International Standards for Food Authenticity and Allergen Detection from ISO TC 34/SC 16

Horizontal Methods for Molecular Biomarker Analysis

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Abstract
ISO Technical Committee 34 “Food Products”/Subcommittee 16 “Horizontal methods for molecular biomarker analysis” works to ensure that standardized biomolecular testing and laboratory criteria are reproducible and technically sound reducing potential disputes between exporting and importing nations and increasing predictability in world trade. Harmonized, easy to handle methods of analysis with defined patterns and known nomenclatures bring more customers to the market. SC 16/TC 34 has increased international stakeholders’ participation in standardizing biomarker testing, improved the quality and relevance of these standards and continues to increase transparency in international markets, particularly for food authenticity, varietal identification and genetically engineered products. ISO standards have been adopted by Codex Alimentarius and many governments throughout the world. The International Organization for Standardization (ISO.org) was formed in 1946. It is an independent, non-governmental voluntary consensus standard body based in Geneva, Switzerland with a membership of 163 national standards bodies. The US ISO member is the American National Standards Institute (ANSI.org) a consortium of US standardization organizations. ISO TC 34/SC 16 was created in 2008. There are 42 participating countries. Its scope is, Standardization of biomolecular testing methods applied to foods, feeds, seeds and other propagules of food and feed crops. The US delegation responsible for developing the US position for standards development in food authenticity and allergen detection is called the US Technical Advisory Group (TAG). It was delegated to the American Oil Chemist’s Society (AOCS.org) by ANSI. AOCS also hosts the TC 34/SC 16 international secretariat. TC 34/SC 16 has published 20 standards with another 16 under development. Recently published standards include: Technical Specification ISO/TS 16393 Molecular biomarker analysis — Determination of the performance characteristics of qualitative measurement methods and validation of methods, International Standard ISO 16578 Molecular biomarker analysis — General definitions and requirements for microarray detection of specific nucleic acid sequences and International Standard ISO 20813 Molecular biomarker analysis — Methods of analysis for the detection and identification of animal species in foods and food products (nucleic acid-based methods) — General requirements and definitions. More on food authenticity and allergen detection work in ISO TC 34/SC 16 is provided.

Poster Category: Authenticity, Fraud, and Adulteration
Poster Number: AUTH-01
Food safety and food authenticity by peptide mass spectrometry – Constitution of new § 64 LFGB working groups for method validation and standardization

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Abstract
The monitoring of food authenticity and food allergens is a crucial task for consumer protection. In annex II of the EU regulation 1169/2011, 12 groups of materials causing most intolerances or allergies in food are listed that have to be labelled on products for the final consumer. The testing for food authenticity as well as food allergens is mostly done by ELISA or PCR. They also have some limitations and drawbacks. ELISA methods often have specificity issues whereas in PCR methods, allergen detection is achieved only indirectly via DNA. Moreover, both technologies have limitations in terms of the number of species or allergens that can be analysed in the same sample in one assay. For ELISA, typically only one species or allergen can be analysed, whereas for PCR, most assays range between one and four allergens. In recent years, new methods for food authenticity have been developed utilising liquid chromatography coupled to mass spectrometry. Since most food allergies are caused by allergenic proteins, the new methods have the potential to detect not only the allergenic ingredient but also the specific protein that causes an allergy. Another major advantage of such methods is the detection of a multitude of allergen-causing proteins in a single analysis. At present, however, these methods generally lack validation let alone standardisation. The Federal Office of Consumer Protection and Food Safety (BVL) in Germany has recognized the potential of such novel technology applications and therefore constituted working groups with the aim to identify appropriate technologies and validate these for official food control. The new working groups, consisting of experts from the field, will focus mainly on the validation and standardisation of methods for allergen detection and food authenticity. The poster will provide an overview over the current and future work of the newly-formed working groups.

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A traceable two-dimensional image analysis method for the characterizing quality parameters in rice-based candidate reference material


Abstract
The following study describes the preliminary results of the implementation of two-dimensional image analysis as a metrological traceable method for the characterization of a candidate reference material (RM) in rice (Oryza sativa L.). Parameters evaluated in the candidate RM were dimensional, physical parameters of quality used by national and international regulations to categorize and determine the content of the whole kernel, broken kernel and chips in rice. Specifically, a RM was simulated by gravimetric mixing of defined portions of whole rice (100%) and small broken rice (100%) to obtain a specific percentage of whole/broken rice. The proposed method consists of three general processes i) sample preparation, ii) capture and image digitization, iii) data processing and analysis. For sample preparation, a representative mass of rice was carefully dispersed in an optical work area while avoiding the occurrence of grain agglomeration. The capture and digitalization process was carried out with a DigiEye system (VeriVide). Metrological traceability was accomplished using an artifact previously calibrated and incorporated into the image digitalization of all samples. ImageJ software was used to investigate grain parameters which include contrast, brightness, outline, as well as dimensional parameters of the grains such as area, perimeter and maximum length. Dimensional parameters data were analyzed using RStudio in order to perform graphical and statistical analysis of the results. The preliminary results demonstrate the applicability of this method to evaluate dimensional specifications of rice that may be subject to international trade. Also, this method provides metrological traceability to the meter unit, improving the limitation of classical methods which are traceable to mass. Efforts are currently underway to establish this method for the characterization of RM for industrial quality control measurements and organization of proficiency testing (PT).

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Meat Authenticity: Does more frequent PT participation improve PT performance?

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Abstract
LGC is a global provider of proficiency testing (PT) schemes in the field of food quality testing. When the horse meat scandal struck Europe in 2013, LGC launched a meat authenticity PT scheme within months. Since then, additional authenticity test materials have been added, including cheese and fish. Estimates from the Grocery Manufacturers Association indicate that food fraud may cost the world economy almost $50 billion per year and that 10% of all food products are likely to be adulterated. While not all food authenticity issues present a health risk to consumers, most will have an impact on brand reputation and consumer confidence. The evolution of molecular methods allows for more frequent and faster analysis of food materials and; therefore, gives the potential for improve transparency in the industry. As we rely on laboratory analytical measurement results to ensure integrity of the food market, we must simultaneously and continuously ensure laboratories entrusted with this analysis are providing accurate measurement results, using robust and reliable methods. One means, arguably the only means, of externally monitoring laboratory performance is through routine proficiency testing. Many requirements and guidance for laboratory analysis in the food industry (e.g., some accrediting bodies, a GFSI recognized certification program, FSIS guidance documents) set a minimum PT participation of once per calendar year, per method or test. As ISO/IEC 17025 moves towards a risk-based approach to determining PT frequency, laboratories may wonder how to optimize PT participation. Using data from the LGC meat authenticity program over the last two years, this poster will demonstrate that laboratories participating two times or more per year achieve higher rates of satisfactory performance than those participating only once per year.

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Poster Number: AUTH-05

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A Rapid, Univariate FT-NIR Procedure to Determine Moisture Concentration, a Quality Parameter, in Olive Oil

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Abstract
We recently observed that the weak near-infrared (NIR) band near 5260 cm\(^{-1}\) was relatively more intense for extra virgin olive oil (EVOO) than for refined olive oil (ROO). We also observed that its intensity was diminished upon heating and erroneously presumed that it may be attributed to volatile carbonyl components in EVOO. In the present study we demonstrate for the first time that this band is primarily attributed to a water O-H combination band. To accurately determine the intensity of this weak band, observed on a shifted and sloping baseline, we measured the peak-to-peak (p-p) height of its first derivative. An exponential calibration curve for p-p height versus gravimetrically-determined concentration of spiked water was satisfactorily generated. The calibration curve was first evaluated by using independent sets of gravimetrically-prepared test samples. Subsequently, it was used to determine the moisture content, a quality parameter, for a limited set of authenticated reference olive oils whose quality and purity were confirmed by official methods. These concentrations, 0.098-0.12% H\(_2\)O (w/w) for EVOO, 0.022-0.030% H\(_2\)O (w/w) for ROO, and 0.028-0.054% H\(_2\)O (w/w) for pomace olive oil (POO), were consistent with those reported in the literature. The lowest moisture concentration obtained for EVOO was subsequently used to segregate a limited set of 88 retail products labeled as EVOO: 42% of these products contained 0.098-0.13% H\(_2\)O (w/w), while the remaining products (58%) yielded lower concentrations between 0.026 and 0.097% H\(_2\)O (w/w). When the purity of these 88 products were determined with official methods, only 3% were found to be adulterated. By contrast, the correlation between moisture content and other olive oil quality parameters has been reported in the literature and has yet to be further investigated and verified based on official methods.

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Analysis of Acylglycerols in Edible Oils by Gas Chromatography Using a Unique Stationary Phase

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Abstract
Characterization of edible oils is essential to the food industry due to the amount of fraudulent activity that surrounds these products. Some edible oils (e.g. Extra Virgin Olive Oil) carry high value, therefore making it an easy target by frauds. By mixing different vegetable oils (e.g. rapeseed, sunflower, etc.) with high quality olive oils, manufacturers increase their oil yields and make larger profits on these counterfeit olive oils. For these reasons, it is important to obtain a triacylglycerol (TAG) fingerprint of edible oils to know that they have not been adulterated with other oils. In addition, the freshness of oils can be determined by looking at the ratio of 1,2 to 1,3-diacylglycerols (DAG). By using a unique gas chromatography (GC) stationary phase without bleed interference and retention time shifting due to phase lost, and is able to resolve TAGs and DAGs, a full analysis of the edible oil can be conducted for oil adulteration and degradation. The analysis and results for these oils will be presented along with and examination of column bleed at high GC operating temperatures.

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Real-Time Authentication of Whiskeys Using DART-QDa Analysis

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Abstract
Whiskey, a popular spirit-based drink, is sold as the product of one distillery or as a blend available worldwide. Analytical methods are required for process control and quality assurance but production is often also strongly regulated with regards to the authenticity of the product. The high popularity of these products generates a high risk of fraud. The characteristics of whiskey are strongly influenced by the cereals used in fermentation and by distillation, maturation and blending regimes. This leads to characteristic profiles exploited for authenticity analysis typically using gas chromatography combined with chemometrics. Such methods can be time-consuming, due to the demands of the chromatographic separation so there is a need for development of tools for more rapid testing. We have demonstrated the potential of Direct Analysis in Real Time (DART) coupled with simple mass detection (QDa) and multivariate statistics for the rapid analysis of whisky samples. Here we report further results of comparative analysis of authentic samples of whiskey brands and determine whether it is possible to distinguish them using LiveID software. LiveID has a visually modern, attractive, web-based interface that offers a workflow-driven process that is easy to learn and use. Initially, authentic reference samples are used to create and validate a statistical model, which are tested with unknown samples to generate live classifications. The output is a simple to interpret yes/no answer delivered in real-time.

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