Wrap-up of the workshop "The milk day"

Trento, 4th February 2015

1. Program

14.00- Registration and welcome

14.10- ***Andrea Adami***, FBK-Technical manager of the SYMPHONY project:

Introduction and presentation of SYMPHONY project

14.30- ***Ulrich Leist***, DRRR, Germany:

Milk quality testing and regulations

14.50- ***Alberto Mattivi***, Prevention Dep. Local Health Trust, Trento, Italy:

Milk and health in the Province of Trento

15.10- ***Nicola Cologna***, CONCAST, Trento:

Milk quality testing in Trentino

15.30- ***Veronica Lattanzio*** ISPA-CNR, Italy:

Rapid Detection of Aflatoxin M1 in Milk: Analytical Challenges and Validation Aspects under EC Perspective

15.50- ***Mark Whatton***, QCL, United Kingdom:

Research & Business - Transitioning Innovation to Commercial Success

16.40- ***Andrea Adami***, FBK-Technical manager of the SYMPHONY project:

MST for food quality and safety

17.00 -Discussion

18.00- Conclusion

2. Workshop introduction and presentation of the SYMPHONY project

Dr. Adami briefly introduces the Symphony project concepts and workshop theme. The workshop, organized in the framework of the SYMPHONY project, aims to discuss relevant open issues in the application of MST in the analysis of milk contaminations.

In dairy industry, one of most pressing unmet needs is the timely detection of contaminants presence in milk, which represent a hazard for human health and an economic loss for the dairy industry. The available technology for aflatoxin detection is laboratory-based, in most cases requires sample preparation and does not provide timely identification of contaminants, thus fails to deliver cost-effective management of milk quality.

The workshop aims at answering the following questions:

- ✓ Can the use of Micro System Technologies and biosensors provide a solution to overcome these limitations?
- ✓ What are the major factors that could jeopardize the integration and automation of current analysis techniques?

Milk chemistry and composition, strict regulations for milk quality, dairy industry requirements, the large diffusion of milk consumers and producers pose substantial challenges, but, in the meanwhile, offer a number of opportunities for exploiting the huge potential of miniaturized systems and sensors.

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 610580

3. Summary of presentations

3.1 Milk quality testing and regulations

Dr. Leist provides an overview of DRRR activities as an accredited body for proficiency testing services. DRRR is a service company for external quality assurance of laboratories of food economy and an accredited inspection body according to DIN EN ISO/IEC 17020: 2004. DRRR offers reference materials, proficiency testing, advising on quality matters and quality assurance training. DRRR's German and international customers are companies of the food and dairy economy and state laboratories as well as food and veterinary inspection services. DRRR provides advice to our customers in all questions of validation of chemical, physical and sensory analysis or statistical problems. DRRR assists customers to build quality management systems according to several international standard like ISO 9001, IFS, DIN EN ISO 17025, DRRR also checks if quality management systems are fit for audits and so on. Furthermore, DRRR is represented in various national and international committees like FIL/IDF, Eurolab and DGQ.

Milk testing procedures in Germany is reviewed in the presentation to highlight the issues and common practices. Milk is principally tested at food producers for two purposes:

1 *testing for milk payment*, where main quality parameters are:

- Fat
- Protein
- Freezing point
- Somatic cell count below 400.000
- Total count of bacteria below 100.000

This is typically performed off-line in large laboratories with very large number of test per day (25000 sample/day) with high throughput instruments like IR spectroscopy

2 *testing for chemical residues, pathogens and contaminants for safety*. Since food manufacturer are responsible for their products, they are in charge for safety enforcement; tests are performed for legal limits of:

- Chloramphenicol
- Mycotoxins
- Aflatoxin M1
- Trichlormethan
- Radionuclides
- Residues
- others

While reference lab only apply reference methods, since testing campaigns are limited and time is not a constraint, reference tests cannot be performed on all samples at the manufacturer site due to the strain on quality lab. It is therefore possible to accredit screening test to have a fast feedback on all samples (or a larger number) if properly calibrated. For this purpose, calibration solutions are required, which is an art by itself since the challenge for test kit producers is to assure sufficient selectivity, sensitivity, accuracy.

Until now, most rapid test kits are applied as a pre-screening. Many kits only test for present-absent while to develop a quantitative test kit is a far bigger challenge.

In addition, food and health authorities test especially on final products for control of food safety.

3.2 Milk and health in the province of Trento

Dr. Mattivi presents and discuss the official controls carried out by the local competent authority about milk safety in application of sector regulations and the results of an interesting research / study on the sanitary aspects of milk processing in the hut.

Statistics of dairies in Trentino region highlight the particular production structure in the region, where farms are very small and mountains huts are used as feeding grounds in the summer. Primary production in Trentino region is the high quality traditional cheeses, which represent an interesting point for safety enforcing with respect to large companies found in Europe and other Italian regions.

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Dr. Mattivi presents the statistics and modalities of official tests in dairy field in Trentino, where the official test by audits and inspections on all production aspects (veterinary, product safety, HACCP, etc.) are performed according to both regulations and internal rules agreed by producers and competent authority, which are often more restrictive than general rules. For instance screening test with Elisa for aflatoxin are required to be confirmed by test on single producers by Elisa and eventually by reference HPLC testing if a value of aflatoxin higher than 35ng/kg is found.

The use of self-control procedures and tests need to be accredited by the Italian National Accreditation Body ACCREDIA (for Italy: http://www.accredia.it/) that performs accreditation with authority derived from the State. The ACCREDIA mission is full compliance of the national accreditation system with the regulation of the European Parliament and Commission n° 765 of July 9, 2008, which is applicable from January 1, 2010, for accreditation and market compliance in all EU countries. This also applies to any new device brought to production lines.

In the Department experience, the majority of non-conformances are related to non-observance of internal rules of the production plant. Interestingly, there may be local issues with particular, new contaminants as in a recent case in Trentino region related to steel manufacturing plant, which resulted in local contamination with dioxins and an intense testing campaign to identify possible contaminated products before they reached the market. This would open the field for new screening test if technically available. The cost per test to identify dioxins is quite high (1300-1400€/sample for reference test, 500-600€/test for screening) with reduced number of molecules identified. Other similar examples of specific needs may be found.

3.3 Milk quality testing in Trentino

Dr. Cologna discuss in his talk the CONCAST structure, including more than 800 associated farmers, 17 dairies and the centralized testing lab. More in detail, Dr. Cologna presented the tests performed at Concast laboratory, which, in addition to common milk quality parameters like fat and protein content, antibiotics, etc. also measures technical properties of milk related to cheese making (e.g. milk coagulation properties, whey starter analysis, presence of spores, particularly important to detect in hard cheese making). Aflatoxin case is discussed and total loss related to the issue in 2013 was about 20k€. A specific case of introduction of a new accredited test for quality is presented, where FTMIR was correlated to milk coagulation properties in order to process the test with fast high-throughput test with respect to traditional method, which required 30 minutes to run. This study was conducted in collaboration with the Department of Agronomy Food Natural resources Animals Environment of the University of Padova (Italy).

3.4 Rapid Detection of Aflatoxin M1 in Milk: Analytical Challenges and Validation Aspects under EC Perspective

Dr. Lattanzio presents the work of ISPA institute on rapid detection of aflatoxin M1 in milk, and an overview of analytical challenges and validation aspects.

Rapid test methods for measuring Aflatoxin M1 (AFM1) in milk are available either as commercial kits or as research methods. Enzyme-linked immunosorbent assays (ELISA), lateral flow tests, immunoaffinity columns coupled with fluorimetric assay are common formats in the current market. The main purpose of screening methods is to detect the presence of a contaminant at level of interest allowing rapid decision making, i.e. in 5 to 20 minutes with respect to about 4 hours/samples in reference methods. An overview of available rapid methods and achievable performances is discussed.

European Union has set a maximum permitted limit of 0.050 µg/kg for AFM1 in milk. Besides the high sensitivity required for AFM1 detection in milk, the major analytical challenge when developing screening tests is to make them reliable and robust, in particular by reducing the false negatives, for laboratory and in-field use. This means to cope with differences from matrix to matrix, environmental conditions, operator skills, lot-to-lot reproducibility. Recent efforts of

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the European Union, for establishing practical guidelines for the generation of fit-for-purposes performance parameters for screening methods for mycotoxins in foods, resulted in the Regulation 519/2014/EC. The EC validation scheme and its practical application to evaluate performances of a commercial kit for AFM1 detection in milk were presented and discussed. A case study related to Fusarium mycotoxins in cereal was presented to illustrate the guidelines for the validation procedure of a rapid screening testing, where tests were performed on different days, on different production lot and milk origins, where spiking was used at different levels to define the system precision and real milk sample to define the accuracy. It is common experience that fat removal can change the calibration curve of a method and sample treatment/clean-up is needed in order to improve the analytical accuracy of the system. Dilution is already a commonly used sample preparation method.

3.5 Research & Business: Transitioning Innovation to Commercial Success

Dr. Whatton discussed in his presentation an opinion on why Micro Systems Technologies, while promising exciting and novel ways to monitor the quality of food and beverages, are difficult to translate in commercial reality.

There are many reasons why promising systems fail to reach the market, but is it possible to understand and mitigate these risks early on? The presentation described the target needs and wants required to hit the market, as evaluated in the development of the Symphony project. Starting from long standing experience of QCL in dairy sector, a view on how research and innovation projects can tackle these challenges and experiences are presented.

One of key points to address is innovation aims need to work with commercialisation aims, since commercial successes are those that provide clear benefits to those who will use them. These benefits can only be known by understanding the target market. Selection of technology and design should target specific requirements from end-users. For aflatoxin testing in milk at dairies, the Symphony project defined the requirements to be achieved, reported in Table 1.

A second key point for successful translation into commercial success is the development phase from a proof-of-concept into a pre-production prototype. An industrial partner focusing on instrument pre-production prototype will understand and work to maximize the major attraction of the innovation, and bring it to final users in such a way it is clearly perceived.

Table 1: User requirements for Aflatoxin detection in milk at dairies

ACCURACY	Test accurately at the legal limits of 25ppt and 50ppt +/-10ppt with a limit of detection of 5-10ppt
EASE OF USE	Analyser system must be very easy to use, ideally with minimal training to operate and maintain. Any sample preparation must be very simple, such as adding reagent X to reagent Y, or avoided altogether.
ROBUST	Robustness was rated as important.
COST PER TEST	The cost per test indicated for dairies/processors was €1-5
TEST TIME	Test time is most important for dairy reception/intake laboratories; Tankers need to be cleared within 5-10 minutes. In other cases test times of up to 20 minutes were acceptable.
ANALYSER COST	Indications of analyser costs varied widely from less than €1000 to over €10,000
SOFTWARE	Must be easy to use and notify the user of any steps they must take
SAMPLE VOLUME	Not too important, typically 100ml would be acceptable.
ANALYSER SIZE	Not considered important but ideals defined

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3.6 MST for food quality and safety

Dr. Adami presents a critical review of advantages of microfluidic devices in food analysis. Among different peculiarities of microfluidics, the major points of interest are mainly the integration of multiple functions in an integrated system, the faster mass and heat transfer in small channels, the possibility to create a laminar flow, multiple parallel testing and high surface interaction. On the other hand, small samples typically used in microfluidics are not a key issue in food analysis, since sampling large volumes are often required for sample representativeness and typically low cost of the sample. Microfluidic devices for food analysis then pose particular challenges for effective analysis. A review of major techniques, concepts and devices of interest in food analysis is presented, including capillary electrophoresis, micro PCR, immunoassay, particle and drop separation and multiplexing techniques.

For contacts:

- **SYMPHONY Project: Integrated SYsteM based on PHOtonic Microresonators and Microfluidic Components for rapid detectioN of toxins in milk and dairY products**

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