DNA analysis, to ascertain the presence and the abundance of any given plant species. New analytical, automated methods are under evaluation within the framework of the EU-funded project FEED-CODE, aimed at the quantitative determination of feed composition. Aspects concerning the way to establish cut-off values for presence/absence determination, and the possibility of a reliable quantitative measurement of the single components, done by DNA analysis, are investigated within the project also with reference to suitable standards.
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International Conference

1st IMEKOFOODS
Metrology Promoting Objective and Measurable Food Quality and Safety

October, 12th - 15th 2014
Rome (Italy)