RICERCHE EFFETTUATE

IGIENE DEGLI ALIMENTI AD USO UMANO

Ballabio D, Robotti E, Grisoni F, Quasso F, Bobba° M, Vercelli S, Gosetti F, Calabrese G, Sangiorgi° E, Orlandi M, Marengo E

Chemical profiling and multivariate data fusion methods for the identification of the botanical origin of honey

The characterization of 72 Italian honey samples from 8 botanical varieties was carried out by a comprehensive approach exploiting data fusion of IR, NIR and Raman spectroscopies, Proton Transfer Reaction – Time of Flight – Mass Spectrometry (PTR-MS) and electronic nose. High-, mid- and low-level data fusion approaches were tested to verify if the combination of several analytical sources can improve the classification ability of honeys from different botanical origins. Classification was performed on the fused data by Partial Least Squares – Discriminant Analysis; a strict validation protocol was used to estimate the predictive performances of the models. The best results were obtained with high-level data fusion combining Raman and NIR spectroscopy and PTR-MS, with classification performances better than those obtained on single analytical sources (accuracy of 99% and 100% on test and training samples respectively). The combination of just three analytical sources assures a limited time of analysis.

Bernini° A, Bolzoni° L, Casagrandi R

Towards a multi-objective approach to design effective biosecurity plans for livestock epidemics

The epidemic spread in livestock farm systems is a major concern, causing the culling of a large number of animals and negative economic and social impacts. Thus, to prevent and control livestock epidemics it is crucial to design effective biosecurity plans, accounting for a multiplicity of objectives. In this context, quantitative epidemiological studies can help explain the possible disease transmission patterns and assess the role of farms in the diffusion process. Here, we evaluated the potential functional role played by farms in shaping the pattern of epidemic spread. As a focal case study, we considered the dairy system in the Emilia Romagna region (Northern Italy). We developed a data-driven daily temporal network model with nodes representing farms, the epidemiological units, and links describing the potential routes of pathogen transmission. We grouped links in two layers, one for direct contacts due to the movement of live cattle and the other for indirect contacts generated by contaminated trucks visiting several farms. We simulated the diffusion of epidemics on the network through a Boolean Susceptible-Infected model. The role of each farm was assessed with respect to a set of objectives focusing on different aspects of the infection process. Then, in a multi-objective space, we identified the set of the most critical farms, where the implementation of biosecurity measures may reveal to be more effective. Trade-offs between the objectives did emerge, showing that the identification of key farms is not trivial and may crucially depend on the specific measures applied.

Bertasi° B, Tilola° M, Benevenia° R, Mangeri° L, Zendrini° A, Scaramagli S,

Delibato E, Gattuso A, Peletto S, Losio° M

Evoluzione delle metodiche molecolari quantitative nell'identificazione di specie per il controllo delle frodi alimentari

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 302-304. - 2 bib ref [Nr. Estr. 8067]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV)   (18.   : Perugia (PG)   : 7-8-9 Novembre 2018)

Food frauds, like species substitution, are a hot topic in the food industry; one of the main issues in this field is to distinguish voluntary adulteration from unintentional trace contamination. To date, control over food fraud is performed mainly with end-point or Real-Time cut-off based PCRs. Advancements in molecular biology techniques allow for the implementation of innovative methods, such as digital PCR or NGS, improve DNA quantification and refine current methods when investigating species substitution. In this work, different digital PCR approaches to obtain absolute quantification were performed on spiked materials to compare results.

Bonan° S, Lepore A, Polonini° G, Caschetto° MG, Caprai° E

Occurrence of citrinin in red yeast rice from Monascus purpureus

 The World Mycotoxin Forum 10th conference "Taking mycotoxin control to the next level" : 12-14 March 2018, Amsterdam, the Netherlands : abstracts of lectures and posters / [s.l. : s.n., 2018]. - p 93-94 (Poster 13) [Nr. Estr. 7845]

The World Mycotoxin Forum Conference "Taking mycotoxin control to the next level"   (10th   : Amsterdam, The Netherlands   : 12-14 March 2018)

Citrinin is a nephrotoxic mycotoxin produced by several species of the genera Aspergillus, Penidihium and Monascus. It is generally formed after harvest and occurs mainly in stored grains, but also in other plant products. Citrinin can also co-occur with other mycotoxins, such as ochratoxin A and patulin. Citrinin is an undesirable contaminant in Monascus purpureus fermentation products (red yeast rice). These products are used for the maintenance of normal blood cholesterol concentrations thanks to the therapeutical action of naturally present monacolin K. Commission Regulation (EU) No 212/2014 sets a limit of 2,000 pg citrinin/kg for food supplements based on red fermented rice. An analytical method for the determination of citrinin in red yeast rice supplements was developed and validated in-house at the Bologna Food Chemical Department of IZSLER (Italy), according to Commission Regulation (EU) No 519/2014. An acidic organic solvent and a mixture of salts were added to the samples to perform the extraction. Identification and determination of citrinin are performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using negative electrospray ionisation. The LOQ value was 50 pg/kg. Suppression or enhancement of the signal due to matrix effects are minimised by using isotope-labelled 13C13-citrinin. Linearity, stability, recovery, repeatability, reproducibility, selectivity, LOQ, and LOD were determined proving that the method was fit-for-purpose. A total of 61 samples were collected from July 2016 to December 2017 to evaluate the occurrence of citrinin in red yeast rice supplements and powder. Red yeast rice powder is used by national industries to produce cholesterol control supplements. Food supplements samples were collected mainly within the Italian market while the raw materials were collected as a result of border inspection activities. One out of six samples revealed a citrinin contamination with huge variabilities in the concentrations of the mycotoxin in individual samples. Occurrence ranged from 60 pg/kg to over 2,000 pg/kg. Two food supplements exceed the limit set and a single sample of red yeast rice powder from China showed a concentration of more than 9,000 pg/kg. A specific limit for raw red yeast rice powder has not been fixed yet. The absence of a reference point for the concentration of citrinin in such matrices could represent a difficulty for border officers to decide on the compliance of the incoming raw materials as no complete data are available on supplements manufacturing.

Bonardi S, Bolzoni° L, Brindani F, Scaltriti° E, Cavallini P, Carnovale G, Pongolini° S

Salmonella detection and counting on pig carcasses and cutting lines in Italian slaughterhouses

During 2014–2015, 300 pig carcasses before chilling and 85 food contact surfaces (FCSs) at cutting lines were tested for Salmonella in three slaughterhouses (namely A, B, and C) of northern Italy. In slaughterhouses A and B, four carcass sites of 100 cm2 each (from both the exterior and interior side) were swabbed with a single sponge. In abattoir C, four 100 cm2 sites of the exterior and the interior sides were swabbed with two independent sponges. The population average prevalence of Salmonella-positive carcasses (which takes into account the structure of the study design, with multiple samples collected in a single day) in slaughterhouses A and B was 12.3%, while in slaughterhouse C it was 11.2%. Presence of Salmonella on exterior and interior sides of carcasses showed a low level of concordance (only 3/12 of the contaminated carcasses were positive on both sides). No significant difference was found for FCSs contamination in the three slaughterhouses, with a population average prevalence of Salmonella-positive FCSs of 19.9%. In addition, we found that the clustering due to the day of sampling account for more than 36% and 60% of the overall prevalence variation on carcasses and FCSs, respectively. Eight serovars were identified, with Salmonella Derby as the most common type. The counting of Salmonella on carcasses showed large variability. It was low (<0.0075 most probable number [MPN]/cm2) in 46.6% of the carcasses and as high as 2.7 MPN/cm2 in 4.7%. Specifically, we found that counts on carcasses fit with ''heavy tailed'' distributions (lognormal and Weibull with a small shape parameter), suggesting not negligible probability of episodes of high Salmonella contamination. The mean values of contamination obtained with the two distributions ranged from 0.235 to 0.435 MPN/cm2.

Bonardi S, Le_Guern AS, Savin C, Pupillo G, Bolzoni° L, Cavalca M, Pongolini° S

Detection, virulence and antimicrobial resistance of Yersinia enterocolitica in bulk tank milk in Italy

In 2016 and 2017, 509 raw milk samples were collected from bulk milk tanks in 315 dairy farms in Parma province, Italy. Yersinia pseudotuberculosis was never detected. Yersinia enterocolitica was isolated from 17 (3.1%) bulk milk samples by culture methods. Estimated apparent prevalence was 3.91% in 2016, and 2.63% in 2017, with no significant difference between the two years (df ¼ 1; p-value ¼ 0.419). Five BT 2/ O:5,27 and 12 BT 1A isolates were detected. The most common virulence genes (ail, inv, virF, yadA, ystA, ystB, myfA, irp2, fyuA) were screened and the genes ail, virF, yadA, ystA were harboured only by 2 O:5,27 isolates. The Italian strains were very close by SNP analysis. The 2 O:5,27 isolates showed a unique antimicrobial resistance pattern, being resistant to amoxicillin, cefoxitin, cephalexin and amoxicillin - clavulanic acid. The study suggests that consumption of raw or improperly pasteurised milk may be hazardous for the consumers.

Bonilauri° P

Prodotti confezionati : misurare, modellare e predire la shelf life

Bonilauri° P

Durabilità utilizzi ed efficacia del trattamento con HHP

Bonilauri° P, Leonelli° R, Ferrarini G, Carobbi D, Ossiprandi MC, Dottori° M, Cuccurese A

Kebab : can the traditional cooking process sanitize a natural contamination by Listeria monocytogenes?

Over the last few years a considerable spread of ethnic foods was observed in Italy. Among them is the Doner kebab. During 2014-2015, in order to evaluate the effectiveness of traditional cooking process, raw product (defrosted), sliced cooked portions cut through electric knife and assembled sandwich were officially sampled in kebab houses and in a local industrial kebab producer in Reggio Emilia (a province in Italy). Microbiological researches for safety and hygienic microbiological indicators were carried out (research of Salmonella, Listeria monoeytogenes, Campylobacter and Shiga toxin-producing Escherichia coli; enumeration mesophilic aerobic bacteria, lactic acid bacteria, sulfite-reducing bacteria growing under anaerobic conditions, yeasts and molds). Between the raw and the cooked product an average of 3 log reduction in mesophilic aerobic bacteria counts was observed. In two out of three kebab houses sampled, which were supplied by the same local industrial producer, the presence of L. monocytogenes was detected. During the official inspection carried out at the production plant a contamination of L. monocytogenes was assessed in both ambient and instruments. Furthermore, 3 lots of products were analyzed and all were found to be contaminated by L. monocytogenes (always above 100 CFU/g). In order to verify the capability of the traditional cooking process to reduce the risk of contamination at an acceptable level, a batch of naturally contaminated kebab (4.5 log CFU/g) was cooked and sliced simulating a day work activity in a kebab shop. The product was then sampled during preparation and enu-meration of L. monocytogenes was obtained. After an hour of cooking, the residual contamination was 1.8 log CFU/g, after two hours and a half L. monocytogenes was no longer detectable in the product, but half an hour later it was again detectable in 25g. At the end of the experiment, the contamination grown up to the same level enu¬merated after an hour of cooking (1.8 log CFU/g). Considering the microbiological results, traditional cooking obtained a rate of -2.40 log CFU/gh-r, a D=26 min that corresponds to a temperature of maximum 60°C (z=6). In conclusion, our experiment demonstrates the traditional kebab cooking process could not always guarantee a complete product decontamination.

Bonilauri° P, Ramini° M, Grisenti S, Cosciani_Cunico° E, Taddei° R, Frustoli A, Daminelli° P, Merialdi° G

Valutazione dell'impiego di HPP come trattamento letale nel processo produttivo di diverse tipologie di salame al fine di implementare i fattori intrinseci ed estrinseci che contribuiscono al controllo di Salmonella spp

Lo scopo del presente lavoro è stata la valutazione dell'abbattimen¬to della concentrazione di Salmonella spp. durante II processo pro¬duttivo seguito da un trattamento ad alte pressioni idrostatiche (HPP) in diverse tipologie di salami at fine di verificare la soddisfa¬zione dell'obiettivo di performance part a 5 log richiesto da FSIS (Salmonella Compliance Guideli-nes for small and very small meat and poultry establishments that produce ready-to-eat, giugno 2017). I dati di abbattimento sono stati ottenuti net corso di attivita sperimentali condotte presso 3 laboratori di

ricerca, due localizzati in IZSLER (BO e BS) e la SSICA di Parma. Le ricette sono state scel¬te tra le maggiormente rappresentative della produzione nazionale tenendo in considerazione la variabilita relativa alle dimensioni della grana, at calibro, alla lunghezza della stagionatura ed alla curva acidificazione in fase di asciugatura. Tutti gli impasti sono stati artificialmente contaminati prima dell'insacco con un mix di almeno tre ceppi di Salmonella. La concentrazione di Salmonella nell'impasto dopo l'aggiunta dell'inoculo (1% v:v) è risultata alme¬no uguale a 5.5 log UFC/g. I salami sono stati quindi sottoposti a cicli differenti di asciugatura e stagionatura. La durata dell'asciugatura 6 risultata compresa tra 4 e 11 giorni, con pH di fine asciugatura tra 4.78 e 5.39. La durata delta stagionatura è stata compresa tra 16 e 90 giorni, con aw finale tra 0.881 e 0.949. Al temine della stagionatura i salami sono stati sottoposti a trattamento con alte pressioni idrostatiche (5930 bar, 300 sec). I valori di pH, aw, e la concentrazione di Salmonella sono stati determinati in almeno 3 repliche a fine asciugatura ed almeno 5 repliche a fine stagionatura e dopo trattamento HPP. Complessivamente sono stati eseguiti 22 challenge test. Tutti i prodotti hanno rispettato II limite di 1200 Degree/hours (GMP: Meat Institute (USA):1995). Nonostante le condizioni di pH, aw e temperatura fossero in asciugatura teoricamente permissive per lo sviluppo di Salmonella, in tutti i processi è stata osservata una riduzione del patogeno, in media uguale a 0.79 (dev.st 0.54) log UFC/g. In accordo con i modelli predittivi disponibili per l'abbattimento non termico di Salmonella (www.combase.cc), ogni giorno passato con aw inferiore a 0,96 determina una condizione sfavorevole al patogeno. E abbattimento osservato a fine stagionatura è risultato in media uguale a 2.33 (dev.st 0.97) log UFC/g. II trattamento di HPP ha comportato il rag¬giungimento per tutti i prodotti del target di 5 log di abbattimenti, con un abbattimento complessivo (processo pit) HPP) minima di 5.12 log ed un massimo di 8.55 log. Le conclusioni del presente lavoro permettono di trarre alcune indicazioni generali sui salami di came suina destinati all'export USA, ma che possono essere ugual¬mente considerate nella gestione del rischio salmonella anche nei prodotti destinati al mercato locale. La durata della stagionatura risulta ii parametro principalmente correlato all'efficacia dell'abbattimento di Salmonella, in particolare II numero di giorni in cui il prodotto permane sotto aw 0.96. Tuttavia, se Si intende sottoporre ii salame al trattamento con HPP va considerato che l'efficacia del trattamento ad alte pressioni idrostatiche si riduce man mano che l'aw nel prodotto decresce.

Borgese L, Dalipi° R, Riboldi A, Bilo F, Zacco A, Federici S, Bettinelli M, Bontempi E, Depero LE

Comprehensive approach to the validation of the standard method for total reflection X-ray fluorescence analysis of water

In this work, we present the validation of the chemical method for total reflection X-ray fluorescence (TXRF) analysis of water, proposed as a standard to the International Standard Organization. The complete experimental procedure to define the linear calibration range, elements sensitivities, limits of detection and quantification, precision and accuracy is presented for a commercial TXRF spectrometer equipped with Mo X-ray tube. Least squares linear regression, including all statistical tests is performed separately for each element of interest to extract sensitivities. Relative sensitivities with respect to Ga, as internal standard, are calculated. Accuracy and precision of the quantification procedure using Ga as internal standard is evaluated with reference water samples. A detailed discussion on the calibration procedure and the limitation of the use of this method for quantitative analysis of water is presented.

Butot S, Ricchi° M, Sevilla I, Michot L, Molina E, Tello M, Russo° S, Tomas D

Estimation of performance characteristics of analytical methods for Mycobacterium avium paratuberculosis (MAP) detection in milk

& abstracts / [s.l. : s.n., 2018]. - p 145 (O-4.2) [Nr. Estr. 7904]

International colloquium on paratubercolosis   (14th   : Riviera Maya, Mexico   : 4-8 June 2018)

Several methods and technologies are published and applied to detect MAP but there is a lack of validation data and performance characteristics obtained following an international recognized approach (ISO 16140-2:2017) to demonstrate these methods are fit-for- purpose. The objective was to estimate performance characteristics for three analytical methods used for MAP detection in three types of milk (heat-treated, powdered and raw milk). Two de-clumping protocols (filtration or sonication) were tested. For each type of milk, samples were spiked with a reference MAP strain at two levels. Samples were stored at -20 °C and distributed to two ISO 17025 accredited laboratories for analysis using three different methods. Both de-clumping protocols showed good performance by microscopy observation. Close results were obtained by culture and phage assay without PMS with value 0.5 Log cells/ml higher for filtration. Before spiking, MAP levels of four pure cultures were estimated by microscopy (Neubauer chamber), phage assay without PMS and Real Time PCR (f57 and IS900) and used to define the spiking levels. MAP levels in milk samples after spiking were estimated by Most Probably Number (MPN) using Real Time PCR (I5900). Values for low levels ranged from 2,500 to 3,100, 7,500 to 15,000 and 7,500 to 48,000 MPN/50nri1 for heat treated milk, powdered milk and raw milk respectively. Sensitivity was estimated by comparing reference values with the results obtained by laboratories performing the three analytical methods: culture, Real Time PCR (IS900 and f57) and Peptide magnetic separation (PMS) + phage assay. Spiked samples showed good homogeneity and stability. Preliminary results obtained in two accredited laboratories showed different performance characteristics (sensitivity) for the three assays tested for MAP detection.

Calò° S, Vitale° N, Galletti° G, Cosciani_Cunico° E, Dalzini° E, Todeschi° S, Tamba° M, Zanoni° M, Daminelli° P

Indagine nazionale sulla temperatura dei frigoriferi domestici in Italia

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 307-308. - 4 bib ref [Nr. Estr. 8068]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV)   (18.   : Perugia (PG)   : 7-8-9 Novembre 2018)

Temperature is one of the most important factors influencing the ability to multiply and survive microorganisms in foods. During the first steps of the chain, the temperature are satisfactorily controlled whereas retail display cabinets and the home refrigerators are less controlled. According to these results, National Reference Center for emerging food safety risks in collaboration with the Istituti Zooprofilattici Sperimentali network has started a research project aimed at studying the temperature in domestic refrigerators. The purposes are: define a national average level of temperature in household refrigerators; define "reasonably foreseeable conditions" under which it will be possible, by the Food Business Operator (FBO), conduct challenge testing of Ready To Eat foods in full compliance with Community requirements and to provide FBO instruments for monitoring the shelf life of food, regardless of the presence of pathogenic microorganisms all along the distribution chain.

Cappa° V, Scaburri° A, Finazzi° G, Bertasi° B, Losio°MN, Vitali A, Avisani° D, Cerioli° M, Zanoni° M

Piano di monitoraggio di Exoli STEC su cagliate : le modalità di gestione degli stabilimenti influenzano la presenza del patogeno

Convegno "Circolazione ed impatto dei patogeni enterici in Italia" : Roma, 18 ottobre 2018 / Istituto Superiore Sanita, Dipartimento Malattie Infettive, Focal Point Italiano dell'Autorità Europea per la Sicurezza degli alimenti (EFSA). - [s.l : s.n, 2018]. - 8127]

Caprai° E, Ammoni E, Polonini° G, Caschetto° MG, Bonan° S

Presence of Pyrrolizidine alkaloids in teas and herbal infusions : preliminary monitoring and risk characterization

Pyrrolizidine alkaloids (PAs) are secondary metabolites produced by over 6000 plant species worldwide, mainly from the botanical families Boraginaceae, Asteraceae and Fabaceae. In animal studies, some PAs have proven to be genotoxic carcinogens. An analytical method for the determination of PAs in teas and herbal infusions was developed in-house and allowed to detect up to 28 PAs. Analysis consist of an acidic/organic extraction and SPE clean up. Identification and determination of PAs are performed by liquid chromatographic separation and mass spectrometric detection. LOQs values for all PAs were 3 Ltg/kg. For a preliminary monitoring of PAs in teas and herbal infusions, 50 samples were collected within the Italian market. Samples included teas and herbs for hot infusion drinks. PAs content of each sample was calculated as the sum of the concentrations of all detected analytes. Concentrations of PAs in individual samples ranged from 3 vg/kg to over 1000 lig PAs/kg of dried matter. Most contaminated samples were black tea and miscellaneous herbal infusions. The main average contributors to the total PAs concentrations in samples were retrorsine, lycopsamine, senecionine isomers and their N-oxide forms. Occurrence resulting from analysis was used to calculate a provisional chronic exposure assessment due to the consumption of PAs containing teas and infusions. Assessment was carried out assuming a daily ingestion of PAs deriving from 2g of tea/infusions. Average and worst case scenarios were assessed for adults and children for a preliminary risk characterization using the MOE approach and assuming equal toxicological potency for all alkaloids.

Caprai° E, Damiano C, Roncada P, Fedrizzi° G

Monitoraggio della presenza di Ocratossina A in salami prodotti artigianalmente nelle regioni Campania e Marche

Ochratoxin A (OTA) is a nephrotoxic mycotoxin produced by several species of the genera Aspergillus and Penicillium. The European Commission fixed maximum OTA levels in several foodstuffs, like cereals, wine, coffee, spices and dried fruits but not in meat-based products. In Italy, the Ministry of Health established a guideline value of 1 µg/kg for pork meat and derived products. Aim of this study was to provide data concerning occurrence of OTA in artisanal salami manufactured in Campania and Marche regions. The analyses were performed on 96 ripened salami and both the inner and the outer portions (casing) were considered. The analytical method was based on a sample preparation procedure with immunoaffinity columns (IACs); identification and determination of OTA was performed by LC-MS/MS. LOQ value was 0.25 µg/kg. Suppression or enhancement of the signal due to matrix effects are minimized by using isotope labelled 13C20-OTA.

Carpenè E, Ferlizza° E, Menotta° S, Cappi° F, Andreani G, Fedrizzi° G, Isani G

Trace elements in "unconventional food"

2018 Scientific Meeting Italian Association for the study of Trace Elements in living organisms - AISETOV "The role of trace elements in health: from healthy environments to healthy living organisms" : Ozzano Emilia, Bologna, October 12, 2018 : abstract book / [s.l. : AISETOV], 2018. - p 22 (O-14). - 1 bib ref [Nr. Estr. 7994]

Scientific Meeting Italian Association for the study of Trace Elements in living organisms (AISETOV) : Ozzano Emilia, Bologna   : October 12, 2018)

Introduction: The human diet is based on myriads of different sources of nutrients, including unconventional food from cyanobacteria to mammals. In most societies, the choice of food is related to historical, religious and psychological groundings that often limit the acceptance of unconventional and exotic meats (Cawthom and Hoffman, 2016). The aim of the present research was addressed to investigate the concentrations of non-essential and essential trace elements in "unconventional food" prepared from samples of venison, snails and mushrooms from the Quaderna Valley (Bologna). Material and Methods: Thirty samples of different cooked food obtained from mammals, birds, snails and mushrooms were included in this study. Nineteen essential and non-essential trace elements (Mn, Fe, Co, Cu, Zn, Se, Mo, Cr, V, Pb, Cd, Hg, As, Al, Ag, Ni, TI, U, Sb) were analysed by ICP-MS after tissue mineralization. Results and Discussion: Trace element concentrations varied widely depending on the investigated samples. Among non-essential elements, the highest levels of Pb were found in samples of woodcock, Scolopax rusticola (2.4 tig/g wet wt) and teal, Anas crecca (4.8 nig wet wt). These high concentrations are related to Pb microscopic fragments present in the meat of venison obtained by local hunters. Meanwhile, in other venison samples from pheasant (Phasianus cokhicus) and hare (Lepus europaeus) Pb concentrations were significantly lower (p<0.05), probably due to the different recipe used for meat cooking. The highest Cd concentration (0.4 nig wet wt) was found in one sample of Roman snail, Helix pomatia, this finding is confirmed by additional analysis on fresh snails. Mushrooms showed Hg levels as high as 0.5 tag/g wet wt. As regards essential elements, in general all the investigated samples were rich in Fe, Zn and Cu. In particular, snails had concentrations of Cu one-two order of magnitude higher, due to the presence of hemocyanin.

Chiscuzzu F, Carta G, Lorenzoni G, Cannella V, Fedrizzi° G, Virgilio S, Guercio A, Nicolussi P

Studio di metodi alternativi all'impiego del mouse test nella determinazione delle biotossine algali PSP nei molluschi eduli lamellibranchi

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 282-283. - 4 bib ref [Nr. Estr. 8064]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV)   (18.   : Perugia (PG)   : 7-8-9 Novembre 2018)

Several microalgae of the genus Alexandrium (Alexandrium minutum and Alexandrium catenella) can produce an algal biotoxin, the Paralytic Shellfish Poison (PSP), that can be accumulated in the shellfish edible tissues and it represents a serious risk to consumer health, particularly for children where it can be fatal. In this study, an in vitro functional assay using cells coltures (Neuro 2a - murine neuroblastoma cells) for a diagnostic method in MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxiphenyl)-2-(4-sulfophenyl)- 2H-tetrazolium was tested in parallel trials with mouse bioassay. As tissue culture of Neuro2a cells are indicated among the most susceptible to PSP neurotoxins, it could be a valid alternative to mouse bioassay. However, it is necessary to improve the purification of the sample to remove the non-specific effect (false positives), probably due to the presence of other environmental contaminants or intrinsic components of the shellfish.

Cirlini M, Demuth TM, Biancardi° A, Rychlik M, Dall'Asta C, Bruni R

Are tropane alkaloids present in organic foods? Detection of scopolamine and atropine in organic buckwheat (Fagopyron esculentum L.) products by UHPLC–MS/MS

A closer monitoring of tropane alkaloids (TA) in foods is now recommended by the European Commission, following a series of alerts related to the contamination of buckwheat with weeds of the genus Datura. A novel, accurate UHPLC–MS/MS method was developed and validated for the rapid detection of scopolamine and atropine in buckwheat foods. A suitable extraction protocol was set up to maximize recoveries and detection limits in different raw, processed and baked foods. The method offers good performances in terms of sensitivity, accuracy and precision, with LOQs at 0.04 and 0.10 mg/kg. The established method is suitable for routine determination of trace levels of TA and was applied to 26 different buckwheat-derived organic foods, detecting TA in 3 samples (13.9–83.9 mg/kg for atropine and 5.7– 10.4 mg/kg for scopolamine). Only in one case the level of contamination was relevant in terms of food safety.

Colombo R, Arghittu M, Daprai L, Tel F, Testa S, Paglialonga F, Ardissino G, Luini° MV, Spelta° C, Bianchini° V, Romanò° A, , Andreoli° M, Losio° MN, Scavia G, Morabito S

Shigatoxin-producing Escherichia coli (STEC) in humans in Northern Italy : an integrated veterinary-medical activity

An integrated veterinary-medical working group started in Lombardy on December 2014 within a One Health project regarding Haemolytic Uremic Syndrome (HUS). The aim was to define serotypes mostly involved in HUS and to compare the molecular profile of human STEC strains versus those isolated from zoonotic source. Feces were enriched in MacConkey Broth, cultured and processed for detection of Shigatoxinl, Shigatoxin2 (stxl, stx2) and eae by means of a multiplex PCR. In case of positive result, isolation and molecular characterization of the STEC were performed. From December 2014/November 2017, 74 STEC strains, 58 stx2-eae or stx2-stxl-eae positive, were isolates from 31 sporadic events and 12 familial clusters or epidemics. Rea!time PCR multiplex for the "top 5" serotypes documented 0157 in 44% of episodes (n=19), and 026, 0111, 0103, 0145 in 21% (n=9), 9% (n=4), 5% and 5%(n=2) respectively. In 7 events the strains isolated were non "top five". Epidemiological investigations were carried out in 22 episodes considering both environment and food samples potentially involved in human infection. In a single case the source of the infection was traced to a cheese called "formaggella di malga" contaminated by serotype 0157-stx 1 -stx2-eae with the same PFGE profile of the strain isolated from infected patient. The intake of cheese was strongly associated with one infection from 026- Stx2-eae and was also probably correlated to a cluster involving 3 cousins eating the same cheese, these two case not having isolated the strains from food. During the same period detailed investigations detected 9 family clusters related with HUS patients, one involving 5 subjects. In 8 out of 9 cases the same serotype and same PFGE profile were observed in the strains isolated from both the patients and relatives (brothers, sisters, parents and grandparents). All the patients with HUS were positive for stx2 except one patient carrying stx 1 . The observation of several family clusters suggests a common source of infection or/and a possible interhuman transmission. The detection of STEC in symptomless relatives should be always worked in or out since the first contact might be a subject other than the one developing HUS. More generally, in this integrated veterinary-medical approach to STEC infection, the surveillance system has not identified any common PFGE profile between human strains and 270 cattle strains obtained in a parallel research in the same area and time period.

Crotta M, Lavazza° A, Mateus A, Guitian J

Quantitative risk assessment of hepatitis E virus : modelling the occurrence of viraemic pigs and the presence of the virus in organs of food safety interest

Hepatitis E virus (HEV) is a zoonotic pathogen with consumption of pork and derived products identified in different countries as a risk factor for human exposure to HEV. Great efforts have been made to understand the dynamics of virus transmission within domestic swine populations through modelling. However, from a food safety prospective, it is critical to integrate the parameters involved in the transmission dynamics with those governing the actual presence of HEV in the bloodstream, the liver, gallbladder or faeces. To date, several aspects related to the pathogenesis of the disease are still unknown or characterized by significant levels of uncertainty, making this conjunction challenging. We used published serological data obtained from pigs in a farrow-to-finish farm to implement an Immune-Susceptible-Infected-Recovered (MSIR) model reproducing the on-farm dynamics that lead to the occurrence of viraemic pigs at slaughter. Expert opinion on the length of time infectious HEV can be detected in liver, gallbladder/bile and faeces after recovery from viraemic status were used to inform a stochastic model aimed at estimating the expected proportion of viraemic pigs, pigs with infectious HEV in liver, gallbladder/bile and faeces entering the slaughterhouse. To simulate the potential effect of on-farm mitigation strategies, we estimated the changes in outcomes of interest as a function of variations in the baseline transmission parameters. The model predicted a proportion of viraemic pigs entering the slaughterhouse of 13.8% while the proportions of, and ranged from 13.8% to 94.4%, 13.8% to 94.7% and from 25.3% to 30.8% respectively, due to the uncertainty surrounding the experts' opinions. Variations in MSIR model's parameters alert of the need to carefully consider the application of mitigation strategies aimed at delaying the decay of maternal immunity or the peak of the within herd transmission. When the rate of decay of maternal immunity and the transmission rate were decreased between 80% and 5% and 40% and 5% from the baseline values respectively, adverse effects on were observed. The model highlights the relevance of specific aspects in the pathogenesis of the disease from a food safety prospective and it was developed to be easily reproducible and updatable as soon as accurate data becomes available. As presented, the model can be directly connected to existing or future pig-related models to estimate the significance of the identified parameters on the risk of human exposure to HEV through consumption of pork products.

Dalipi° R, Berneri° R, Curatolo° M, Borgese L, Depero LE, Sangiorgi° E

Total reflection X-ray fluorescence used to distinguish mechanically separated from non-mechanically separated meat

This paper presents the successful application of total reflection X-ray fluorescence spectroscopy (TXRF) for the identification of products obtained from mechanically separated meat (MSM), a very important issue for food quality and safety. According to the European Food Safety Authority, one of the most important parameters to distinguish MSM is the content of Ca. Consequently, the development of reliable and cost-effective analytical tools is very important to monitor the chemical composition of these foods. In this study, we have developed an analytical method for elemental analysis of meat samples based on total reflection X-ray fluorescence. A simple sample preparation by suspending the grinded meat sample in a solution of diluted Triton X-100 and polyvinyl alcohol in water showed to be the best for this kind of samples. Fresh chicken meat, chicken meat with different percentage of MSM, pure MSM and meat products prepared with MSM were analyzed. The content of K, Ca, Fe, Cu and Zn was determined. Results show that Ca, but also K and Fe are

significant markers to distinguish MSM from fresh meat. A limit of 40% MSM for differentiation was achieved by applying principal component analysis. The method accuracy was evaluated comparing the obtained results with those obtained after acidic digestion and ICP-MS analysis.

Dalzini° E, Monastero° P, Panteghini° C, Daminelli° P, Bertasi° B, Losio° MN

Strain variability : kinetics and genotypical characterization of Listeria spp. isolated from food

IAFP's European Symposium on Food Safety : 25-27 April 2018, Stockholm, Sweden / [s.l. : International Association of Food Protection, 2018]. - p 111 (poster P2-57) [Nr. Estr. 7843]

European Symposium on Food Safety (IAFP)   : Stockholm, Sweden   : 25-27 April 2018)

Listeria monocytogenes is a foodborne pathogen that can resist disinfectants, form biofilms, and survive under extreme physicochemical situations, such as dry environments, different temperatures, a wide range of pH values, and high salt concentrations. All these conditions promote the proliferation of the pathogen in a large variety of food matrices. Purpose: The aims of this study were: (i) to evaluate the kinetics properties (growth and death rates) of Listeria spp. isolated from food and (ii) to type Listeria spp. isolates by pulsed field gel electrophoresis (PFGE). Methods: A total 22 strains (17 L. monocytogenes and six L. innocua) previously isolated from different food matrices (vegetables, dairy, meat, and fish) and environmental samples were cultivated in brain heart infusion broth at 25°C to calculate the growth rates and at 55°C to evaluate the death rates. All the experiments were performed in duplicate. PFGE fingerprints were obtained with the restriction enzyme Apal according to PulseNet protocol. Results: A wide strain variability was found in relation to the kinetics properties: The growth rates ranged from 0.25 to 0.46 log CFU/ml, while the death rates ranged from -1.02 to -4.61 log CFU/ml, confirming that, in stress conditions, the strains could assume different stress-response behavior. Two strains of L. monocyto genes isolated from fillet trout and bacon showed both the highest and the lower values for the growth and the death rates, respectively. PFGE subtyping of all the isolates showed 19 different PFGE types, proving a wide genomic variability. No correlation between food matrices, kinetics properties, and genotype was found. Significance: These results may improve knowledge about the strains' behavior in optimal or stressful condition and may lead to the creation of a strain collection to use for challenge tests in different food matrices and in different conditions.

Daminelli° P, Cosciani_Cunico° E, Dalzini° E, Monastero° P, Losio° MN

Validazione di processo : l'esperienza dell'IZSLER nell'applicazione delle linee guida FSIS

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 75-76. - 5 bib ref [Nr. Estr. 8050]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV)   (18.   : Perugia (PG)   : 7-8-9 Novembre 2018)

The validation is a necessary step to demonstrate that the HACCP system, designed for a specific food process by the Food Business Operator, is able to adequately control the potential microbiological risks to produce safe food. According to the international guidelines, the production of safe Uncooked Comminuted Fermented Meat Products (UCFM) is largely based on the prevention of pathogen growth during the fermentation process and passes through the maximization of their death during the maturation and storage of the product. The time to reach a pH of 5.3 during fermentation / drying, may represent a critical limit within a HACCP plan. In this study we reported two examples of characterization and validation of Milan-style salami and Cacciatore type. From the present study it has emerged that the characteristics of the products or of the relative processes considered are such as to allow to consider the processes "under control" from safety point of view.

Daminelli° P, Dalzini° E, Cosciani-Cunico° E, Monastero° P, Pavoni° E, Losio° MN

Foodborne pathogens : inactivation in protected designation of origin Coppa and Pancetta Piacentina during the process and after high-pressure processing

Foodborne pathogens such as Escherichia coil, Listeria monocyto genes, and Salmonella spp. may occur in dry cured meat products via contaminated raw meat, ingredients, processing equipment, and/or as a consequence of post-processing contamination, and they have also been Purpose: The objective of this study was to evaluate pathogen inactivation in two typical Italian protected designation of origin meat products, Coppa and Pancetta Piacentina. We also validated the integrated lethality of high-pressure processing (HPP) as a post-processing intervention to eliminate pathogens in meat cured products. Methods: Three multi-strain cocktails of E. coli 0157, Listeria innocua, and Salmonella Typhimurium were used to contaminate separately a total of 36 Coppa samples (on the surface) and 30 Pancetta samples (in the core and on the surface) just before putting them into the casings for curing (180 days for Coppa and 120 days for Pancetta). Control products were inoculated with sterile solution to evaluate the physical-chemical changes during the process. HPP treatment (600 MPa for 5 min) was applied after curing on six replicates. Microbial survivals were estimated by plate count method. Results: The long curing times, combined with unfavorable conditions, allow for pathogen reduction during the process. With HPP treatment, the results showed that on Coppa it was possible to achieve a total reduction of about 6.2, 5.5, and 7.0 log CFU/g for E. coli, L. innocua, and Salmonella spp., respectively. For Pancetta, it was possible to achieve a total reduction of about 7.0, 4.7, and 5.3 log CFU/g (on the surface) and 6.9, 4.6, and 5.1 log CFU/g (in depth) for E. coli, L. innocua, and Salmonella spp., respectively. Significance: These data showed that adding HPP to the production process could be used to reach the performance standards for traditional Italian dry-cured meat products.

De_Medici D, Schembri P, Ciccaglione AR, De_Santis P, Di_Bartolo I, Di_Pasquale S, Guercio A, La_Rosa G, Losio° MN, Scavia G, Tosti ME, Alfonsi V, Bilei S, Bruni R, Chelli E, Iaconelli M, Moro O, Pavoni° E, Purpari G, D'Amato S, Suffredini E

'One health' approach for risk assessment of foodborne transmission of hepatitis E virus : preliminary results from the CCM 2016 program

Background: Autochthonous Hepatitis E is an increasing concern. The infection is mostly associated to consumption of raw/undercooked pork or wild boar meat, but the virus has been detected also in other food matrices. To better understand the epidemiology of Hepatitis E and virus circulation in the interface man-environment-animal-food in Italy, a nationwide project (Fig. 1) was initiated. Material and Methods: Data on HEV infections in Italy were collected through two pathways: a) sera of patients diagnosed with acute hepatitis conferred to the NRL for viral hepatitis (coverage: 8 Regions); b) epidemiological data provided to SEIEVA (coverage: 85% of Local Health Units). Estimate of HEV circulation in the population, including asymptomatic infection, was ensured through sewage monitoring (coverage: 19 wastewater treatment plants in 8 Regions). Data to assess the risk of foodborne transmission were gathered by analysis of different food categories and, for meat product, by evaluating the probability of contamination during animal slaughtering. Stratified sampling and harmonized methods were used for these analyses. A risk ranking tool will allow combining data available in scientific and grey literature, information of food production chains, and data collected within the project in order to provide a risk ranking prioritization for food involved

in HEV transmission in Italy. Results: Since March 2017, 79 cases of nonA-nonC acute hepatitis were reported to the NRL. HEV acute infection was confirmed for 20 cases through detection of IgM or HEV-RNA, 15 of which characterized as 3c (n=6), 3e (n=2) and 3f (n=7). In the same period 49 cases were reported to SEIEVA with peaks of notification in late spring 2017 and early spring 2018. Virus circulation was confirmed by environmental surveillance: 11/201 wastewater samples (5.5%; 6 in Lazio, 4 in Abruzzo and 1 in Emilia-Romagna) were found positive for HEV genotype 3. Prevalence of HEV in food producing animals was low: despite high seroprevalence in tested pigs (73.4% on average), the virus was detected in 2/215 (0.9%) pork livers at slaughtering, but not in muscles from the infected animals. As for food, 302 samples were analysed: 74 pork meat products (25 of which containing liver), 65 leafy vegetables, 66 bivalve shellfish and 91 raw milk samples. HEV was detected only in one sample, a fresh pork sausage, with a low viral load (-40 genome copies/g). Overall, HEV prevalence was 0.3% (CI 95%: 0.0%-2.0%) in all foods and 1.4% (CI 95%: 0.0%-8.0%) for pork products. Conclusions: The complex flow of HEV among humans, animals and environment demands a One-Health approach to investigate transmission routes. The data of this study will help defining a risk ranking for foodborne transmission of HEV and the planning of effective controls.

De_Medici D, Schembri P, Guercio A, Losio° MN, De_Santis P, Purpari G, Pavoni° E, Bilei S, Di_Pasquale S, D'Amato S, Suffredini E

Hepatitis E virus : a nationwide study for risk assessment of foodborne transmission

Di_Ciccio PA, Colagiorgi A, Bruini I, Zanardi E, Ghidini S, Filipello° V, Finazzi° G, Ianieri A

Biofilm formation by different genotypes of dairy Staphylococcus aureus isolates from Northern Italy

Staphylococcus aureus (S. aureus) is a pathogen that causes several serious diseases in both humans and animals worldwide. It represents a great concern in the dairy production chain, since it is often isolated from raw milk and dairy products. Furthermore, it is considered a major issue for the food processing industry due to its ability to form biofilms, that can become persisting sources of contamination. It has been observed that different genotypes of S. aureus differ in their contagiosity and pathogenicity In particular, S. aureus genotype B (GTB) is associated with high within-herd prevalence. This indicates an increased contagious and virulence potential compared with other genotypes (OGTs). Furthermore, in a recent study dairy S. aureus GTB from Switzerland showed significantly higher biofilm formation compared with OGTs. The aim of this study was to evaluate the biofilm-forming abilities of different genotypes of dairy S. aureus strains isolated from Italy (Piedmont and Emilia Romagna). A total of 49 dairy isolates were genotyped by using ribosomal spacer PCR and MLST. Biofilm formation was evaluated under static conditions on six-wells polystyrene plates (37°C, 24 h). 15 out of 49 (30,6%) isolates were found to be GTB, whereas the remaining isolates (=34) belong to other ten different genotypes. Six of GTB isolates (40%) shown biofilm forming ability In particular, four isolates were classified as weak and two as moderate biofilm producers. As OGTs isolates concern, 13 strains (38.2%) resulted capable to form biofilm: five weak, six moderate, and two strong biofilm producers. The majority of STs found are previously reported to be bovine-associated. In contrast with previous findings, in this work differences in biofilm forming ability between S. aureus GTB and OGTs were not observed. Interestingly, both the strong biofilm producers were not GTB (AA, ND). In summary, no specific genotype or MLST could be categorized

significantly into one level of biofilm formation. Further studies are necessary to confirm these initial findings.

Di_Nardo° S

Acute exposure of the Italian young population to tropane alkaloids through cereal-based food consumption : a preliminary estimate at a local level

Tropane alkaloids (TAs) are secondary metabolites of several plants, characterised by an acute toxicity on the nervous system. EFSA established a group ARfD of 0.016 pg/kg bw expressed as the sum of (-)-hyoscyamine and (-)-scopolamine, assuming equivalent potency. The present study was carried out on 82 cereal-based food samples collected in Lombardia and Emilia-Romagna regions during 2016-2017 and analysed for TAs by the Food Chemical Department of the IZSLER using a validated LC-MS/MS method. LOQ value was 0.3 pg/kg for cereals. Most sample concentrations (97.6%) were below the LOQ. Four samples showed the presence of atropine or scopolamine, namely: a rice-based cream and a maize-based cream intended for infant consumption, and two buckwheat products. A preliminary investigation was performed on the acute exposure to the sum of atropine and scopolamine by infants, young children and teenagers consuming cereal products. Two scenarios were assessed. First scenario considered real data for mean and high consumers. Infant exposure ranged from 0.0008 to 0.0104 pg/kg b.w./day; children exposure ranged from 0.0012 to 0.0045 pg/kg bw/day; teenagers exposure ranged from 0.0011 to 0.0031 pg/ kg bw/d ay. A worst-case scenario was calculated taking into account left-censored data in an UB approach, assuming a concentration of 0.29 pg/kg for samples below the LOQ. Infants exposure ranged from 0.0011 to 0.0431 pg/kg b.w./day; children exposure ranged from 0.0006 to 0.0089 pg/kg b.w./day; teenagers exposure ranged from 0.0004 to 0.0066 pg/kg b.w./day. The preliminary study shows that the group ARfD is not exceeded within the first scenario. If left-censored data are assessed, the acute exposure of infants to the sum of TAs could exceed the ARfD. However, the worst-case assessment was very conservative and affected by severe uncertainties, mostly related to a large number of left-censored data and the lack of updated consumption information.

Diletti G, Scortichini G, Abete MC, Binato G, Candeloro L, Ceci   R, Chessa G, Conte A, Di_Sandro A, Esposito M, Fedrizzi° G, Ferrantelli   V, Ferretti° E, Menotta° S, Nardelli V, Neri B, Piersanti A, Roberti F, Ubaldi A, Brambilla G

Intake estimates of dioxins and dioxin-like polychlorobiphenyls in the Italian general population from the 2013-2016 results of official monitoring plans in food

The implementation of the European Union strategy for polychlorodibenzo-dioxins and -furans (PCDD/Fs), and dioxinlike polychlorobiphenyls (DL-PCBs) is determining a general reduction of their presence in the environment and in the food chain. The most important route for human exposure to these substances is food consumption and, as a consequence, a progressive decrease of their dietary intake has been observed in the last decades. In this context, it seemed worth updating the PCDD/F and DL-PCB intake estimation for the Italian population. A total of 2659 samples of food of animal and vegetable origin analyzed for PCDD/Fs and DL-PCBs in the period 2013–2016 by accredited official laboratories and the national food consumption database were considered for the dietary intake assessment in different age groups of the Italian general population The median cumulative intake estimates expressed as pgWHO-TEQ/kg body weight per day and computed with a deterministic and a probabilistic approach were 1.40–1.52 for children, 0.82–0.85 for adolescents, and 0.64–0.61 for adults, respectively. Such results confirmthe decreasing trend of PCDD/F and

DL-PCB dietary intakeeventhoughtheTolerableDailyIntake(TDI)valueof2WHO-TEQ/kgbodyweight perday is exceededat the 95th percentile for all age groups, with children as sensitive group.Most contributing food categories to the intake resulted fish, food of vegetable origin, and cheese. A sensitivity analysiswas also performed to calculate the target contamination levels able to keep the dietary exposure belowthe TDI. Computed target levels fall between P50 and P97 of the occurrence distribution of the main food groups, meaning that most of the Italian food production can be considered safe.

Ferro GL, Pellicciotti° S, Ostorero F, Gili M

Simultaneous determination of natural steroids residues in blood serum by LC-MS/MS

8th International symposium on hormone and veterinary drug residue analysis : Ghent, Belgium, 22-25 May, 2018 : abstract book / organized by Faculty of Veterinary Medicine, Faculty of Pharmaceutical Sciences, Ghent University, Belgium and Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium. - [Ghent : Ghent University, 2018]. - p 95 (Poster P.27) [Nr. Estr. 8140]

International symposium on hormone and veterinary drug residue analysis   (8th   : Ghent, Belgium : 22-25 May, 2018)

Among forbidden substances included in the EU legislation, steroid compounds and, particularly, endogenous steroids, (i.e., androgens, estrogens, progestins) constitute a challenge in the framework of veterinary official monitoring plans. Indeed, these molecules can be naturally present at variable levels in body fluids depending on the species, sex and age of the animal and usually are detected using different analytical methods. The aim of this study was to develop a multiresidue, rapid and very effective analytical procedure for quantitative determination of progesterone, testosterone, 1713-estradiol, estriol and estrone in bovine blood serum. This study describes a comparison between different approaches for sample preparation: liquid-liquid extraction with TBME, protein precipitation with $ZnSO4$ / methanol mixture, clean up with C18 (500 mg/6 ml), NH2 (500 mg/3 ml), Oasis® MAX (60 mg/3 ml), Oasis® PRIME HLB (200 mg/6 ml) and AFFINMIP® SPE cartridges respectively. The analytes were detected by LC-MS/MS in ESI positive and negative mode; Chromatographic separation was performed on a Waters XSelect HSS T3 XP (3 x 150 mm; 2,5 Lim) column, using a gradient elution with A] NI-14F 1 mM in water and B] methanol. Estradiol-d4, testosterone-d3 and progesterone-d9 were used as internal standards.

Filipello° V, Benevenia° R, Finazzi° G, Zanardi° G,   Bertasi° B

Quantifying LAB populations in raw milk and dairy products through a Real Time PCR assay

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International ICFMH symposium   (26th   : Berlin   : 3-6 September 2018)

Raw milk, and its derived products, are a complex environment with microbial populations belonging to several different taxonomic groups. Until now, knowledge on the identification of microorganisms in these matrices has been gained mainly through culturing methods and, possibly, with phenotypic typing analysis. However, often these techniques lack a sufficient discrimination power and do not allow to consider those bacteria adverse to cultural growth, leading to problems of underestimation of the microbial populations. In the last years, NGS allowed a more accurate estimation of the microbial diversity. However, this technology requires costs and times of analysis that not all the laboratories are able to bear. For these reasons we developed a qReal Time FOR assay to allow the rapid quantitative estimation of the main LAB populations that are naturally present in raw milk and have beneficial effects on the cheesemaking process. In particular, we focused on Streptococcus thermophilus, Leuconostoc mesenteroides, Lactococcus lactis, Lactococcus garviae, and Lactobacillus spp., and consequently five pairs of specific primers were identified after bibliographic

research and in silico testing. The reactions were initially tested on certified strains grown at their optimum conditions to ensure an acceptable CFU/mL quantitation. The reactions were carried out using the SsoAdvancedTM Universal SYBR® Green Supermix (Biorad). Evaluation of positive reactions was pointed out by observation of amplification plot and melting curve data. Standard curves were generated by plotting the cycle threshold values (Ct) of the qPCR perfomed on diluition series of purified DNA from the different strains against their correspondent log input cells mL-1. Cells concentrations were calculated by the viable cell plate count method, plating tenfold diluition of the cultures on BHI, incubating for 2 days and determining CFU in duplicate. Subsequently the assay was tested on field samples of raw milk, curd, and cheese in different moments of the aging. The developed method will have the double function of i) assessment of the suitability of raw milk for cheesemaking due to the presence of non-starter LAB populations beneficial for the process, ii) monitoring of the dynamics of the considered populations throughout the aging, to help understanding how they may influence the final quality of the product.

Filipello° V, Benevenia° R, Mangeri° L, Galuppini° E, Losio° MN, Zanardi° G, Bertasi° B

Protocollo di screening per la valutazione della flora filo- ed anti-casearia nel latte crudo da destinare alla produzione di formaggio

Il latte crudo rappresenta un ambiente complesso di popolazioni microbiche che include microorganismi di rilevanza tecnologica e di grande interesse in termini di qualita alimentare. Le popolazioni dominanti sono rappresentate dalla flora lattica (LAB - lactic acid bacteria), un gruppo di batteri in grado di fermentare ii lattosio coinvolto massivamente nei processi di caseificazione, maturazione e sviluppo delle qualita organolettiche dei formaggi. I LAB piu comuni nel latte crudo appartengono ai generi Lactococcus, Lactobacillus, Leuconostoc, e Streptococcus; essi determinano effetti positivi sul prodotto finito, e per questo rappresentano la flora filo-casearia. I LAB svolgono inoltre un importante ruolo nel¬l'inibizione della proliferazione di batted patogeni. La flora anticasearia è invece rappresentata principalmente da batted sporigeni appartenenti at genere Clostridium; C. tyrobutyricum e la specie maggiormente coinvolta in fenomeni di alterazione tardiva del formaggio causando ingenti perdite economiche per la formazione di fessurazioni, gonfiori e gas maleodoranti. Queste popolazioni hanno quindi un impatto diretto sullo sviluppo dei prodotti lattiero caseari, e meritano un'accurata valutazione. Ad oggi, la rilevazione di questi microorganismi Si basa principalmente su metodi colturali, che sono laboriosi, richiedono lunghi tempi di analisi (fino a sette giorni), non hanno un sufficiente potere discriminante e non permettono di considerare alcune specie di lattici the crescono con difficolta in laboratorio, generando problemi di sottostima. Per questi motivi abbiamo sviluppato un protocollo di Real Time PCR per la valutazione della flora fib- ed anti-casearia nel latte destinato alla produ-zione di formaggio, che permetta di valutare rapidamente la propor¬zione delle popolazioni batteriche che possono influenzare i processi di caseificazione. A seguito di ricerca bibliografica, sono state selezionate sette coppie di primers per la ricerca di S. thermophilus, L. mesenteroides, L. lactis, L. garviae, Lactobacillus spp., C. tyrobutyricum, e C. sporogenes. Le condizioni di amplificazione sono state ottimizzate a partire da DNA estratto da ceppi certificati coltivati rispettando l'optimum di crescita. Le reazioni di PCR sono state effettuate con SsoAdvanced Universal SYBR Green Supermix (Biorad) e la positivita del campioni è stata identificata osservando le curve di amplificazione e di melting. Per la quantificazione sono state costruite curve standard riportando su un grafico i treshold cycles di diluizioni seriali del DNA dei diversi ceppi e ii corrispettivo valore logaritmico di UFC/mL. Tutte le specie in esame sono state identificate univocamente con curve di melting specifiche. 'noltre, sono stati testati 56 cannpioni di latte crudo. I LAB del genere Lactobacillus rappresentano la popolazione prevalente e sono stati identificati net 96% (95CI 79-99%) del campioni, seguiti da Lactococcus spp. (39%) e L. mesenteroides (13%). C. tyrobutyricum e stato indentificato nel 10% del campioni (95CI

5-21%), ma livello di contaminazione è risultato inferiore a 2 UFC/ml in tutti i campioni positivi. La possibilita di valutare rapidamente l'idoneita del latte destinato alla caseificazione attraverso questo protocollo potrebbe avere notevoli ricadute economiche per gli operatori del settore lattiero-caseario, e grandi potenzialita applicative net pagamento del latte secondo qualita.

Finazzi° G, Bertasi° B, Pavoni° E, Andreoli° G, Sacchi° C, Colmegna° S, Bertoletti° I, Losio° MN

Hygiene and health features of edible bivalve molluscs traded in Lombardy Region of Italy in 2017

Edible bivalve molluscs are frequently the cause of very serious outbreaks of food poisoning. Microbiological suitability for human consumption, according to European Union (EU) Regulation 2073, is based on two fecal bacteriological contamination parameters (Escherichia colt and Salmonella), but it is fundamental to consider other microorganisms which are naturally present within the marine environment and are potentially pathogenic, such as viruses.   Purpose: The object of this work was to analyze the data obtained from samples collected by public health officers in food stores of the Lombardy region of Italy in 2017, and to perform an overview of the hygienic condition of this food matrix. Methods: A total of 307 samples (232 mussels, 48 clams, 25 oysters, 2 others) were collected in one- or five-sample units (s.u.). A total of 1,060 s.u. were analyzed. The detection of Salmonella with RT-PCR technique and further confirmation of positivity with culturing method was performed on 1,040 s.u., and 1,048 s.u. were analyzed for E. coli with most probable number (MPN) count. A total of 33 samples (41 s.u.) were also analyzed for the hepatitis A virus and for norovirus detection with RT-PCR technique. Results: Salmonella, serotyped as S. rissen, was found in one s.u. Three samples had unsatisfactory results for E. coli, according to EU Regulation: one had a value > 700 MPN/100 g in one s.u., and two had a value between 230 and 700 in 2 of the 5 s.u.. Remarkably, 938 s.u. (89.5%) had < 18 E. colt MPN/100 g. One sample was positive for norovirus G1. Significance: In general, the edible bivalve molluscs marketed in Lombardy showed a satisfactory level of hygiene, both for the microbial and the viral parameters. The low level of E. coil contamination suggests it would be profitable to replace the MPN count with the more cost   effective and less laborious pour plate count method.

Finazzi° G, Filipello° V, Bertoletti° I, Zecca O, Marchetti M, Losio° MN

Foodborne outbreak of gastroenteritis caused by Staphyilococcal enterotoxin in Northern Italy

Staphylococcal Food Poisoning (SFP) is one of the most common foodborne diseases worldwide. SFP is caused by the ingestion of food contaminated with preformed Staphylococcal Enterotcodns (SEs) produced by S. aureus. In alpine regions raw milk dairy products are hand-crafted in small scale artisanal dairies built in the pastures. Traditional cheeses represent appealing products to the new trends for the search of natural and authentic foods. Sometimes the lack of proper hygienic measures during food processing increases the probability of contamination with S. aureus Description of the problem: On August 2017 a case of gastroenteritis was reported to the local hospital of Sondrio (Northern Italy). The outbreak involved subjects from 2 different family units who participated together to an excursion in the mountains. Symptoms (abdominal pain, vomiting,

diarrhoea) were developed by 5 subjects within 2 hours from lunch. The meal consumed by all the symptomatic people included sandwiches prepared with a traditional cheese bought in a pasture. Leftover cheese, and another batch collected in the same pasture, were screened for Salmonella spp., STEC, B. cereus, S. aureus and its related enterotoxins. Results: Both batches of cheese, showed a high level of contamination of coagulase positive Staphylococci (from 3 to 6 log cfu/g detected in different samples) and were positive for staphylococcal enterotoxins (type D). All samples were negative for all other investigated hazards. Based on the epidemiological investigation, cheese was identified as the source of infection. Lessons: The technologies used in alpine pastures dairies could be not effective in hindering and limiting the proliferation of S. aureus in the early phases of production. The application of basic good manufacturing practices, starting from control of the health status of cows and milking hygiene, combined with the surveillance on products are fundamental to prevent situations that can cause foodborne outbreaks. Key messages: Raw milk dairy products are expression of the alpine culture. The complex pasture system is prime to safeguard the environmental, economical and touristic development of the alpine regions. Good hygienic practices for food production and manipulation tion are the main important tools to prevent and control foodborne outbreaks.

Finazzi° G, Pavoni° E, Bertasi° B, Andreoli° G, Sacchi° C, Colmegna° S, Bertoletti° I, Losio° MN

I molluschi bivalvi vivi consumati in Lombardia sono sani e sicuri? Risultati del biennio 2017-18 secondo le indicazioni dell'Accordo Stato Regioni del 10 Novembre 2016

I molluschi bivalvi vivi (MBV) sono spesso indicati come causa di episodi di tossinfezione alimentare. Canalisi dei dati ottenuti dal campionamento eseguito nell'ambito del Piano Regionale Integrato del Controlli ha permesso di avere un quadro generale sul livello igienico sanitario dei MBV presenti nel circuito della grande distribuzione in Lombardia nel 2017 e nei primi 5 mesi del 2018.Icriteri indicati dal Reg. 2073/05CE prevedono ricerca di Salmonella spp. e numerazione di E. coli, ma 6 fondamentale considerare altri agenti potenzialmente patogeni che possono essere naturalmente presenti negli ambient' marini. Le Linee Guida per i Controlli Ufficiali, riportate negli Atti 212 dell'Accordo Stato Regioni del 10/11/16, suggeriscono infatti di considerare anche altri parametri tra cui virus e Vibrio patogeni. Nel 2017 sono stati prelevati dalle ATS della Lombardia, in 1 o 5 unita campionarie, 307 campioni (232 cozze, 48 vongole, 25 ostriche, 2 altre specie) per un totale di 1060 u.c., e nei prinni 5 mesi del 2018, 139 campioni (77 cozze, 55 vongole, 6 ostriche, 1 altre specie) per un totale di 427 u.c. La ricerca di Salmonella spp. è stata eseguita mediante screening con Real Time PCR e conferma dei positivi mediante metodo ISO 6579-1 su 1040 u.c. nel 2017 e 358 u.c. nel 2018. La nunnerazione di E. coil con metodo ISO 16649-3 6 stata eseguita su 1048 u.c. nel 2017 e su 368 u.c. nel 2018. 33 cannpioni sono stati analiEati nel 2017 per ricerca del virus dell'epatite A (HAV) e Norovirus (NoV) con metodo ISO/TS 15216-2, 46 nel 2018. Solo nel 2018 è stata eseguita la ricerca di Vibrio patogeni con metodo ISO/TS 21872-1 su 9 cam¬pioni. Nel periodo considerato sono stati isolati 2 ceppi di Salmonella, una tipizzata S. rissen nel 2017 e una S. schwarzen-grund nel 2018. Ire campioni prelevati nel 2017 hanno data risultati non conformi per il parametro E. coil secondo le indicazioni del Reg. 2073/05 CE (1 con valore >700 MPN/100 g; 2 per valori compresi nel range m=230 e M=700 in piO u.c.); mentre nel 2018 sono stati 7 (5 per valore >700 MPN/100 g.; 2 per valori compresi   net range m e M). Un campione è risultato positivo per NoV GI net 2017 nnentre net 2018 ben 24 sono risultati positivi per NoV Gil di cut 5 anche per NoV Gt. Nel 2018 è stato isolato un ceppo di V. parahaemolyticus risultato pert) privo dei fattori di patogenicita. It livello igienico del MBV commercializzati in Lombardia era apparso soddisfacente net 2017, con solo 4 campioni non conformi (1,3%). Il quadro net primi mesi del 2018 appare peggiorato con 8 campioni non conformi per i Criteri di Sicureua del Reg. 2073/05 CE (9,5% del totale), a cut si aggiungono 24 campioni con positivita virologica (52,2%) 2 del quail ostriche, normalmente consumate crude.

numento di positivita virologiche è probabilmente legato a fattori ambientali che net mesi invernali possono aver favorito un rimescolamento delle acque a livello di estuari e foci fluviali, e quindi anche una maggior ridistribuzione del virus nelle acque di miticoltura. Questa ipotesi è supportata dal fatto the 22 positivita virologiche sono state riscontrate in vongole, organismi che vivono in stretta simbiosi cot fondali marini. Questi dati evidenziano l'impor¬tanza di considerare anche altri fattori di potenziale patogenicita, come virus e Vibrio patogeni, per una corretta valutazione igienico sanitaria del MBV e per rispondere all'esigenza di una maggiore tutela del consumatore.

Gaidella L, Rosignoli° C

Un caso di salmonellosi in ambiente domestico "rurale"

Settim Vet. - Vol. no 1036 (2018). - p 28 [Nr. Estr. 7840]

Gamba° V, Bolzoni° G, Monastero° P, Daminelli° P

Effect of antibiotic residues on the survival of starter whey cultures and persistence of B-lactam antibiotics in milk products during the cheese production process

8th International symposium on hormone and veterinary drug residue analysis : Ghent, Belgium, 22-25 May, 2018 : abstract book / organized by Faculty of Veterinary Medicine, Faculty of Pharmaceutical Sciences, Ghent University, Belgium and Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Belgium. - [Ghent : Faculty of Pharmaceutical Sciences, 2018]. - p 47 [Nr. Estr. 7875]

International symposium on hormone and veterinary drug residue analysis   (8th   : Ghent, Belgium : 22-25 May, 2018)

The production of semi-hard cheese from raw milk requires the addition of natural whey starter, which contains a complex microflora of lactic acid bacteria. Milk may contain antibiotic residues, which can interfere with the growth of starter cultures. Here, we have analysed the effect of 9 common antibiotic residues on starter whey cultures when present at concentrations equal to the Maximum Residue Limits (MRLs) established by the European Union. By assessing the survival of mesophilic lactobacilli and determining the pH and titratable acidity of milk over time, we show that Penicillin G, Cefquinome, Ceftiofur and Desfuroylceftiofur had an inhibitory effect, while Sulfadimethoxine, Enrofloxacin, Oxytetracycline, Amoxicillin or Monensin did not. Furthermore, we produced semi-soft cheese from raw milk and analysed the persistence of Cefquinome in milk products, i.e. whey, curd and cheese, after spiking milk at an antibiotic concentration double than the MRL. We show that almost 90% of Cefquinome residues remain in whey, while the rest concentrates in curd, reaching concentrations similar to the one initially present in spiked milk. In ripening cheese, residues decreased approximately 6- fold in a week, and continued to decrease over time, reaching our limit of detection (0.5 ppb) after three weeks. Upon visual inspection, cheese made from spiked milk showed clear alterations in texture, i.e. large holes, compared to control cheese wheels, suggesting possible proliferation of non-starter bacterial species.

Gamba° V, Daminelli° P, Zanardi° G

Evaluation of the distribution of drug residues in different dairy products during the cheese production process

The 30th World Buiatrics Congress : August 28 to September 1, 2018 : Sapporo, Japan : abstract book, keynote lectures, oral and poster presentations / [s.l. : s.n., 2018]. - p 371 [Nr. Estr. 8084]

World Buiatrics Congress (WBC)   (30th   : Sapporo, Japan   : August 28 to September 1, 2018)

Gasparini° M, Cancemi° G, Ferretti° E

Confirmatory method for the determination of Amitraz residue in food of animal origin sample by liquid chromatography-tandem mass spectrometry

As a consequence of Fipronil emergency in eggs during 2017, Italian Health Ministry indicated to Official Laboratories to start Amitraz analysis in poultry meat, eggs and poultry and bovine fats. Amitraz is used as veterinary drug on beehives to control the parasite Varroa jacobsoni destructor, and also it could be used as acaricide. The amitraz molecule is very unstable and can hydrolyze through a series of intermediate compounds to form other stable but toxic molecules. Therefore in EU, Maximum Residue Limit was expressed as Amitraz including the metabolites containing the 2,4-dimethylaniline (DMA) moiety. Under these premises a confirmatory method was developed for the simul¬taneous determination of Amitraz, N-2,4-dimethylphenyl-N-methyl-formami¬dine (DMPF), N-2,4-dimethylphenyl-formamide (DMF) and 2,4-dimethylaniline (DMA) in food samples of animal origin by liquid chromatography coupled with electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). After a solvent extraction and a purification step, analytes were detected and quantified using an internal standard approach versus deuterium-labelled Amitraz standard. During the method development we observed that some preparation conditions result very critical for Amitraz molecule and it is necessary to use different types of sample preparation both in terms of extraction and purification condi¬tions, depending on matrix type analyzed. European regulations 396/2005 state that analytical methods for the determination of pesticide residues in animal products must be able to reveal concentrations lower than the maximum allowed residue levels (MRLs) and have to be in compliance with the "Working document on the summing up of LOQs in case of complex residue definitions". The developed method is in compliance with European Regulations and achieved sensitivity fit for purpose. The method was validated as a quantitative confirmatory method according to the Document N° SANTE/11813/2017. The results obtained showed that the method is suitable for statutory residues testing regarding the following performances characteristics: instrumental linearity, matrix matched calibration, Limit of Quantification (LOQ), specificity, precision, trueness and uncertainty. Precision and trueness was checked at LOQ, 3-15xL0Q and 15-75xL0Q depending on the matrix considered; mean re¬coveries (trueness) ranged between 60 and 86% for every compound and the RSDr to determine the repeatability was below 20%. Uncertainty evaluated was below 50% for every analyte. The results appeared very satisfying and coherent with the criteria indicated in the Document N' SANTE/11813/2017.

Gazzola° A, Romanò A, Maisano° AM, Spelta° C, Bertocchi° L, Ferrara° G, Finazzi° G, Filipello° V, Decastelli L, Bergagna S, Cremonesi P, Vezzoli° F, Graber HU, Luini° MV

Genotipizzazione di S. aureus isolati da latte bovino di aziende lombarde

Staphylococcus aureus strains (n=1559) isolated from North Italian dairy herds were genotyped by RS-PCR. A selection of these strains (84 from bulk tank milk and 91 from individual cow milk) was also analyzed for the presence of adlb gene, which is considered a possible marker of contagiousness. The adlb gene amplification was included in a multiplex PCR also targeting nuc and mecA genes. RS-PCR identified 83 different genotypes combined into 48 genotypic clusters, among which genotype B with his variants (GTB) was the most prevalent (n=491). A clonal diffusion of a single genotype was shown in most farms (373/458), especially when GTB was involved. Most of

Staph. aureus belonging to GTB carried the adlb gene (38/43), as well as very few strains belonging to a different genotype. When investigated, all of them were associated with high prevalence in the herds, confirming that RS-PCR and adlb PCR could be useful tools in predicting the clinical properties of isolates.

Grisendi° A,   Listorti° V, Defilippo° F, Fedrizzi° G, Dottori° M, Bonilauri° P

Detection of aflatoxin B1 in different life stages of Black Soldier Fly (Hermetia illucens)

Mycotoxin-contaminated cereals (e.g., maize, wheat, barley) and nuts (e.g., peanut, walnuts) can be deleterious to humans and can reduce animal health and production. The mycotoxin presence in these products is monitored and, when concentrations are above maximum allowable limits, products are downgraded or removed from the food chain. Insects can be used as food by both humans and animals so they contribute to food security. There is growing interest in larvae of the black soldier fly (Hermetia illucans, BSF). BSF larvae can be sustainable proteinaceous feed ingredients for pigs, poultry, and fish, as well as alternatives to conventional sources (soybean meal and fishmeal) with associated environmental and societal issues. The mycotoxin tolerance of these insect species is unknown. In nature, the larvae of BSF usually thrive in various decomposing materials and are commonly reared on diets of approximately 70% moisture, and kept at 28°C. Both these conditions promote bacterial and fungal growth, therefore the current study investigated the potential accumulation of Aflatoxin B1 (AfB1) in larvae (L3 and L4), pre-pupae and pupae of the BSF. Insects were reared under controlled conditions (RH 70%, photoperiod 14:10 h (L:D) and temperature of 25°C) on two substrates: Gainesville diet for the control group and a mycotoxin contaminated diet, enriched with peanuts containing naturally high concentration of AfB1 (38 pg/kg), for the contaminated groups. AfB1 concentration in contaminated diet was of 3 pg/kg. At the end of the exposure period the insects were separated from the residual material, washed, killed by freezing and analyzed (LC-MS/MS). The results showed that the concentrations of AfB1 on the larvae reared on the contam-inated diet remain far below 1 pg/kg (L3 = 0.2 pg/kg, L4 = 0.1pg/kg) and in pre-pupal and pupal stage the concentration of mycotoxin further decrease under the detection limit of the method (< 0.05 pg/kg). Finally, in the residual diet high levels of AfB1 were detected (from 5.1 to 6.5 pg/kg).The applied rearing conditions were successful in supporting the development of BSF with high survival rates. The BSF larvae do not accumulate AfB1, this confirms the results obtained in previous studies and leads us to suppose that mycotoxins are present only in the gut and not in other insects tissues. The slightly higher concentration of AfB1 in the diet at the end of the experiment could have been due to the fact that larvae not accumulating AfB1 and reducing the substrate during their growing, have produced a concentration of the AfB1 in the residual diet.

Lomonaco S, Gallina S, Filipello° V, Leon MS, Kastanis GJ, Allard M, Brown E, Amato E, Pontello M, Decastelli L

Draft genome sequences of 510 Listeria monocytogenes strains from food isolates and human listeriosis cases from northern Italy

Listeriosis outbreaks are frequently multistate/multicountry outbreaks, underlining the importance of molecular typing data for several diverse and well-characterized isolates. Large-scale whole-genome sequencing studies on Listeria monocytogenes isolates from non-U.S. locations have been limited. Herein, we describe the draft genome sequences of 510 L. monocytogenes isolates

from northern Italy from different sources.

Losio° MN, Pavoni° E, Finazzi° G, Agostoni C, Daminelli° P, Dalzini° E, Varisco° G, Cinotti° S.

Preparation of powdered infant formula : could product's safety be improved?

The recent outbreak of Salmonella Agona linked to the consumption of infant formula (powdered formula) has rekindled the attention about the correct procedures for preparation and use of these products. International guidelines have already been published so far, particularly in association with Cronobacter sakazakii in early 2000s. FAO/WHO suggested to reconstitute formula with water at no less than 70°C. We therefore contaminated powdered formula with low levels of Salmonella spp and C sakazakii to evaluate the pathogens inactivation during the formula preparation using water at 70°C. In these conditions we observed a survival of both pathogens, indicating that the suggested recommendations may be not enough to guarantee the safety of this product. Higher temperatures are needed to reduce the biological risk, even if it may be not easily realized in actual domestic conditions. Moreover, the impact on the nutritional value of reconstituted formulas should be evaluated.

Malacarne M, Visentin G, Summer A, Cassandro M, Penasa M,   Bolzoni° G, Zanardi° G, De_Marchi M

Investigation on the effectiveness of mid-infrared spectroscopy to predict detailed mineral composition of bulk milk

This Research Communication investigated the potential of mid-infrared spectroscopy to predict detailed mineral composition of bovine milk. A total of 153 bulk milk samples were analysed for contents of Ca, Cl, Cu, Fe, K, Mg, Na, P and Zn. Also, soluble and colloidal fractions of Ca, Mg and P were quantified. For each milk sample the mid-infrared spectrum was captured and stored. Prediction models were developed using partial least squares regression and the accuracy of prediction was evaluated using both cross- and external validation. The proportion of variance explained by the prediction models in cross-validation ranged from 34% (Na) to 77% (total P), and it ranged from 13% (soluble Mg) to 54% (Cl-) in external validation. The ratio of the standard deviation of each trait to the standard error of prediction in external validation, which is an indicator of the practical utility of the prediction model, was low and never greater than 2. Results from the current study supported the limited usefulness of mid-infrared spectroscopy to predict minerals present in low concentration in bulk milk. For major mineral components, results from the present research did not match previous findings demonstrating the need for further studies using larger reference datasets.

Mazzone P, Di_Paolo A, Curcio L, Corneli S, Ortenzi R, Sebastiani C, Ciullo M, Spaccini G, Biagetti M, Valiani A, Pezzotti G, Cammi° G, Arrigoni° N

Valutazione della sopravvivenza di Mycobacterium avium subsp. paratuberculosis (MAP) ai processi di trasformazione del latte ovino

Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 92-93. - 6 bib ref [Nr. Estr. 8052]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV)   (18.   : Perugia (PG)   : 7-8-9 Novembre 2018)

Mycobacterium avium subsp. paratuberculosis (MAP) is the causative agent of Paratuberculosis, a chronic enteritis of ruminants. MAP is also suspected to be involved in Crohn's disease in humans; although there is still no conclusive evidence, many surveys have evidenced MAP contamination in the food chain. Here we describe a challenge study investigating MAP survival in the production process of pecorino, an Italian cheese made from raw sheep milk. Three batches of pecorino were produced from milk spiked with MAP. During the different phases of manufacturing, ten samples were collected for microbiological and chemical-physical analysis. MAP load, determined with Plate Count and qPCR, was almost stable during salting, drying and ripening (60 days), decreasing only one Log10, if compared to the initial level. Based on these results, if high MAP contamination is presumed in raw milk, an initial pasteurization treatment of milk, at 72°C for at least 25 seconds, is highly recommended.

Meletti° F, Bertasi° B, Suffredini E, Boniotti° B, Di_Pasquale S, Lovari S, De_Medici D, Losio° MN

Applicazione della digital PCR a supporto della metodica ISO/TS 15216 per la quantificazione del virus dell'epatite A

XVIII Congresso Nazionale SIDiLV : Perugia (PG), 7-8-9 Novembre 2018 : volume degli atti / [s.l. : Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV), 2018]. - p 345-347. - 4 bib ref [Nr. Estr. 8070]

Congresso Nazionale Societa' Italiana Diagnostica di Laboratorio Veterinaria (SIDiLV)   (18.   : Perugia (PG)   : 7-8-9 Novembre 2018)

Hepatitis A is a virus (HAV) involved in water and foodborne hepatitis in humans; consumption of different types of food causes outbreaks worldwide. Up to now, qualitative molecular biology methods have been applied to detect viruses and the current official method to detect HAV in food matrices is stated in the ISO 15216-2 (2), which is based on qualitative real-time PCR. However, it can be expected that quantitative analytical protocols (e.g. ISO 15216-1) (1) will become a requirement in the next future. Recently new technologies of DNA quantification, as digital PCR (dPCR) have been introduced. The topic of this work was the setting up of different digital PCR systems and the comparison among each of them and real-time PCR, to obtain a valid support to ISO quantitative method.

Merialdi° G, Ramini° M, Grisenti S, Dalzini° E, Bardasi° L, Frustoli A, Daminelli° P, Bonilauri° P

Valutazione dell'impiego di HPP come trattamento letale net processo produttivo di diverse tipologie di salame al fine di implementare i fattori intrinseci ed estrinseci che contribuiscono al controllo di Listeria monocytogenes

Attualita nell'igiene degli alimenti: stato dell'arte e prospettive future : XXVIII Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI) : Milano,12-14 Settembre 2018 / [s.l. : s.n., 2018]. - p 27 (P008) [Nr. Estr. 7929]

Convegno Nazionale Associazione Italiana Veterinari Igienisti (AIVI)   (28.   : Milano   : 12-14 Settembre 2018)

Lo scopo del lavoro b la valutazione dell'abbattimento di L. monocyotgenes durante il processo produttivo seguito da un trattamento ad alte pressioni idrostatiche (HPP) in diverse tipologie di salami al fine di soddisfare dell'obiettivo di performance pan i a 3 log richiesto da FSIS Salmonella Compliance Guidelines for small and very small meat and poultry establishments that produce ready-to-eat (RTE) products, giugno 2017 e net DGI-SAN 0036882-P- Trattamenti di Alte Pressioni (HPP) in prodotti alimentari destinati all'esportazione verso i Paesi Terzi - Chiarimenti applicativi,

29/09/2014. I dati di abbattimento sono stati ottenuti net corso di attivita sperimentali condotte presso 3 laboratori, due localizzati in IZSLER (BO e BS) e la SSICA di Parma. Le ricette sono state scelte tra le maggiormente rappresentative della produzione nazionale tenendo in conside-razione la variabilita relativa alle dimensioni della grana, al calibro, alla lungherza della stagionatura ed alla curva di acidificazione in fase di asciugatura. Tutti gli impasti sono stati artificialmente contaminati prima dell'insacco con un mix di almeno tre ceppi di L. monocyto genes o di L. innocua come suo surrogato. La concentrazione di Listeria spp. nell'impasto dopo l'aggiunta dell'inoculo (1% v:v) risultata almeno uguale a 5 log UFC/g. I salami sono stati quindi sottoposti a cicli differenti di asciugatura e stagionatura. La durata dell'asciugatura è stata compresa tra 5 e 11 giorni, con pH a fine asciugatura compreso tra 4.78 e 5.41. La durata della stagionatura è stata compresa tra 21 e 90 giorni, con aw finale tra 0.880 e 0.951. Al temine della stagionatura prevista per ogni sin-gala prodotto i salami sono stati sottoposti a trattamenti con alte pressioni idrostatiche (5930 bar, 300 sec). I valori di pH, aw, e concentrazione di Listeria sono stati determinati in almeno 3 repliche a fine asciugatura ed almeno 5 repliche a fine stagionatura e dopo trattamento HPR Complessivamente sono stati eseguiti 18 challenge test. Tutti i prodotti hanno rispettato il limite di 1200 Degree/hours. Nonostante le condizioni, di pH, aw e temperatura fossero in asciugatura teoricamente permissive per lo svi¬luppo di L. monocyogenes; in tutti i processi b stato osservata una riduzione del patogeno in media uguale a -0.90 (dev.st 0.46) log UFC/g. In accordo con i modelli predittivi disponibili (www.combase.cc) ogni giorno passato con aw inferiore a 0.96 determina una condizione sfavorevole al patogeno. Lfabbattimento osservato a fine stagionatura é risultato in media uguale a -1.64 (dev.st 0.78) log uFyg. II trattamento di HPP ha comportato il raggiungimento per tutti i prodotti del target di -3 log, con un abbattimento complessivo (processo pib HPP) minima di -3.17 log ed un nnassimo di -4.89 log. Le conclusioni del presente lavoro permettono di trarre alcune indicazioni generali sui salami di suino destinati all'export USA, ma che possono essere considerate nella gestione del rischio L. monocytogens anche nei prodotti destinati al mercato locale. Il pH di fine asciugatura e la durata della stagionatura risultano i parametri principalmente determinanti per l'abbattimento del patogeno. Tuttavia, se si intende sottoporre ii salame al trattamento con HPP va conside¬rato che l'efficacia del trattamento ad alte pressioni idrosta-tiche si riduce man mano che l'aw nel prodotto decresce.

Merla° C, Andreoli° G, Garino C, Vicari° N, Tosi° G, Guglielminetti ML, Moretti° A, Biancardi° A, Arlorio M, Fabbi° M

Monitoring of ochratoxin A and ochratoxin-producing fungi in traditional salami manufactured in Northern Italy

Fungi have a crucial role in the correct maturation of salami, but special attention should be addressed to the production of the nephrotoxic, immunotoxic, and carcinogenic mycotoxin ochratoxin A (OTA). In a monitoring study conducted in Northern Italy, OTA was detected by liquid chromatography coupled with mass spectrometry in 13 out 133 samples of traditional salami (9.8% of the total count). Mycological analysis of these samples yielded 247 fungal isolates Which were identified to species level. The most frequent species were Penieilliunt nalgiovense, P. solitum, and P. cluysogenum. P nordiennt, an OTA-producing species commonly found in proteinaceous food, was not found in these samples. Three isolates were found to be Aspergillus westerdijkiae, an OTA-producing species. In order to check the results of the microbiological identification, 19 different strains of Aspeigillus and 94 of Peniedlianz were tested for the presence of a sequence common to OTA-producing fungi by real-time PCR. None of the studied isolates, including the three A. westerdijkiae, possessed the otanpsPN target which is common to OTA¬producing strains. Two out of three isolates of the A. westerdijkiae were also PCR-negative for the otanpsPN gene and did not produce OTA in culture. Conversely, this target sequence was amplified from the DNA purified from 14 salami casings including three casings harboring A. westerdijkiae. The amplification of sequences specific for OTA-producing strains performed on total genomic DNA extracted directly from salami casings provided a more suitable approach than PCR analysis of isolates from salami for the OTA-related otanpsPN gene to evaluate the risk of OTA contamination.

Morganti° M, Bolzoni° L, Scaltriti° E, Casadei° G, Carra° E, Rossi L, Gherardi P, Faccini F, Arrigoni° N, Sacchi AR, Delledonne M, Pongolini° S

Rise and fall of outbreak-specific clone inside endemic pulsotype of Salmonella 4,[5],12:i:-; insights from high-resolution molecular surveillance in Emilia-Romagna, Italy, 2012 to 2015

Ntuli V, Njage PMK, Bonilauri° P, Serraino A, Buys EM

Quantitative risk assessment of hemolytic uremic syndrome associated with consumption of bulk milk sold directly from producer to consumer in South Africa

This study was conducted to estimate the hemolytic uremic syndrome (HUS) risk associated with consumption of producer-distributor bulk milk (PDBM) contaminated with Shiga toxin-producing Escherichia coli (STEC) in South Africa. Data were obtained from recently completed studies in South Africa taking into account prior collected prevalence data of STEC in raw and pasteurized PDBM and survey information from producer-distributor outlets and households. Inputs for the models were complemented with data from published and unpublished literature. A probabilistic exposure model was developed with Monte Carlo simulation in Excel add-in software using @Risk software. Hazard characterization was based on an exponential dose-response model to calculate the probability of illness from STEC infection in individuals 5 years and younger and individuals older than 5 years. The estimated mean STEC level was 0.12 CFU/mL (95% confidence interval [CI]: 0 to 1.2; a- = 0.34) for raw PDBM and 0.08 CFU/mL (95% CI: 0 to 1; rr = 0.27) for pasteurized PDBM. A higher risk of HUS cases per year was recorded in raw than in pasteurized PDBM and also in individuals younger than 5 years of age. For every 100,000 servings consumed, the expected median numbers of HUS cases per year from raw PDBM were 52 for 5 years and younger and 3.2 for older than 5 years. The median numbers of cases per year for pasteurized PDBM were 47 for 5 years and younger and 2.9 for older than 5 years. Sensitivity analysis revealed that serving volume and time taken to sell PDBM at producer-distributor outlets were the factors with the greatest impact on probability of illness. The models developed in this study are an example of risk assessments for milk produced and marketed from similar scenarios across the globe.

Pavoni° E, Barbieri° I, Colombrita D, Di_Bartolo I, Caccuri F, Ruggeri FM, Caruso A, Losio° MN

Detection of norovirus in children with acute gastroenteritis in a hospital of Italy

Background: Noroviruses (NoV) are the main non-bacterial agents of acute gastroenteritis (AGE). Norovirus infection is characterized by a high attack rate due to a low infectious dose. The virus can be transmitted through droplets and fomites. To better understand the role and the incidence of NoV, laboratory-based surveillance was conducted on paediatric patients affected by GE admitted to the main hospital in Brescia (northern Italy). Methods: Over two years 448 stool samples (1 per patient) were collected from pediatric patients (except 4 nurses) affected by GE and admitted at the hospital of Brescia. Samples were processed for routinary tests (Salmonella spp., Campylobacter spp., rota-virus, adenovirus, astrovirus). Negative samples were further analyzed for NoV by random reverse transcription (cDNA) followed by 2 PCRs targeting both ORF1 and ORF1/ORF2 overlapping region. All PCR-positive samples were sequenced. Multiple infections were not investigated.

Results: Of 448 stool samples, 227 proved negative for enteric bacteria, rotavirus, adenovirus and astrovirus. Among these, 88 (38.8%) were positive for NoV. Based on sequencing, 1 NoV GI and 7 NoV GII genotypes were identified. The most common was GII.4. GII.P4 v. Den Haag 2006b was detected in 4 nurses and shared 100% nt identity with the NoV strain detected in 5 young patients. Epidemiological investigation revealed that these patients acquired infections >24h after hospital admission. They were monitored by nurses that became sick (AGE) in the same week as the young patients. Conclusions: This study confirmed that NoV circulates among AGE-affected children and adults. Person to person transmission could have occurred between children and nurses. GII.4 is the most common genotype. Key messages: NoV is an important issue among AGE-affected children and adults. Specific guidelines for hygiene management are needful measures to reduce the impact of nosocomial NoV outbreaks.

Pavoni° E, Cosciani_Cunico° E, Dalzini° E, Monastero° P, Galuppini° E, Daminelli° P, Losio° MN

Murine calicivirus inactivation during the process of fermented sausages

Introduction: Italian salami is a raw fermented sausage that locally can be produced with pork meat, fat and liver. A preliminary study was performed to investigate the survival of murine calicivirus during the process of salami. This microorganism was used as a surrogate of the zoonotic hepatitis E virus (HEV). Material and method: Continuous RAW264.7 cell line (mouse monocyte macrophage) was used in order to verify virus viability/ infectivity. The titer (TCID50/mL) was calculated according to Reed & Muench formula. Batter was either inoculated with 1% v/w viral suspension (starting titre 7 Log TCID50/mL) or not (negative control). 3 replicates from one batch were analyzed at each sampling time. Process followed company specification. Samples (50 g) were blended and 2 g were lysed with glass beads, and centrifuged. The supernatants were filtered (0.22 pm) and inoculated on RAW264.7 cell line. The viral titre was determined both by visualization on 24-well plates (100X optical microscope), and by end point-PCR on the samples criolysates. Batter/salami were homogenized in peptone water (1/10 v/w). Mesophilic lactic acid bacteria (LAB) were enumerated by plate count. Temperature, pH and aw were monitored at each sampling time. Virus concentration was reported as log TCID50/mL; microbiological count (CFU/g) was reported as log CFU/g. The average and standard deviation values were determined on 3 samples. Significance was statistically analysed by Student t-test at a 95% confidence interval (P< 0.05). Results: Results showed that after inoculum the viral titre was about 4 Log TCID50/mL while, after 49 days, the concentration was 1 log TC1D50/mL. LAB count increased in the first 3 days (fermentation) from 6.2 to 8.6 Log CFU/g and pH dropped from 5.65±0.02 to 4.95±0.03; while no significant differences were observed in LAB concentration, pH showed a linear increase (5.57±0.20) until the end of process. The avalues decreased from 0.96±0.01 to 0.90±0.02. Discussion: The obtained results in this preliminary study showed that the salami process inactivated murine calicivirus (HEV surrogate) significantly.

Rubini° S, Berardelli C, Tosi A, Gigliotti° D, Cozzi L, Suffredini E

I controlli ufficiali sui molluschi bivalvi dopo le modifiche introdotte dal Reg. (UE) 2015/2285

Il Regolamento (UE) 2015/2285 della Connmissione dell'8 dicembre 2015 (Reg. 2015/2285) introduce modifiche sostanziali all'allegato II del Reg. (CE) n. 854/2004 (organizzazione del controlli ufficiali relativamente al molluschi bivalvi vivi) e all'allegato I del Reg. (CE) n. 2073/2005 (criteri microbiologici applicabili ai prodotti alimentari) uniformando, a decorrere dal 10 gennaio 2017, il criterio Escherichia coli per i prodotti immessi sul mercato a quanto definito nel Codex Alimentarius. Tale approccio consiste in un piano di campionamento a tre classi (n=5, c=1, m=230 e M=700 E. coli MPN/100 g), in luogo del precedente piano a due classi (n=1, c=0, M=230 E. coli MPN/100 g). Scopo del presente lavoro è stato la valutazione dell'innpatto di tale nnodifica sulle attivita di controllo dei nnolluschi prodotti nella Regione Emilia Romagna nnediante la revisione critica dei risultati acquisiti nelle deternninazioni di E. coli nel corso degli ultimi 4 anni.

The Regulation (EU) 2015/2285 has amended the Reg. 854/2004 with regard to the sampling criteria. According to that, the enumeration of E. coli must be performed on five sample units, in agreement with what set in Codex Alimentarius. Molluscs are suitable for human consumption if all the five values observed are 230 MPN/100 g of flesh and intravalvular liquid or if one of the five values observed is between 230 and 700 MPN/100 g of flesh and intravalvular liquid. Our data show that, in Emilia Romagna, the prevalence of noncompliant bivalve molluscs samples did not varied significantly with the introduction of Reg. 2015/2285. However, the revision of legislation reduced the risk of marketing bivalves characterized by higher contamination levels, therefore guarantying a higher level of protection of consumer health.

Rubini° S, Cozzi L, Losardo M, Berardelli C, Losio° MN, Pavoni° E, Bertasi° B, Suffredini E

Rilevazione e caratterizzazione molecolare e sierologica di Vibrio parahaemolyticus in molluschi bivalvi del Nord Adriatico (2011-2016)

Vibrio parahaemolyticus e un microrganismo autoctono dell'ambiente marino, occasionalnnente responsabile di gastroenterite nell'uomo come conseguenza del consumo di prodotti ittici crudi o poco cotti o in relazione ad eventi di cross-contaminazione di prodotti cotti. Diversi fattori di patogenicita sono stati descritti in V. parahaemolyticus ed in particolare due emolisine (TDH e TRH, codificate rispettivamente dai geni tdh e trh) sono state associate all'instaurarsi di sintonnatologia gastroenterica nell'uomo. Più recentemente, è stata sottolineata la rilevanza del sistenni di secrezione di tipo III (T3SS) al fini della patogenicita. Scopo del presente lavoro è stata la valutazione della prevalenza di V. parahaemolyticus e la caratterizzazione della sua potenziale patogenicita, in nnolluschi bivalvi raccolti dal Nord del Mare Adriatico nel periodo compreso fra il 2011 e il 2016.

Vibrio parahaemolyticus is a microorganism occasionally responsible for gastroenteritis associated to seafood products. Several pathogenicity characters (tdh, trh, T3SS) have been described in V. parahaemolyticus. Aim of this study was to assess the prevalence of V. parahaemolyticus in bivalves from the Northern Adriatic Sea and to characterize the distribution of pathogenicity markers in the collected isolates. A total of 1262 bivalve samples were collected over 6 years (2011-2016). V. parahaemolyticus was detected in 33.4% of the samples and 272 isolates were characterized. The gene tdh was never detected, while 4.0% of the isolates carried the trh gene (trh2 variant) and 16 isolates (5.9%) carried Type III Secretion Systems. The 16 potentially pathogenic isolates showed diversity of serotypes and Sequence Types and were mostly isolated during spring and summer of the different years.

Rubini° S, Galletti° G, Barbieri S, Menotta° S, Montanari° S, Merialdi° G, Govoni G, Boschetti L, Pompei M, Milandri A

Monitoring programme of Shellfish in North Adriatic Sea (Italy) : old and new marine biotoxins

European Public Health Conference   (11th   : Ljubljana, Slovenia   : 28 November - 1 December 2018)

The Shellfish Monitoring Programme (SMP) in Emilia Romagna (Italy) has been carried out according to European Legislation (EL) since 1997. The area considered in the present study is situated in the North-Western Adriatic Sea, between Po river delta and Reno river estuary. Biotoxins included in the EL are: ASP (Amnesic Shellfish Poison), PSP (Paralytic Shellfish Poison), and Lipophilic toxins (including Diarrhetic Shellfish Poison). Methods: From 1997 to 2017, 7126 bivalve mollusks (4337 Mediterranean mussel; 2040 Manila clams; 592 oysters; 157 striped venus) were analyzed with the EU reference methods. The mouse bioassay was used for lipophilic toxins from 1997 to March 2012, and replaced by LC-MS/MS method from April 2012. The mouse bioassay, supported by the chemical method, has been used for PSP toxins determination and HPLC-UV for the ASP toxins. Results: The marine biotoxins found were mainly lipophilic (okaidates ans yessotoxins groups): 536 (7,52%) samples were positive, while 501 (7,03%) contain toxins but below the EL limit. ASP toxins have never been found and PSP toxins have been detected only in 2007 but lower than EL limit. Conclusions: The presence of biotoxins follows a constant seasonal trend in the 21 years of monitoring. In recent years some significant facts have been observed: (i) yessotoxins group in mussels stays longer than past years even though below the legal threshold; (ii) spirolides (toxic macrocyclic imines), never previously reported in the area, appeared in mussels in 2003, following significant blooming of Alexandrium ostenfeldii. In 2017, tetrodotoxins were found northward of the study area and the Adriatic sea currents direction could suggest that TTXs will soon reach the Po delta. To safeguard public health, it is crucial to implement the SMP for both mollusks and phytoplankton and to develop reliable, fast and economical methods of analysis. Key messages: The presence of lipophilic biotoxins is increasing in North Adriatic Sea. New marine biotoxins have been found in North Adriatic Sea.

Rubini° S, Galletti° G, D'Incau° M, Govoni G, Boschetti L, Berardelli C, Barbieri S, Merialdi° G, Formaglio A, Guidi E, Bergamini M, Piva S, Serraino A, Giacometti F

Occurrence of Salmonella enterica subsp. enterica in bivalve molluscs and associations with Escherichia coli in molluscs and faecal coliforms in seawater

The objectives of this study were to present data on the presence of Salmonella enterica subsp. enterica and on the enumeration of Escherichia coli and faecal coliforms respectively in different species of bivalve molluscs and seawater and to conduct a retrospective evaluation to assess the capacity of E. coli in molluscs and faecal coliforms and S. enterica subsp. enterica in sea and brackish water to predict the presence of S. enterica subsp. enterica in bivalve molluscs, and therefore, the risk of exposure for consumers. Data were collected from 4972 seawater samples and 5785 live bivalve molluscs samples (2877 Ruditapes philippinarum, 2177 Mytilus galloprovincialis, 256 Chamelae gallina and 475 C. gigas and O. edulis) collected in the molluscs production area of Ferrara, Northern Italy, from 1997 to 2015. An overall S. enterica subsp. enterica occurrence of 2.2% was reported in water and molluscs, with percentages varying depending on the type of sample and on the classification areas. All the 237 Salmonella strains were identified as genus Salmonella and a total of 53 different serovars were observed. Significant associations between the fecal indicators and presence of S. enterica subsp. enterica were observed both applying EU and USA criteria, but, it should be noted that the EU approach seems to be more stringent achieving the goal of identifying the most critical batches (94 out of the 100) whereas, following the USA approach, a not negligible and higher number of batches compliant for faecal coliforms but contaminated by S. enterica subsp. enterica has to be mentioned. In any case, the faecal indicators E. coli in molluscs and faecal coliforms in seawaters reflect only in part the presence of S. enterica subsp. enterica in

molluscs and the consequent potential risk for consumers. Microbiological evaluation of seawaters seems to have a minor impact into the prediction of S. enterica subsp. enterica presence in molluscs.

Rubini° S, Losardo M, De_Magistris I, Barbieri S, Bolognesi° E, Berardelli C, Losio° MN, Pavoni° E, Bertasi° B, Suffredini E

Occurrence and characterization of Vibrio parahaemolyticus in bivalves from the Adriatic Sea, Italy

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European Public Health Conference   (11th   : Ljubljana, Slovenia   : 28 November - 1 December 2018)

Sangiorgi° E,   Rovellini P, Piro R, Berneri° R, Miano B

Screening and confirmatory methods to assess palm oil addition in food

42nd International Symposium on Capillary Chromatography and 15th GCxGC Symposium : 13-18 May, 2018, Riva del Garda, Italy : abstract book / [s.l. : s.n., 2018]. - p 471 (P.15) [Nr. Estr. 7877]

International Symposium on Capillary Chromatography : 42nd GCxGC Symposium : 15th   : Riva del Garda, Italy   : 13-18 May, 2018)

Palm oil (PO) is the most widely consumed vegetable oil on the planet and the demand for PO has increased steeply in last time as substitute of animal fat and as an alternative to hydrogenated vegetable oils. Its rapid expansion threatens some of the planet's most important and sensitive habitats. Recently the problems concerning food safety have evidenced the dangerous content of 3-MCPD in it [1]. The consciousness of this facts has led the consumers to request food with no PO as ingredient. Starting from December 2014, all food contain palm oil sold in Europe must state it in the ingredient list [2]. Because of its low price and rheological and stability properties it is possible its use without a label declaration. To avoid and prevent this food fraud it is important to have reliable and easy analytical methods to verify this statement. This presentation investigates different methods to determine the presence of PO in food, from chromatographic separations to fast DART-MS approach. Different mixture of sunflower oil and rapeseed oil with PO were used to determine a LOD of PO addition. With DART-ion trap in MS-MS mode (PPP as precursor ion) it was possible to detect PO presence up to 2%. Using chemometric evaluation (Metaboanalyst programs) on DART-HRMS and the ratio PP0/000 data it was possible to define a threshold TAGs ratio for the presence of PO in the most of food products with more than 1% of PO. DART-MS could be considered screening methods. For the determination of tocopherols and tocotrienols an HPLC with fluorescence detector was used; for TAGs, DAGs and MAGs an HPLC coupled with RI detector was used. The tocopherols and tocotrienols analysis along with TAGs profile allowed to distinguish the PO presence in the samples at 1% and could act as confirmatory analysis.

Scaltriti° E, . Bolzoni° L, Vocale C, Morganti° M, De_Flaviis° M, Casadei° G, Re M, Pongolini° S

WGS-based retrospective analysis for surveillance of L. monocytogenes infections in Emilia-Romagna - Italy

ASM conference on rapid applied microbial next-generation sequencing and bioinformatic pipelines : September 23-26, 2018 Tysons, VA / [Washington : ASM, 2018]. - 1 p (Poster 43) [Nr. Estr. 8034]

ASM conference on rapid applied microbial next-generation sequencing and bioinformatic pipelines : Tysons, VA   : September 23-26, 2018)

Background: Whole Genome Sequencing (WGS) of clinical, food and environmental isolates is often performed for detecting and tracing of L. monocyto genes outbreaks. Nevertheless, due to the long incubation of listeriosis, most outbreak sources remain undetected with the consequence that clusters of genomically-similar isolates remain only presumptive outbreaks. In this condition of missing epidemiological confirmation, the identification of a cut-off of genomic similarity leading to a confident definition of the outbreak borders is crucial for the attribution of isolates to the outbreaks. In this retrospective study, we analyzed human isolates collected in Emilia-Romagna region of Italy from 2012 to 2017 to look for possible outbreaks. Methods: A total of 119 L. monocytogenes isolates belonging to serogroup IIa were subjected to WGS with 250x2bp paired-end runs on a Miseq Platform (Illumina). Genonnes were de-novo assembled with SPAdes and core-genome multi-locus sequence typing (cgMLST) was performed using the BigsDB allele scheme (Institut Pasteur). Genomic clusters were identified based on the number of pairwise allele differences between isolates according to the cut-off proposed by ECDC. For long-lasting clusters, phylogenetic analysis based on SNPs (CFSAN pipeline, US FDA) was performed through molecular clock models in a Bayesian framework (BEAST). Statistical analyses were performed to detect the presence of within-cluster evolutionary signals against date-randomized datasets. Results: The analysis performed with cgMLST highlighted the presence of 13 clusters, which included between 2 and 21 cases and lasted between 3 months and 5 years. Despite the relatively small time span between isolations, we found that the larger cluster detected showed enough evolutionary signal to allow phylogenetic inference. We estimated a very small effective population size confirming the clonal origin of the infection. We also found that including late isolates (excluded from the cluster based on allele differences) did not change the size of the population, suggesting a common origin. The presence within the cluster of isolates with identical sequences did not allow defining a fully resolved tree. However, by comparing the trees produced by the Bayesian simulations with date-randomized trees, we found a high level of population drift (represented by tree imbalance), suggesting that the source of infection is under high selective pressure. Conclusions: Our study suggests that allele thresholds should not be used as the only parameter to define L. monocytogenes clusters. It is critical to complement allele counts with clustering generated by phylogenetically meaningful algorithms. This approach can help retrospectively evaluate cluster membership (in particular with regard to late isolates of an outbreak) and identify persistent strains in food facilities.

Scaltriti° E, Bolzoni° L, Vocale C, Morganti° M, De_Flaviis° M, Casadei° G, Re MC, Pongolini° S

WGS-based retrospective analysis for surveillance of L. monocytogenes infections in Emilia-Romagna (Italy)

BACKGROUND. Whole Genome Sequencing (WGS) of clinical, food and environmental isolates is often performed for detecting and tracing of L. monocytogenes outbreaks. Nevertheless, due to the long incubation of listeriosis, most outbreak sources remain undetected with the consequence that clusters of genomically-similar isolates remain only presumptive outbreaks. In this condition of missing epidemiological confirmation, the identification of a cut-off of genomic similarity leading to a confident definition of the outbreak borders is crucial for the attribution of isolates to the outbreaks. In this retrospective study, we analyzed human isolates collected in Emilia-Romagna region of Italy From 2012 to 2017 to look for possible outbreaks. METHODS. A total of 119 L. monocytogenes isolates belonging to serogroup IIa were subjected to WGS with 250x2bp paired-end runs on a Miseq Platform. Genomes were de-novo assembled and cgMLST was performed using the BigsDB allele scheme. Genomic clusters were identified according to the cutoff proposed by ECDC. For long-lasting clusters, phylogenetic analysis based on SNPs was performed through molecular clock models in BEAST. Statistical analyses were performed to detect the presence of within-cluster evolutionary signals against date-randomized datasets. RESULTS. The analysis performed with

cgMLST highlighted the presence of 13 clusters, which included between 2 and 21 cases and lasted between 3 months and 5 years. We found that the larger cluster detected showed enough evolutionary signal to allow phylogenetic inference. We estimated a very small effective population size confirming the clonal origin of the infection. We also Found that including late isolates did not change the size of the population, suggesting a common origin. The presence within the cluster of isolates with identical sequences did not allow defining a fully resolved tree. By comparing the trees produced by the Bayesian simulations with date-randomized trees, we found a high level of population drift, suggesting that the source of infection is under high selective pressure. CONCLUSIONS. Our study suggests that allele thresholds should not be used as the only parameter to define L. monocytogenes clusters. It is critical to complement allele counts with clustering generated by phylogenetically meaningful algorithms. This approach can help retrospectively evaluate cluster membership and identify persistent strains in food facilities.

Schiavo MR, Vodret B, Altissimi S, Bogdanova T, Defilippo° F, Fraulo P, Gallina S, Montarsi F, Pagani M, Somenzi C, Tolone M, Tilocca MG, Zimmardi A, Bonilauri° P, Haouet MN

Studio interlaboratorio per la ricerca di impurità solide in alimenti mediante il filth test nei prodotti cerealicoli

The study was aimed to the characterization of the Italian official method for the detection of solid foreign bodies (filth test) in cereals by means of proficiency testings. The study was performed by ten laboratories on wheat flour samples contaminated with insect fragments. The overall results (sensibility 100%, specificity 95%, accuracy 98%, false positives 5%, LOD = 1) show that the method carried out by the working group laboratories is high performing.

Spelta° C, Bianchini° V, Zugnoni° A, Romanò° A, Maisano° A, Ardissino G, Tel F, Bono P, Colombo R, Panteghini° C, Bertasi° B, Morabito S, Scavia G, Luini° M

Characterization of Shiga-toxin-producing Escherichia coli (STEC) from dairy came of Northern Italy : an integrated veterinary-medical activity

Cattle are considered the main reservoir of STEC, but the assessment of the virulence profile (eae, stx1/2, stx subtype and accessory virulence factors) of isolates is crucial to define STEC pathogenic potential. In the framework of an integrated veterinary-medical collaboration, a survey was conducted in cattle with the aim to compare the molecular profiles of animal and human STECs. An active survey was conducted between July and October 2016 in 62 intensive cattle farms in Lombardy and additional samples were collected from calves passively referred for routine diagnosis. Feces were enriched in 0.1% peptone water (PW). STEC isolation was attempted from Real-Time PCR stx-positive samples by analyzing 50 typical colonies. Stx positive colonies were analyzed with multiplex PCR for 13 serogroups more frequently associated with human infection, for stx subtypes and for the virulence genes subAB, saa, tia, ckf, adf0, hiyA, efall-lifA5'3' and toxB. A total of 270 strains were obtained from the active survey (N=157) and from the routine diagnostic activity (N=113). Most of the strains were eae-negative (N=150) and out of the 120 eae-positive STEC, the strains carrying the stx1, stx2, or both ski -stx2 were 82, 26 and 12 respectively. The 38

stx2-eae positive strains were mostly represented (N=26) by serogroups unrelated with the 13 investigated (Ond), while only 12 strains were recognized as top five STEC: 0157 (N=7), 0111 (N=2), 0113 (N=2) and 0145 (N=1). Overall, these results are in line with the ones reported at European level in the joint EFSA/ECDC report 2017. Stx genes subtyping revealed the presence of stxla in 105 strains (97.2%), and stxl d in just 3 strains. A greater variability in the stx2 was observed with 2a and 2c detected in more than 70% of the stx2 isolates. A significant correlation between the presence of the eae gene and the positivity for at least one of the virulence genes adf0, ckf, efal¬lifA 5'-3 'and toxB was observed. Overall, 187 strains were genoiyped by PFGE and a wide genetic variability was found. The strains from the same farm tended to fall in the same cluster, with general evidence of a single profile circulating in an entire herd or in a single group (calves, heifers, cows). A few STEC showing the same pulsoiype were observed in more than one farm. No common PFGE profiles were observed between the isolates from cattle and 74 clittlical isolates obtained in a parallel monitoring of STEC infections in human operated in the same region by the veterinary-medical collaborating group. Our study revealed a high genetic variability of STEC in the cattle population with some strains harboring the typical virulence asset of STEC causing illness to humans. This findings strenghten the importance of maintaining an integrated multi-disciplinary approach to STEC surveillance.

Spelta° C, Bianchini° V, Zugnoni° A, Romanò° A, Maisano° AM, Gazzola° A, Ardissino G, Tel F, Dodaro A, Vignati C, Panteghini° C, Bertasi° B, Morabito S, Scavia G, Luini° MV

Caratteri di virulenza di Escherichia coli produttori di Shiga-tossine (STEC) isolati in allevamenti bovini del Nord Italia

A total of 270 bovine STEC strains, isolated from an active survey and from the diagnostic activity, were characterized. The great majority of the strains were eae- (n=150) or eae+ stx1 (n=43) belonging to non-Top5 serogroups. Only 12 out of 38 eae+ stx2 strains were recognized as Top5: O157 (n=7), O111 (n=2), O113 (n=2) and O145 (n=1). The stx1a subtype was found in all strains except one, while the subtype stx2a was prevalent (56%), followed by 2c, 2d, 2g and 2b. Eight additional virulence factors were investigated and a good relation between eae gene and adfO, ckf, efa1-lifA 5'-3 'and toxB was found. The PFGE profiles showed that strains from the same farm tended to fall in the same cluster, with general evidence of a single profile circulating in a group. Not only the serogroup, but also the virulence profile (eae, the type and subtype of stx and the accessory virulence factors) should be taken into account in view of considering STECs as pathogens for humans.

Taddei° R, Bardasi° L, Fontana° MC, Mengoli° M, Bassi° P, Merialdi° G

Ricerca di Salmonella spp in campioni di spezie mediante metodo ISO 6579-1:2017 e valutazione dei protocolli di preparazione del campione secondo norma ISO 6887-4:2017

Salmonella outbreaks are increasingly associated with the consumption of contaminated spices. Compounds with antimicrobial effect present in spices can interfere with pathogenic bacteria

detection, increasing the risk of false negative results. Salmonella detection was carried out in 46 spice samples: oregano (3), black pepper in granules (9), minced black pepper (7), chilli pepper (7), flaked garlic (9), dry garlic (2), rosemary (2), curry (3), turmeric (1), cinnamon (1), minced cinnamon (2). ISO 6579-1: 2017 method was performed, validating each result by analyzing the sample artificially contaminated with S. Agbeni. In case of inhibitory effect of the matrix, the sample preparation protocols were modified by adding K2SO3 or by increasing the ratio of the initial dilution, according to ISO 6887-4: 2017. One sample of pepper in granules resulted positive for the presence of Salmonella. Oregano, minced cinnamon and garlic showed inhibitory effect on Salmonella multiplication..

Varisco° G, Finazzi° G, Daminelli° P, Bolzoni° G, Losio° MN

Quantitative assessment of exposure to milk pathogens

Direct raw milk selling has been allowed in Italy since 2004. The presence of pathogenic bacteria in raw milk has been well documented both in Europe and in USA, and the isolation rate varies considerably from study to study. The main objective of this study was to estimate the risk of different pathogens, such as Salmonella, Listeria monocyto genes, Campylobacterjejuni, E. coli VTEC due to consumption of raw cow's milk purchased from vending machines located in different Italian regions. The development and implementation of different quantitative risk assessment (QRA) models is one means to ensure food safety control. The QRA considered the presence of these pathogens in dairy farms, the field handling conditions of raw milk during distribution and delivery to the consumer, consumer habits and the behaviour of pathogens throughout the food chain. Data were collected from a previous survey that gathered together microbiological records of official controls monitoring raw milk samples from self service vending machine in different regions of Italy performed by the regional veterinary authorities from 2008 to 2011 One hundred seventy-eight of 60,907 samples were positive for one of the four foodborne pathogens investigated: 18 samples were positive for Salmonella, 83 for L. monocytogenes, 24 for E. coil 0157:H7, and 53 for C. jejuni in the seven regions investigated. No significant differences in prevalence were found among regions, but a significant increase in C. jejuni prevalence was observed over the years of the study. Data on consumer habits revealed that some behaviors may enhance the risk of infection linked to raw milk consumption: 37% of consumers did not boil milk before consumption, 93% never used an insulated bag to transport raw milk home, and raw milk was consumed by children younger than 5 years of age The RA models predicted no human listeriosis cases per year either in the best or worst storage conditions and with or without boiling raw milk, whereas the annual estimated cases of salmonellosis depend on the dose-response relationships used in the model, the milk storage conditions, and consumer behavior in relation to boiling raw milk or not. Considering from 10,000 up to 20,000 consumers each year, the models predicted for the best and worst storage conditions (4°C and 12° C), respectively, 2.12 and 1.14 campylobacteriosis cases and 0.02 and 0.09 HUS cases in the 0- to 5-year age group and 0.1 and 0.5 HUS cases in the over 5-year age group Quantitative risk assessment related to raw milk consumption is necessary from a public health perspective and the proposed RA model represents a useful and flexible tool to perform future RAs based on local consumer habits to support decision-making on safety policies. Further educational programmes for raw milk consumers or potential raw milk consumers are required to encourage consumers to boil milk to reduce the associated risk of illness.

Zanardi° G, Losio° MN, Cosciani_Cunico° E, Dalzini° E, Daminelli° P

Effetto delle popolazioni lattiche sulla crescita di Listeria monocytogenes in latte crudo d'asina e di bovino

Donkey's milk is an important food from a medical and nutritional point of view. The study aims to investigate the microbial quality of raw donkey milk and to evaluate the behavior of L. monocytogenes during the milk storage at 8°C. The physical, chemical and microbiological properties of donkey and caw milk were significantly different, and that influence the behavior of foodborne pathogens such as L. monocytogenes. The results that have emerged to this preliminary study highlight the need to deepen the safety properties about raw donkey milk, even considering its increasing demand by consumers.