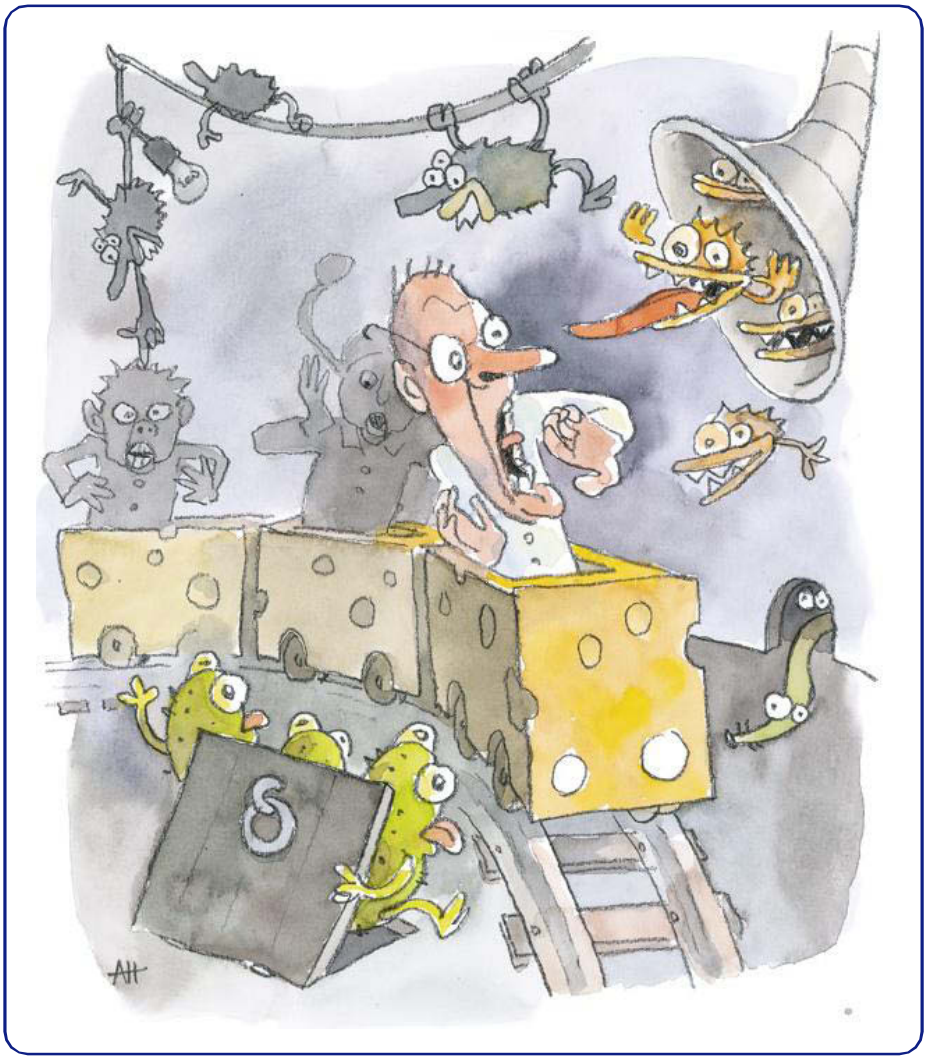


RISK ASSESSMENT OF MICROBIAL PROBLEMS AND PREVENTIVE ACTIONS IN FOOD INDUSTRY

2nd Open Seminar arranged by SAFOODNET – Food Safety and Hygiene Networking within New Member States and Associated Candidate Countries

VTT SYMPOSIUM 251

Keywords: risk assessment, food processing, good hygiene practices, hygienic design, contamination routes, pathogens, microbial contaminants, biofilm, cleaning, disinfection, antimicrobial effects

RISK ASSESSMENT OF MICROBIAL PROBLEMS AND PREVENTIVE ACTIONS IN FOOD INDUSTRY

2ND OPEN SEMINAR ARRANGED BY SAFOODNET – FOOD SAFETY AND HYGIENE NETWORKING WITHIN NEW MEMBER STATES AND ASSOCIATED CANDIDATE COUNTRIES; FP6-022808-2006

ISTANBUL, TURKEY, OCTOBER 22–23, 2007

EDITED BY
GUN WIRTANEN & SATU SALO
VTT TECHNICAL RESEARCH CENTRE OF FINLAND

ISBN 978-951-38-6325-8 (soft back ed.)
ISSN 0357-9387 (soft back ed.)

ISBN 978-951-38-6326-5 (URL: http://www.vtt.fi/publications/index.jsp)
ISSN 1455-0873 (URL: http://www.vtt.fi/publications/index.jsp)

JULKAISIJA – UTGIVARE – PUBLISHER

VTT, Vuorimiehentie 3, PL 1000, 02044 VTT
puh. vaihde 020 722 111, faksi 020 722 4374

VTT, Bergsmansvägen 3, PB 1000, 02044 VTT
tel. växel 020 722 111, fax 020 722 4374

VTT Technical Research Centre of Finland
Vuorimiehentie 3, P.O. Box 1000, FI-02044 VTT, Finland
phone internat. +358 20 722 111, fax +358 20 722 4374

VTT, Tietotie 2, PL 1000, 02044 VTT
puh. vaihde 020 722 111, faksi 020 722 7071

VTT, Datavägen 2, PB 1000, 02044 VTT
tel. växel 020 722 111, fax 020 722 7071

VTT Technical Research Centre of Finland, Tietotie 2, P.O. Box 1000, FI-02044 VTT, Finland
phone internat. +358 20 722 111, fax +358 20 722 7071

Cover picture Antti Huovinen

Edita Prima Oy, Helsinki 2008

PREFACE

Food Safety and Hygiene Networking within New EU Member States and Associated Candidate Countries (SAFOODNET FP6-022808) is a specific support action EU-project building-up a sustainable network in food safety. It aims at knowledge sharing to prevent risks related to microbial hazards, to find future RTD needs and apply for RTD funding in food processing and packaging safety. The action focuses towards Czech Republic, Denmark, Estonia, Finland, Hungary, Latvia, Slovenia and Turkey in the pilot actions, seminars, and workshops on process hygiene and end product safety. Interested researchers and SME representatives from other new EU countries and ACCs are encouraged to participate in the activities. The objectives of SAFOODNET are to: 1) disseminate knowledge from national and international food safety projects in open seminars, workshops, practical exercises, RTD activities and pilot actions resulting in new research projects for food industry especially SMEs; 2) establish an expert group (EG) in which authorities, scientists, industrial representatives build-up or strengthen existing networks and identify specific needs for future RTD activities in food safety and 3) bridge networks within the new EU, fostering scientific co-operation and knowledge transfer in food safety.

The 2nd SAFOODNET seminar was arranged to a targeted audience of young scientists and junior employees in food processing industry, food hygiene and safety research and authority. The title of the seminar was "Risk assessment of microbiological problems and preventive actions in food industry" and it was dealing with microbial risk assessment, Hazard Analysis Critical Control Point (HACCP), Good Manufacturing Practises (GMP) and Good Hygiene Practices (GHP), which all are primary tools in controlling microbial hazards in the food processing. The microbial control procedures in a food processing line are set to prevent both the entrance and the establishment of harmful microbes into the process and to limit the microbes by controlling deposit formation on surfaces and activity in products. Identification of definitive critical control points, however, is difficult and the implementation of effective HACCP programs can be complex. The HACCP programmes have been implemented in food processing and packaging for years and recently also in lubricant industry. Thus there is significant amount of experience that can be distributed to the audience coming mainly from food research organisations and enterprises in the new EU.

The forthcoming activities in SAFOODNET-project are an open seminar on risk management and two workshops incl. practical exercises in microbial risk assessment and management to disseminate methodologies and methods; networking through project web-site (http://safoodnet.vtt.fi); establishment of an expert group to suggest and evaluate pilot actions for future RTD needs in food safety; performance of food safety pilot actions in food industry especially in SMEs and building-up a new channel for co-operation within the new EU.

CONTENTS

LECTURES

BIOFILM PROBLEMS OF PATHOGENS IN FOOD PROCESSING LINES

Gun Wirtanen
VTT Technical Research Centre of Finland, Espoo, Finland

According to the literature biofilm problems caused by both pathogens and food spoilage microbes have been found e.g. in air handling systems, in cooling systems, in milk transfer lines, on conveyors, in packaging machines, on vegetable processing surfaces, on heat exchanger surfaces, on ultrafiltration and reverse osmosis membranes, in blancher extractors, on mixers, on slicers, on gaskets, on floors and in drains. We can see from the list that problems generating from biofilm can occur anywhere in the food process if the design and maintenance is improper. Therefore, equipment design plays the most important role in combating biofilm formations. Dead ends, corners, cracks, crevices, gaskets, valves and joints are vulnerable points for biofilm accumulation. Poorly designed sampling valves can destroy an entire process or give rise to incorrect information due to biofilm effects at measuring points. The choice of materials and their surface treatments, e.g. grinding and polishing, are important factors in inhibiting the formation of biofilm and in promoting the cleanability of surfaces. The surface structure of stainless steel is very important in avoiding biofilm formation; it has been reported that although the grain boundaries of AISI 316L stainless steel constitute 3–20% of the total surface area, over 90% of the adherent bacteria were found attached to the grain boundaries.

The equipment surfaces in the food industry provide the microbes growing in biofilms with liquids and excess of nutrients. This type of phenomena should be combated using efficient cleaning procedures to improve process hygiene. Biofilm formation in these systems is a symptom of disturbance in the process. Biofilms are less likely to accumulate in well-designed systems which are effectively cleaned. Biofilm formation causes problems in many areas such as industrial water systems, medicine and in the food processing industry. Biofilm can generally be produced by any microbes under suitable conditions, although some microbes naturally have a higher tendency to produce biofilm than others. The

autoclaving time required for successful sterilization to several hours due to the bacterial slime of a *Bacillus* strain, which improved the heat resistance of the bacterium. The slime-forming microbial flora involved in biofilm formation in manufacturing of packaging material can be divided into primary, e.g. bacteria of the *Bacillus*, *Pseudomonas* and *Enterobacter* strains and fungi of the *Aspergillus*, *Mucor* and *Penicillium* strains, and secondary slime-formers, e.g. bacteria of the *Alcaligenes, Flavobacterium, Klebsiella, Micrococcus* and *Staphylococcus* strains and fungi of the *Paecilomyces* and *Tricoderma* strains. Other contaminants on food contact surfaces are enterobacteria, lactic acid bacteria, micrococci, thermophilic streptococci and *P. fragi*.

Common foodborne pathogens e.g. *Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Mycobacterium paratuberculosis, Legionella pneumophila Clostridium perfringens, Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Campylobacter jejuni,* and *Yersinia enterocolitica* have readily been found to produce biofilms on surfaces. From a hygienic and health point of view infection and disease problems with e.g. *L. pneumophila* biofilms can occur in hot water systems. *Salmonella* spp. form biofilms on food contact surfaces in which the cells are much more resistant to sanitisers than the planktonic cells. Acid-adapted *E. coli* O157:H7 has shown enhanced survival and prevalence in biofilms on stainless steel surfaces. Campylobacter is able to form a biofilm on stainless steel and glass in only 2 days and the determinations have shown that *C. jejuni* cells form a viable but nonculturable state within the biofilm. Food residues or moisture improve the chances of survival by campylobacter on surfaces. Experiments have been carried out with *L. monocytogenes*, *P. fluorescens* and *Y. enterocolitica*, which cause contamination of materials used in gaskets. The lubricants used in conveyors are a problem, especially in dairies and breweries. *L. monocytogenes*, an opportunistic pathogen, has been isolated from a variety of food-processing and can persist in the equipment for years. It has been isolated from lubricants in dairies. *L. monocytogenes* has been found to form biofilms on common food contact surfaces e.g. plastic, polypropylene, rubber, stainless steel and glass. Sources of *Listeria* in food plants are conveyor belts, cutters, slicers, coolers, freezers, brining and packaging machines and drains. It can also survive in lubricants especially when the lubricants are contaminated with organic material and water. *L. monocytogenes* has also been shown to survive e.g. in butter, which was the vehicle in a Finnish *Listeria* epidemic in 1998–1999.

In the food industry yeasts are best known for their beneficial role in the production of fermented products and bread. However, an abundant growth of unwanted yeasts in processing can lead to problems in quality and safety with significant financial losses. Yeasts can proliferate in a greater selection of food than earlier thought including dairy products such as cheese, butter, cream and yoghurt; meat products such as sausages; sugar, syrups, honey, berries and fruit products, vegetables including pickled cabbage and pickled cucumbers; juices, soft drinks, alcoholic beverages and wines, salad dressings, mayonnaise, confectioneries, jams and jellies as well as bread. The most important intrinsic factors determining the susceptibility of a product to yeast spoilage are water activity, nutrients and acidity. The most common spoilage effects are off-flavour e.g. souring; gas production e.g. discolouration and swelling of containers; and textural changes e.g. sediment formation and surface growth. Yeasts belonging to *Saccharomyces*, *Candida* and *Rhodotorula* have been isolated from biofilms on conveyor tracks and can and bottle warmers in packaging departments of the beverage industry. Most publications dealing with food processing biofilms are concerned with bacterial biofilms. There are very few published studies concerning yeast biofilms in food processing. Generally, foods are not believed to transfer yeast infections although there are occasional reports of gastro-enteritis or allergic reactions in which yeasts were suspected to be the causative agent. At least 200 species of yeasts have been found in food. The following strains have been found to spoil food products *Brettanomyces bruxellensis, Candida krusei, C. parapsilosis, Debaryomyces hansenii, Kloeckera apiculata, Pichia membranaefaciens, Rhodotorula mucilaginosa, R. glutinis, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Torulopsis holmii, Zygosaccharomyces bailii, Z. bisporus* and *Z. rouxii*. Some spoilage species are also opportunistic pathogens that are able to cause various types of infections in susceptible people.

References to this extended abstract can be found in the publications:

Salo, S. & Wirtanen, G. 2005. Disinfectant efficacy of foodborne spoilage yeast strains. Transactions of the Institution of Chemical Engineers, Part C: Food and Bioproducts Processing, 83, C4:288–296.

Wirtanen, G. & Salo, S. 2005. Biofilm risks. In: Lelieveld, H., Mostert, T. & Holah, J. (Eds.). Handbook of hygiene control in the food industry. Cambridge: Woodhead Publishing Ltd. Pp. 46–68. ISBN 1-85573-957-7.

MODELLING AS A TOOL IN EVALUATING HYGIENIC DESIGN OF FOOD PROCESSING EQUIPMENT

Bo B. B. Jensen
BioCentrum-DTU, Kgs. Lyngby, Denmark

Modelling is a buzz word these days in science. A lot of modelling is done in almost any aspect of science increase the understanding of economics, processes and products and their interaction. One aspect of modelling is the prediction of conditions inside products, inside processing equipment and on surfaces of processing equipment. This can be utilised for evaluating the hygienic design of especially closed processing equipment. Making it possible to for equipment manufactures to evaluate hygienic design of processing equipment provides the opportunity to redesign in the early stages of designing with respect to hygienic design (e.g. cleanability). Earlier the first indications of the level of cleanability were not given until after cleaning trail testing on the prototype of a component. Modelling can be used to evaluate the hygienic design because the cleanability in closed processing equipment is carried out using Cleaning In Place (CIP) procedures and that sterilisation depends on the ability to reach a certain temperature for a given time on all surface to kill the micro-organisms.

Generally speaking CIP consist of pumping water or detergent through the process line. The bond between soil and product surface is weakened by the detergent, heat and the contact time, whereas the removal it self is a consequence of the force the moving liquid exerts on the soil attached to the surface. Also the movement of the liquid provides the transportation of chemicals and heat through out the system. From this it is evident that different degree of cleaning in close systems is primarily related to the movement of liquid. This movement can be modelled using Computational Fluid Dynamics (CFD) and thereby cleanability can be predicted if threshold values for different flow parameters are known. Modelling the sterilisation effect is commonly done for products, and here it is well known that the temperature distribution in the product is of great importance. This is not always recognised when sterilising processing plants as “cold” spots can arise from the design of the equipment. The temperature

distribution on surface in crevice and scratches is related to, not so much the convective heat transfer from the steam lead through the system, but more importantly the conduction through stainless steel, gasket material or soil not properly removed by the cleaning procedure. In this case modelling can be used to show the importance of a good hygienic design.

Unpublished work shows that combining knowledge of the wall shear stress threshold value, wall shear stresses predicted using CFD and qualitative knowledge of local fluid exchange predicted using CFD provides very good predictions of areas with different degree of cleaning. The weakness of this, to be applicable to industry, is the fact that no threshold value has yet been found for the fluid exchange and an approach to quantify fluid exchange is still work in progress. In addition transient simulations of flow is a time consuming exercise, which also has to be taken into consideration. A study at INRA aimed at measuring wall shear stress in discrete points using the electrochemical censors in pieces of equipment and comparing the measured values with microbial counts from cleaning test performed in similar equipment to that used for measuring wall shear stress. Areas of different degree of cleanability was seen in areas exposed to identical, on a time average, wall shear stresses but also better cleaning was seen in areas exposed to low wall shear stresses than in other areas exposed to high wall shear stresses. INRA explained these differences by the fluctuation of the measured signal from the censors. High fluctuations provide better cleaning than low fluctuations. What they actually measured was mass transfer to the probe.

As previously mentioned the influence of different fluid parameters on cleaning is only an interesting finding if the level of the important parameter, in this case the fluctuations, can be predicted or measured. Measuring is an option, but only in discrete points and only using destructive methods. It has been shown that steady state CFD analysis can be applied to predict areas exposed to high or low fluctuations. The fluctuations are not predicted directly, but visualising the turbulence intensity (T_i) (see Equation 1) provides a good prediction.

$$T_i = \sqrt{\frac{2}{3} \cdot \frac{k}{U^2}} = \frac{\sqrt{u'^2}}{U} \qquad (1)$$

Where k is the turbulent kinetic energy, U is the velocity and u' is the fluctuating component of the velocity. Values are taken in the computational cell closets to the wall. At present turbulence intensity can be used to give a qualitative prediction of high and low (relatively) fluctuations in a geometry. However, for this parameter to be an applicable for actual equipment design a scale defining low and high fluctuations are needed. Prediction of the actual level of fluctuation can turn out to be very difficult due as:

- the fluctuations is not was is found from the CFD analysis but the turbulence intensity can be used to produce a qualitative prediction.
- calibration of the CFD model might be possible with respect to finding the same levels of turbulence intensity as the level of fluctuation. However, a more obvious approach is to have a constant conversion between turbulence intensity predicted by CFD and the fluctuations found from the electrochemical measurements.
- levels of turbulence intensity and turbulent kinetic energy dissipation in the inlet, pressure outlet and initially in the CFD flow domain might have large influences on not only the magnitude of the predicted turbulence intensity, but also on the location of local extremes.

Having obtained data on important hydrodynamics parameters in cleaning it is possible, not only to evaluate the cleanability of entire pieces of equipment, but also make additional verification of these parameters by comparison between CFD predicted values and results from cleaning tests such as the European Hygienic Engineering and Design Group (EHEDG) test, the TTC agar test or even discrete enumeration of micro-organisms on the surfaces in discrete points.

GOOD HYGIENE PRACTICES AND HYGIENIC DESIGN

Alpay Seyhan
JohnsonDiversey, Istanbul, Turkey

Safe Food is food which is free of contaminants and will not cause illness or harm. People working with food have special responsibilities for safeguarding the health of consumers. Food Hygiene includes all conditions and measures necessary to ensure the safety and suitability of food at all stages of the food chain. Any person who directly handles packaged or unpackaged food, food equipment and utensils or food contact surfaces and is therefore expected to comply with food hygiene requirements is responsible for good hygiene practices. The main principles of Good Food Hygiene Practices (GHP) are to prevent food-borne illness by controlling the growth of micro-organisms or by stopping cross contamination, to destroy any pathogenic micro-organisms through the application of appropriate techniques such as cooking, processing or irradiation, and to discard any unfit or contaminated food.

Personal hygiene is one of the most important practices in preventing spread of *Shigella* sp., *Staphylococcus aureus*, Norwalk virus, *Campylobacter* sp. and *Salmonella* sp. “Hand Washing Stations” must be equipped with hot and cold running water (preferably non hand contact taps), antibacterial soap, method to dry hands (preferably single use disposable towel), waste container and nail brush. Hand sinks should not be used for anything other than washing hands. Protective clothing is also important for good hygiene practices. Clothes should be washable, light coloured and without external pockets. Protective clothing should cover food handlers clothing and is worn to protect food from contamination. Food handler should have clean hair and body, short and clean fingernails without polish, should not carry any jewellery.

Points to consider for hygienic design are: materials of construction, hygienic construction, airflow and temperature, drains, ring mains, changing procedures, hand washing and washrooms. Materials should be capable of withstanding chemicals and processes during production. Materials should be easy to clean

and jointed hygienically to prevent soil ingress. Joins with the floor should be rounded and in one piece to prevent trapping of soil/water. Vertical surfaces should be smooth to minimise soil adhesion. Materials should have non-painted surfaces to prevent foreign body contamination. Materials should not contain any substances capable of tainting food. Any internal construction such as platforms should be designed for easy access. Materials should be easy to clean and undamaged by chemicals used. Internal construction should allow easy access to other areas e.g. overheads should not restrict ceiling cleaning etc.

Production equipment should be easy to clean. Components should be chemically resistant. Electrical components should have the appropriate Ingress Protection (IP) rating (usually IP65). The machine should be simple to breakdown. Any valves in CIP (cleaning in place) systems should be easy to dismantle and hygienically designed to prevent "dirt traps". Equipment manufacturers should be able to provide detailed information on cleaning and machine breakdown. When purchasing major pieces of equipment it may be a good idea to send someone from hygiene to ask relevant questions. Food contact surfaces should be easily accessible (if possible) for cleaning during breaks. Electrical motors should be easy to cover to protect from water. Control panels should be easy to clean and water resistant.

It is important to consider airflow, especially in high risk areas. Air should move from high risk to low risk areas to minimise cross-contamination. Areas generating aerosols during cleaning of equipment (washrooms) should be isolated from production. If this is not possible extraction and airflow systems should minimise flow out of the area. Lowering temperature reduces microbiological growth rates. Suitable disinfectants should be used that can function at low temperatures.

Drains should flow away from high risk areas. They should be easy to access. Covers should be easy to remove and clean. Note that never pressure to drains during or after disinfection! Ring-mains should be installed with drop points covering all areas of the factory that require cleaning. The type of ring-main needs to be decided upon e.g. water, chemical. Locations for bulk tanks will also be required with bunds to capture possible spillage. Washrooms should be equipped with sinks (detergent – rinse – disinfectant) soak tanks, hygienic utensil storage, stainless steel tables to clean items off the floor, racks to store

cleaned and disinfected items. Air-flow should not be directed towards production.

Cleaning is the systematic application of energy to a surface or substance with the intention of removing dirt. Energy can be kinetic – physical, mechanical, turbulence, thermal or chemical. Chemicals that help to dissolve grease and remove dirt are available as powder, liquid, gel or foam. Foam cleaning produces effective clean, hits hard to reach places, reduces cleaning time and improves safety. Gel cleaning allows much longer contact time, by sticking the detergent to the surface with a gelling agent. This is very useful for high soil situations as contact times can be extended to hours, and high soil applications like smoke house cleaning become feasible. The new generation form of chemical is "thixo-foam" where the detergent and gelling agent concentrates are "water thin". When blended with water to the correct dilution they became gels, and became water thin again when rinsed. They are generated and applied as a foam, but collapse on the surface to leave a gel. Thus the combine the ease and visibility of foam application, with the extended contact times and cleaning power of gels. They are easy to rinse too. The design of pressure cleaning system can be in four different types:

1. De-central (localised) systems, where detergent is placed underneath each cleaning point;
2. Central systems, where detergent is placed at a central point, is pre-diluted and pumped to each cleaning point;
3. Automatic systems, where the complete cleaning sequence is controlled via Programmable Logic Controller (PLC) and
4. Mobile systems where the cleaning units are not statically installed.

Choosing and settling of the system is very important, because it has a direct effect on cleaning performance, safety and hygiene cost. Cleaning pressure is another point to pay attention. If applied a high pressure, aerosols can be a serious problem for cross contamination. Process and CIP design are important to delivering CIP effectiveness or quality. When processing equipment is purchased, there is a specification for both the grade of material to be used e.g. 304 stainless steel, 316L stainless steel, and the surface finish e.g. 2B, electro polished. The surface finish defines how smooth the surface is. Surface finish is

important as it affects how soiling is attached to surfaces and the ability of a CIP system to mechanically remove the soil. Whilst surface finish is usually correct when equipment is new, over time the surface can become damaged by equipment repair and grinding or corrosion of the surface producing “pits”. From a quality perspective poor welds have much the same affect as poor surface finish. This is quite apart from the mechanical issues of weld strength etc. Poor welding typically either leaves an excess of weld material on the surface (overfilled) or too little (undercut). Evidence of poor welding on the outside of a pipe is almost a sure sign of poor welding inside.

A process pipe work dead leg, is any “T” piece where the tee pipe length is more than ½ a pipe diameter in the “non flow” direction, or 1 pipe diameter in the “flow” direction. If the dead leg exceeds these dimensions there will not be sufficient CIP mechanical action in the dead leg to remove soiling, and the sanitizer may not be able to contact the surfaces during the disinfection step, causing contamination in the next product processed. If the dead legs are excessively long or configured downwards, then they may remain full of “stagnant” product. There are two primary ways of creating mechanical action in vessel cleaning: a) Using a low pressure spray ball to create a “falling film” of liquid cascading down the tank walls under gravity and b) Using a high pressure rotary jet device to create “jetting impact” progressively on all tank surfaces.

Spray balls typically deliver a high volume of liquid, at low pressure (1–2.5 bar), to the upper circumference of a vessel. The liquid cascades down the vessel walls under gravity, creating mechanical action. It is important to ensure that all flow rates are correct coming into vessels. It is also important that the spray balls are not only the correct types for the size and shape of the vessel, but also the holes are not blocked.

All of these situations are some examples to create an effective hygienic design at a food processing plant. In addition, there are a lot of examples for different industries or applications. Please do not forget that all necessities of plant should be established according to HACCP principles. As a consequence of poor hygienic design inhibition of microbial growth can be very difficult or even impossible.

EXPERIMENTAL DATA AND MODELLING IN OPTIMISATION OF TANK CLEANING

Satu Salo
VTT Technical Research Centre of Finland, Espoo, Finland

Maintaining the hygiene of tanks is an important issue in dairies and breweries. Cleaning of milk or beer fermentation tanks is challenging since neither insufficient nor excessive cleaning are optimal and economical. Especially cleaning of the bottom of tanks is difficult to optimise because mechanical cleaning effect is weak depending only on liquid film falling in the lower parts of the tank. The spray ball, which sprays liquid on the tank walls, is often placed in the top of tank. Thus the sprayed cleaning solution does not reach the lower parts of a large tank. Also the use of hot water and strong chemicals is limited in several practical cleaning systems. The size of a fermentation tank can be several hundred cubic meters limiting the use of many traditional sampling methods since the reachable areas are scarce compared to the entire tank surface. Therefore validation of the cleaning procedure is potentially problematic in closed processes, since most of the interior in big tanks are unreachable.

Pilot scale tanks were used to test the applicability of various methods for evaluation of the cleanliness of fermentation tanks. The aims of this study were to find appropriate methods to detect cleanliness in pilot scale tanks, to find out which parts of the tank were difficult to clean and to optimise the cleaning procedure. The test equipment was an 80 l stainless steel tank with a spray ball in the tank lid. Cleaning tests were performed in the tank soiled by spreading sour milk containing *Bacillus stearothermophilus* spores (VTT E-88318) on tank walls and another type of soil was obtained when the tank was filled with beer. Two different type of cleaning protocols was used. In the first mild cleaning procedure water was pumped for 20 min to the tank through the rotating spray ball. A more severe cleaning procedure was adapted from the guideline on testing cleanability of closed equipment published by European Hygienic Engineering and Design Group (EHEDG). This cleaning procedure consisted of 1 min pre-rinsing with cold water, 20 min alkali cleaning performed by

circulating 60 °C 1% EHEDG test cleaner PD332 (Lever Industrial, the Netherlands) liquid in the system and rinsing with cold water for 1 min. The velocity of cleaning liquid in the pipeline before spray ball was adjusted to 1.5 m/s in both cleaning set-ups.

The methods found to be suitable for validation of the cleanliness were visual observation of a fluorescent indicator using UV-light, a contact agar method and culturing based on swabbing and swiping with non-woven cloths. According to the contact agar results the lower parts of tank wall were the most difficult to clean when the mild rinsing procedure was used. The upper parts of tank wall as well as the smooth parts of the lid were the best cleanable parts. The ATP-method was used for measuring organic load including microbes from swabbed samples. However, these results did not reveal differences between cleaned areas nor cleaning procedures. Informative microscope and image analysis results revealing area fraction of surface covered with microbes and other debris was expected to obtain from stained stainless steel plates attached on tank wall. No conclusions concerning the cleanliness of various areas of a tank could be made based on microscopying, because the plates detached from tank wall during cleaning.

The validation of the cleaning procedure and the design of a proper cleaning system can be supported and improved using computational fluid dynamics, which through a simulation reveals areas not easily covered by the cleaning fluid. Microbial results from pilot scale studies are needed to interpret the simulation results properly. Using various methods simultaneously improves the interpretation of cleanability of tanks.

In the following research the pilot study set-up was arranged to reveal differences in cleaning results with various angles of inclination and volumetric flow rates. The test set-up was built to simulate an inclined tank bottom. Cleanability was detected with contact agars, agar moulding method, visual observation using UV-light and epifluorescent microscopying combined with image analysis. The flow of water was characterised by calculating average velocities of the flow and the thicknesses of the water layer in various positions in the test system. The information obtained from calculations based on fluid mechanics was verified and visualized using computational fluid dynamics (CFD) simulations.

The microbiological cultivation results showed that the plates remained dirty in all rinsing procedures while the microscope results indicated difference between rinsed and unrinsed test plates. The microscope results revealed that 20–30% of the surface originally fully covered with biofilm became clean in this rinsing procedure. The deviation in the microscope results between repeated samples and parallel positions on the surface was high and disturbed the interpretation of these results. However, the trends drawn from all data points were similar between all tested inclinations indicating the degree of inclination having minor effect on the cleaning result for the cleaning procedure tested in this work. The results from the pilot scale experiments studying a simple cleaning case supported well the simple case flow study performed. This supports the hypothesis that a combination of knowledge in fluid dynamics and microbiology gives an excellent base for hygienic design, hygienic integration of tanks into the system and for evaluation of CIP cleaning systems.

References to this extended abstract can be found in the publications:

Salo, S. 2006. Evaluating hygiene and cleaning efficiency of food process surfaces based on experimental data and modelling. Lyngby: Technical University of Denmark. 55 p. + appendices (189 p.).

Salo, S., Friis, A. & Wirtanen, G. 2005. Improving the cleaning of tanks. In: Lelieveld, H., Mostert, T. & Holah, J. (Eds.). Handbook of hygiene control in the food industry. Cambridge: Woodhead Publishing Ltd. Pp. 497–506. ISBN 1-85573-957-7.

EFFICACY OF CLEANING AGENTS AND DISINFECTANTS USED IN DECONTAMINATION PROCEDURES IN FOOD INDUSTRY

Gun Wirtanen
VTT Technical Research Centre of Finland, Espoo, Finland

Cleaning and disinfection is carried out in order to produce safe products with acceptable shelf life and quality. In the food industry there is a trend towards longer production runs with short intervals for sanitation. The cleaning programmes should be performed as cost-effectively and safely as possible, which means as infrequently as possible, in the shortest possible time, with low chemical, energy and labour costs, producing as little waste as possible and with no damage to the equipment. The mechanical and chemical power, temperature and contact time in the cleaning regime should be carefully chosen to achieve an adequate cleaning effect. The use of effective cleaning agents and disinfectants on surface-attached microbes minimises contamination of the product, enhances shelf life and reduces the risks of foodborne illness. A prolonged exposure of the surfaces to cleaning agents and disinfectants enhances the removal. Attention should also be paid to the quality of the processing water, steam and other additives. Using additives of poor quality easily spoils the process. Furthermore, the tools and methods used must also suit the process and the personnel must be properly trained and responsible to maintain a good level of plant hygiene.

Physical, chemical and microbiological cleanliness are essential in food plants. Factors governing the selection of detergents and disinfectants in the food industry are that the agent should be efficient, safe, not damage or corrode equipment, be easily rinsable and not affect the sensory values of the product produced. Physical cleanliness means that there is no visible waste, foreign matter or slime on the equipment surfaces. Chemically clean surfaces are surfaces from which undesirable chemical residues have been removed, whereas microbiologically clean surfaces imply freedom from spoilage microbes and pathogens. Attached bacteria or bacteria in biofilms can be a problem in food processing; because they adhere to the surfaces and if the cleaning is insufficient the remaining bacteria start to grow and

multiply after the cleaning and contaminate the product. Once a biofilm is firmly established, cleaning and disinfection becomes much more difficult. Inadequate cleaning and sanitation of surfaces coated with biofilm cause contamination, because the biofilm protects the microbes against both cleaning agents and disinfectants.

An efficient cleaning and disinfection procedure consists of a sequence of rinses and detergent and disinfectant applications in various combinations of temperature and concentration. In a wet open process the gross soil should be removed by dry methods, e.g. brushing, scraping or vacuuming and visible soil rinsed off with low-pressure water. Using water of sufficient volume and temperature increases the cleaning effect. However, a pure water washing system is not practical due to ineffectiveness and cost limitation. Surfactants, which suspend the adhered particles and microbes from the surfaces in the water, are added to increase the washing effect. After a production run the equipment should be dismantled and the cleaned utensils should be stored on racks and tables, not on the floor. The cleaning of open process surfaces and surfaces in the processing environment is carried out using either foam or gel cleaning. The foam-units are constructed to form foam of varying wetness and durability depending on the cleaning to be performed. The application of gels extends the contact time with a soiled surface and can be used with low-pressure system. The cleaning is mostly carried out in combination with a final disinfection, because there are likely to be viable microbes on the surfaces that could harm continued production. Furthermore, good ventilation in the process facilities is needed to enable drying of the process equipment and process lines.

In the cleaning of closed processes, prerinsing with cold water is carried out to remove loose soil. The CIP treatment is normally performed using hot cleaning solutions, but cold solutions can also be used in the processing of fat-free products. The warm alkaline cleaning solution, normally of 1–2% sodium hydroxide, is heated to 75–80°C and the cleaning time is 15–20 min. The equipment is rinsed with cold water before the acid treatment is performed at approximately 60°C for 5 min. The cleaning solutions should not be reused in processes aiming at total sterility because the reused cleaning solution can contaminate the equipment. The design of the tank should ensure that also parts directly above the spray ball also are cleaned. Drainage, minimisation of internal probes, crevices and stagnant areas, arrangement of valves, couplings and instrument ports and instrumentation should

be planned carefully so that the equipment is easily cleanable. Problems caused by equipment constructions and materials cannot be eliminated with CIP, because the CIP treatment was not designed to eliminate biofilms.

Cleaning agents are applied to remove soil, microbes and biofilms from surfaces. The cleaning agents should be surface active, soluble, non-toxic, rinsable, non-corrosive and easy to use. The effects of the cleaning agents on biofilms have been thoroughly investigated. Removal of biofilm is important for the maintenance of the equipment, since the debris left on surfaces can act as nutrients for the build-up of new biofilm. In order to minimize biofouling, it is best to clean the equipment at frequent and regular intervals using suitable, efficient cleaning procedures for the process before the biofilm has the opportunity to develop. Metal chelators have been used in the breakage of the biofilm layers. Cleaning agents containing chelators and surfactants take part in detachment of the biofilm matrix from surfaces. Chelators bind magnesium and calcium ions, thereby destabilizing the outer membranes of microbial cells. The cleaning effect ceases when the surfactant molecules are fully deployed in tying up the soil and therefore it is important to maintain a sufficient concentrations of chelators and surfactants in the cleaning solution.

The disinfectants have been developed to destroy microbes. Microbes have nevertheless been found in disinfectant solutions, which is due to their ability to form resistant strains and build-up of protective biofilms. This means that microbial contaminants can be spread on the surface to be cleaned instead of being cleaned. Disinfection is required in food plant operations where wet surfaces provide favourable conditions for the growth of microbes. The aim of disinfection is to reduce the surface population of viable microbes after cleaning and to prevent microbial growth on surfaces before restart of production. Disinfectants do not penetrate the biofilm matrix left on the surfaces after an ineffective cleaning procedure very well, and thus do not destroy all the living cells in biofilms. Disinfectants are most effective in the absence of organic material, e.g. fat-, sugar- and protein-based materials. Interfering organic substances, pH, temperature, concentration and contact time generally control the efficiency of disinfectants. The disinfectants must be effective, safe and easy to use, and easily rinsed off surfaces, leaving no toxic residues or residues that affect the sensory values of the product. The use of disinfectants in food plants depends on the material used and the adhering microbes. Disinfectants approved for use in the food industry are alcohols, chlorine-based compounds, quaternary ammonium compounds, oxidants (peracetic

acid, hydrogen peroxide and ozone), persulphates, surfactants and iodophors. They should be chosen based on the process:

- Is the agent effective in the pH range used?
- Is the agent stable when diluted? Does it vaporize?
- Is the agent toxic, safe or irritating?
- What is the spectrum of the agent?
- How does temperature affect the activity of the agent?
- Is the agent corrosive on the surface?
- Is the agent surface active?
- Is the agent stable when reacting with organic material?
- Is the agent effective, and what are the costs?

Available reports about the susceptibility of foodborne spoilage microbes and pathogens to various chemicals used in the food industry are sporadic and have focused on only a few spoilage species, food processes or cleaning agents and disinfectants. Information about the efficacy of various cleaning agents and disinfectants against forborne spoilage microbes is needed in order to be able to choose the appropriate chemicals for the decontamination of process equipment and utensils. NOTE! Cleaning and disinfection in the food industry should be based on systematic planning.

References to this extended abstract can be found in the publications:

Salo, S. & Wirtanen, G. 2005. Disinfectant efficacy on foodborne spoilage yeast strains. Transactions of the Institution of Chemical Engineers, Part C: Food and Bioproducts Processing, 83, C4:288–296.

Wirtanen, G. & Salo, S. 2003. Disinfection in food processing – Efficacy testing of disinfectants. Reviews in Environmental Science and Bio/Technology, 2, 293–306.

Wirtanen, G., Storgårds, E. & Mattila-Sandholm, T. 2003. Biofilms. In: Caballero, B., Trugo, L. & Finglas, P. (Eds.) Encyclopedia of Food Sciences and Nutrition. London: Academic Press. Pp. 484–489.

Wirtanen, G. & Salo, S. (Eds.) 2004. DairyNET – Hygiene control in Nordic dairies. VTT Publications 545. Espoo: VTT. 253 p. + 63 p. ISBN 951-38-6408-1.

DETECTION OF *SALMONELLA* IN POULTRY – MEASURES PERFORMED WITHIN RISK ASSESSMENT IN TURKEY

Ayşegül Eyigör & Kamil Tayfun Carli
Uludağ University, Bursa, Turkey

The Food and Agriculture Organisation / World Health Organization Codex Alimentarius Commission defines risk assessment as a scientifically based process consisting of four steps: 1. Hazard identification, 2. Hazard characterization, 3. Exposure assessment, and 4. Risk characterization. In hazard identification, we aim to find the biological agent that may be present in a particular food, which has the potential to cause adverse health effects to humans (WHO, 2002). Here, we give information on the involvement of our laboratory in the detection of *Salmonella* from poultry, the hazard identification step of risk assessment process, and an update on *Salmonella* profile in chickens in Turkey. Our laboratory has been working on the determination of the presence of *Salmonella* in poultry samples by using the following universally recognized bacteriological methods: the 'United States Department of Agriculture Animal and Plant Health Inspection Service-USDA, NPIP, 1996' for intestinal samples, cloacal swabs, drag swabs, litter samples, chick dust etc., and the 'United States Food And Drug Administration- Bacteriological Analytical Manual *Salmonella* part (FDA-BAM Salm; Wallace *et al*., 1999)' for poultry meat and related samples. We have also been working on the development and/or implementation of alternative PCR-based methods to bacteriology for rapid and reliable detection of *Salmonella* in poultry samples.

In 1999, we developed a method, detecting salmonellae in tetrathionate broth (TTB) enriched chicken faeces by capillary polymerase chain reaction (cPCR, using *inv*A-specific primers), followed by capillary gel electrophoresis (Carli *et al*., 2001b). We had used pure *Salmonella enterica* serovar Enteritidis 64K, reisolated and detected it by capillary PCR after buffered peptone water and nutrient broth, tetrathionate broth base Hajna (TTBH), and tetrathionate broth (TTB) preenrichments. When the same culture was mixed with intestinal

homogenate, bacteriological reisolation and capillary PCR detection was achieved only by TTBH and TTB preenrichments. Capillary gel electrophoresis revealed that a *Salmonella* genus-specific 281-bp PCR product was detected with high specificity and sensitivity when *Salmonella* strains but not non-*Salmonella* strains were tested. We compared the results of the capillary PCR and bacteriological examination from the natural samples in that study and found that 35 of 53 naturally contaminated samples produced a specific PCR product. In 9 of the 35 PCR-positive samples, *Salmonella* could not be detected bacteriologically either by PE or a primary and delayed secondary enrichment (DSE) combination. In the 18 PCR-negative samples, 4 samples were found to harbour *Salmonella* by both PE and DSE and 14 samples were positive after DSE. Fifty-three additional intestinal homogenate samples, which were negative by their PE and DSE in bacteriological examination, were found to be also negative by their PCRs. The total time required to detect *Salmonella* with the capillary PCR method we used was approximately 20 h. If samples are from clinically diseased birds, the total time for PCR and detection is reduced to 2 h since the 18-h PE is not required. Results indicated that TTB enrichment, bacterial lysis, and genus-specific capillary PCR combined with capillary gel electrophoresis constituted a sensitive and selective procedure with a potential to rapidly identify *Salmonella*-infected flocks.

Further in 2001, we implemented a SYBR green based real-time PCR to TTB enrichment step of *Salmonella* detection as a rapid primary screening to determine *Salmonella*-infected flocks. Four hundred and ninety two intestinal homogenates and 27 drag swabs from 47 poultry flocks were sampled (Eyigor *et al*., 2002). The number of positive individual samples by real-time PCR and culture method was 65 (12.5%) and 35 (6.8%), respectively. The number of *Salmonella*-positive flocks was 13 (27.7%) by both methods. Melting curve analysis revealed the Tm for *Salmonella*-specific PCR product as 87.1 °C. *Salmonella* isolation was carried out with PCR to determine the serovar, as well. With this study we showed that real-time PCR is a powerful tool in rapid and accurate *Salmonella* monitoring in poultry companies, together with standard bacteriology. Advantages were: simultaneous, 18 h 25 min *Salmonella* detection time of 32 samples per run in LightCycler (LC, Roche Diagnostics, Mannheim, Germany), simultaneous amplification and analysis, specificity determined by melting curve analysis. In 2002, we developed a probe-specific real-time PCR assay, applying fluorescence resonance energy transfer assay, for routine

monitoring and detection of *Salmonella* (*Salmonella inv*A gene-based PCR products) in chicken faeces and carcass samples (Eyigor & Carli, 2003). The sensitivity and the specificity of this system were determined as 3 colony forming units ml^{-1} and 100%, respectively. Overnight TTB enrichment cultures of chicken feces and carcass samples were used in template preparation for PCR. Also, USDA-NPIP and FDA-BAM Salm was used for confirmation/strain identification purposes. Seventy-two cloacal swab, 147 intestine, and 50 carcass (neck) samples were examined. Thirteen (8.8%) and 25 (17%) of the intestinal samples were found to harbour *Salmonella* by bacteriology and PCR, respectively. Forty five (45) of 50 (90%) carcass samples were *Salmonella* positive by both methods. *Salmonella* was not detected from cloacal swab samples. Results indicated that the assay had the potential for use in routine monitoring and detection of *Salmonella* in infected flocks and carcasses.

In 2007, we implemented a cPCR procedure for *Salmonella* detection from poultry meat (Gunaydin *et al.*, 2007). *Salmonella* detection limits of this optimized cPCR were determined with DNA templates from the samples of TTB, Rappaport Vassiliadis broth (RVB) and selenite cystine broth (SCB) artificially contaminated with 10-fold dilutions of 6×10^8 CFU ml^{-1} of pure *Salmonella* Enteritidis 64K stock culture. Detection limits of cPCR from TTB, RVB and SCB were found as 6, 6×10^1 and 6×10^4 CFU ml^{-1}, respectively. In addition, detection limits of bacteriology were also determined as 6 CFU ml^{-1} with TTB and SCB, and 6×10^1 CFU ml^{-1} with RVB. A total of 200 samples, consisting of 100 chicken and 100 turkey meat samples, were tested with optimized cPCR and bacteriology. Eight and six per cent of the chicken meat samples *Salmonella* isolates, four belonged to serogroup D, two to serogroup B. The TTB cultures of both artificially and naturally contaminated samples were found to be superior to those of RVB and SCB cultures in their cPCR results. This cPCR, utilizing template from 18-h TTB primary enrichment broth culture of poultry meat enabled rapid detection of Salmonella in laboratories with low sample throughput and limited budget. In studies performed in the 1980s, *Salmonella enterica* subsp. *enterica* serovar Gallinarum (*S.* Gallinarum) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) were reported as the dominant serovars in chicken flocks in Turkey, while by the beginning of 1990s *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis) started to replace these aforementioned serovars (Carli, 1990; Carli *et al*., 2001a). For example, in our previous report in 2001 (Carli *et al*., 2001a)

out of 814 ileocecal intestinal samples that were examined by bacteriology, 151 (18.6%) samples were found positive for *Salmonella*. The serovar breakdown for these positive samples were reported as 81.5% for *S*. Enteritidis, 10.1% for *Salmonella enterica* subsp. *enterica* serovar Thompson, 7.6% for *Salmonella enterica* subsp. *enterica* serovar Agona, and 0.8% for *Salmonella enterica* subsp. *enterica* serovar Sarajane in the same study (Carli *et al*., 2001a). Between the years of 2000–2005, we have analysed a total of 2128 samples (n) as follows: Intestinal samples (1218), Cloacal swabs (532), Drag swabs (64), Litter samples (58), Chick dust (3), Embrionated egg (20), Feaces (70), Intestine, ileocecal junction (3), Chick (6), Chicken meat (25), Chicken carcass (15), Chicken neck skin (30), Chicken drip water after scalding (3), Chicken scalding water (3), Turkey meat (17), Turkey carcass (15), Turkey neck skin (30), Turkey drip water after scalding (3), Turkey scalding water (3). We have isolated 168 *Salmonella* spp. (7.9%) with either of the aforementioned PCR methodologies combined with bacteriology and partially published it in 2005 (Eyigor *et al*., 2005). The dominant *Salmonella* serovar was determined as *S*. Enteritidis, while serogroup C1 and C2 in 2001 and serogroup E1 in 2002 were isolated as additional serovars. We can say that *S*. Enteritidis seems to be the major problem in poultry breeding flocks in Turkey. Currently, part of our work is on determining the detection efficacies of probe specific PCR (LC PCR) and two bacteriological methods (modified FDA-BAM Salm and the International Standards Organisation 6579-2002 method) on poultry meat and red meat samples.

References:

Carli, K.T. 1990. Bacteriological and serological studies on the Salmonella *strains isolated from chickens (broiler and layer) in Bursa district. Turk. J. Vet. Animal Sci., 14, 428–438.*

Carli, K.T., Eyigor, A. & Caner, V. 2001a. Prevalence of Salmonella *serovars in chickens in Turkey. J. Food Prot., 64, 1832–1835.*

Carli, K.T., Unal, C.B., Caner, V. & Eyigor, A. 2001b. Detection of Salmonella *in chicken faeces by a combination of tetrathionate broth enrichment, capillary PCR and capillary gel electrophoresis. Journal of Clinical Microbiology, 39, 1871–1876.*

Eyigor, A., Carli, K.T. & Unal, C.B. 2002. Implementation of real time PCR to tetrathionate broth enrichment step of Salmonella *detection in poultry. Lett. Appl. Microbiol., 34, 37–41.*

Eyigor, A. & Carli, K.T. 2003. Rapid detection of Salmonella *from poultry by real-time polymerase chain reaction with fluorescent hybridization probes. Avian Dis., 47, 380–386.*

Eyigor, A., Goncagul, G., Gunaydin, E. & Carli, K.T. 2005. Salmonella *profile in chickens determined by real-time polymerase chain reaction and bacteriology from years 2000 to 2003 in Turkey. Avian Pathol., 34, 101–105.*

Gunaydin, E., Eyigor, A. & Carli, K.T. 2007. A capillary polymerase chain reaction for Salmonella *detection from poultry meat. Lett. Appl. Microbiol., 44, 24–29.*

United States Department of Agriculture Animal and Plant Health Inspection Service. 1996. The National Poultry Improvement Plan CFR Part 147. Auxiliary Provisions on National Poultry Improvement Plan. Subpart B. Bacteriological examination procedure. § 147-11. Laboratory procedure recommended for the bacteriological examination of Salmonella *(pp. 14–19). Washington, DC: United States Department of Agriculture.*

Wallace, H.A., June, G., Sherrod, P., Hammack, T.S. & Amaguana, R.M. 1999. Salmonella*. In: Tomlinson, L.A. (Ed.) Food and Administration. Bacteriological Analytical Manual.*
Hypertext Source: http://vm.cfsan.fda.gov/_comm/bam-5.html#Intro.

World Health Organization, Food and Agriculture Organization of the United Nations. 2002. Risk assessments of Salmonella *in eggs and broiler chickens. Microbiological Risk Assessment Series, 2, p. 1.*

PREVALENCE AND MECHANISMS OF ANTIBIOTIC RESISTANCE OF *CAMPYLOBACTER* SPP. FROM WATER AND POULTRY MEAT SAMPLES

Sonja Smole Možina, Marija Kurinčič, Ana Kramar, Tina Zorman, Katja Zelenik*, Mojca Lušicky* & Selma Uzunović-Kamberović[#]
University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia
* Institute of Public Health Maribor, Maribor, Slovenia
[#] Cantonal Public Health Institution Zenica, Zenica, Slovenia

Thermotolerant campylobacters are currently recognized as a leading cause of foodborne illness in many developed countries worldwide. They are usually transmitted by contaminated food and drinking water, but there is still much unknown in their epidemiology. Additional problem is the emergence and spread of their antimicrobial resistance. We studied the prevalence and the mechanisms involved in resistance against ciprofloxacin, erythromycin and tetracycline, antibiotics of choice in human and veterinary medicine, in *Campylobacter* spp. isolates, which were collected from retail poultry meat and surface or drinking water from different Slovene and Bosnian regions in the years 2001–2007.

Isolation of bacterial strains from poultry meat and water samples Two hundred seven (207) samples of poultry meat from 16 different suppliers on Slovenian and Bosnian market were investigated with the ISO 10272 guideline for the presence of thermotolerant campylobacters in the period 2001–2003. One hundred eighteen (118) strains were long-term stored at –80 °C in culture collection ZIM, BF, Ljubljana, for further studies. During the years 2004–2007, 592 samples of surface or drinking water from different regions in Zenica-Doboj Canton, BiH, were examined for thermotolerant campylobacters at the Public Health Institute in Zenica, BiH. In 2006–2007, 336 water samples from Slovenia were included in monitoring at the Public Health Institute in Maribor. In total, 58 presumably thermotolerant *Campylobacter* water strains were sent to the Biotechnical Faculty and stored for testing described below.

Species identification *C. jejuni* and *C. coli* were identified by standard (ISO) and polymerase chain reaction (PCR) procedures as described previously. Additionally, the identity of poultry meat isolates was confirmed by PFGE typing using *Sma*I and CHEF mapper XA System (Bio-Rad).

Determination of antibiotic resistance and mechanisms involved Antimicrobial resistance testing was first performed using disk diffusion method and Epsilometer test as described previously. Minimal inhibitory concentrations (MICs) of ciprofloxacin, erythromycin and tetracycline were confirmed by broth microdilution method with CellTiter-Blue® reagent and automated fluorescence signal detection as described by Kurincic *et al.* (2007). The resistance to ciprofloxacin was confirmed also by mismatch amplification mutation assay (MAMA-PCR) detection of mutations in quinolone resistance determining region (QRDR) of the *gyr*A gene. Mutations in 23S rRNA and TetO genes were studied by PCR-RFLP and PCR, respectively. The involvement of efflux pumps was evaluated by measurements with/without the efflux pump inhibitor phenylalanine-arginine ß-naphthylamide (PAßN) (Kurincic *et al*., 2007).

Isolation of thermotolerant **Campylobacter spp.** ***from poultry meat and water samples*** In total, 90% of Slovene and 74% of Bosnian tested fresh retail poultry meat samples were found positive for thermotolerant campylobacters. Among 928 water samples, surface and drinking waters were tested. In total, 55 out of 679 (55/679) surface water and 3 out of 249 (3/249) drinking water samples were found positive for thermotolerant campylobacters.

Species identification One hundred twelve (112) meat isolates and 54 water isolates survived long-term freezing and were included in PCR species identity confirmation. We found high proportion of *C. coli* among thermotolerant campylobacters from poultry meat and water samples (64/112% and 38/50, respectively). Only 6/50 water isolates were confirmed as *C. jejuni*, some were confirmed to belong to the genus *Campylobacter*, while four isolates were not confirmed as campylobacters and were excluded from the further testing.

Antimicrobial resistance and mechanisms involved in **Campylobacter** ***meat and water isolates*** We compared the occurrence of antimicrobial resistance to ciprofloxacin, erythromycin and tetracycline among 112 poultry meat and 50 water isolates, identified as *C. coli*, *C. jejuni* or *Campylobacter* spp. Resistance

to ciprofloxacin was more frequent among the meat isolates (43.8% versus 26.0% among water isolates). In contrast, resistance to erythromycin was much more frequent among the water isolates (44.0% versus 21.4% among meat isolates). However, the erythromycin resistance rates of meat and water isolates were very high, comparing to reports from some other European countries. Tetracycline resistance was rare among water isolates, but quite frequent among the meat isolates (6.0% versus 18.8%, respectively). With the aim to study the mechanisms involved, the resistance of 30 isolates to erythromycin, ciprofloxacin and tetracycline was studied in the absence and presence of efflux pumps inhibitor phenylalanine-arginine ß-naphthylamide (PAβN), which affected the efflux pump CmeABC. As the result of PAβN presence, the susceptibility to erythromycin was in average increased 24-fold. The presence of efflux pumps activity at the isolates with mutations in target genes was also observed and the sinergistic activity of these two drug resistance mechanisms was proved. The smaller effect of the PAβN was observed when used in the presence of ciprofloxacin and tetracycline.

Conclusions An inevitable side effect of the use of antimicrobials is the emergence and dissemination of resistant bacteria. Because of the global increasing of antimicrobial resistance and the potential public health consequences of the transmission of resistant bacteria through the food chain there is an urgent need for monitoring antimicrobial resistance at the local, regional and international level. We need a monitoring system of the prevalence and antibiotic resistance of zoonotic bacteria from human, animal, food and environmental samples at different levels to understand the epidemiology of resistant strains and to assure the safety of our food.

References to this extended abstract can be found in the publications:

Kurincic, M., Botteldoorn, N., Herman, L. & Smole Možina, S. 2007. Mechanisms of erythromycin resistance of Campylobacter *spp. isolated from food, animals and humans. Int. J. Food Microbiol., Vol. 120, Issues 1–2, pp. 186–190. Available online: http://dx.doi.org/10.1016/j.ijfoodmicro.2007.03.012*.

LISTERIA IN READY-TO-EAT PRODUCTS

Renata Karpíšková
National Institute of Public Health, Brno, Czech Republic

Listeria monocytogenes is a Gram-positive intracellular foodborne pathogen that can cause sometimes very serious and fatal disease among high risk human population. Listeriosis represents both sporadic cases as well as foodborne listeriosis outbreaks. While a number of listeriosis outbreaks has been reported and linked to a specific food source, the majority of reported human cases are classified as sporadic cases for which no source is identified. The difficulty to detect human listeriosis outbreaks can be linked to a number of factors, including the long incubation period of listeriosis (up to 90 days and the occurrence of linked cases over long time period), the rare occurrence of cases, and the geographical dispersion of cases. *L. monocytogenes* is the only one of the six recognised listeria species associated with human infection. However the other listeria species co-exist in foods with *L. monocytogenes* in mixed populations and thus can be used as sensitive indicator of the presence of *L. monocytogenes*. Among 13 serotypes of *L. monocytogenes*, strains belonging to specific serotypes (4b, 1/2a and 1/2b) are associated with majority of listerioses cases. *L. monocytogenes* can be isolated from many different sources. Raw food of both the animal and plant origin including the food processing plant environment can be the source of contamination of ready-to-eat (RTE) foods. The principal reservoir of *Listeria* is soil, plants (forage) and water. Other reservoirs include infected domestic and wild animals. The main route of transmission to both humans and animals is through consumption of contaminated food or feed.

L. monocytogenes is relatively resistant micro-organism able to multiply at refrigeration temperatures. The limits for growth are ranging from 1–45 °C with the optimum at 30–37 °C and the organism can survive at –18 °C for several weeks. It is not usually heat resistant and should not survive milk pasteurization treatment unless very high initial numbers are present (10^5–10^6 CFU.ml^{-1}). Cooking kills *Listeria*, but the bacteria are known to multiply at low

temperatures which make its occurrence in ready-to-eat foods with a relatively long shelf life particularly important. *L. monocytogenes* has a lower a_w limit for growth at approximately 0.92. Subjection to low temperature and low water activity enhances the bacteriostatic effect. *L. monocytogenes* has a broad pH range for growth with the upper limit at about pH 9.2 and the lower at pH 4.6. The organism grows well under aerobic, microaerobic and anaerobic conditions. According to the Commission Regulation on microbiological criteria of foodstuffs (EC) 2073/2005 the food business operators are responsible for the determination of safe shelf-life, where reasonably foreseeable conditions of distribution, storage and use have to be taken into account. Although in practice shelf-life is usually designed for legal temperature limits and proper consumer practices, while significant deviations exist in product composition. There are also temperature fluctuations in the cold chain, false consumer practices are frequent and decision about safety is generally based on visual observation of spoilage. The impact of temperature fluctuations during distribution and storage is higher than that of the composition parameters. Findings of *L. monocytogenes* in foodstuffs are important from the two main points of view:

- Presence of *L. monocytogenes* in RTE foods that are able to support growth of the bacterium.
- Findings of *L. monocytogenes* in concentrations greater than 10^2 CFU.g^{-1} (ml^{-1}) in food. This concentration is regarded as a direct risk for human health, whereas concentrations less than 10^2 CFU.g^{-1} (ml^{-1}) are usually not considered significant for human disease, except in vulnerable population groups.

Data Published in the Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the EU in 2005, published by EFSA support that the most frequently contaminated RTE foods with *L. monocytogenes* were fishery products (7.5% out of 8,155 samples examined), followed by meat products (2.7% out of 13,481 samples), fruits and vegetables (0.6% out of 865 samples), dairy products other than cheeses (0.8% out of 10,048 samples) and cheeses (0.6% out of 16,885 samples). Some human listeriosis cases can be traced back to contaminated foods that did not undergo a listericidal treatment (e.g., cold smoked fish, raw milk, and raw milk cheeses). But most human listeriosis appears to be caused by RTE foods that have undergone a listericidal treatment (e.g., pasteurization).

There is considerable evidence that contamination of RTE foods often occurs during post-processing stages in the processing plant, even though other post-processing sources (e.g., at retail, in consumer homes) may also contribute to *L. monocytogenes* contamination of this sort of foods.

PREVENTION OF TOXIGENIC MOULDS IN GRAINS, NUTS AND DRIED FOODS

Güner Özay
Tübitak, Gebze, Kocaeli, Turkey

Moulds are the most frequently occurring micro-organisms on earth. They absorb nutrients nutrient from a living or death host material including food substrates instead of producing their own food. Reproduction of moulds occurs through spores. Mould spores can be airborne and able to move by wind, insects, birds, etc. from one plant to another. Mould spore infection can occur at any stage of crop production in plats, in the field and it can infect healthy products during, pre and post harvest stages including storage. They are invisible to the naked eye and examined under microscope except for the high contamination level. Some of the moulds produce secondary metabolites known as mycotoxins (by-products of growth) that are toxic to human and animals. Moulds are the major cause of spoilage in some crops in the world. Mycotoxin contamination of crops has been a worldwide problem. It is estimated that 25% of the world's food crops are affected by mycotoxins during cultivation and storage.

It is estimated that there are more than 300 mycotoxins presently identified. The most frequently found mycotoxins are aflatoxins B1, B2, G1 and G2, ochratoxin A, deoxynivalenol (DON), fumonisin, zearalenon (ZEA) and T2 toxin. Health hazards of mycotoxins can be acute and cronical and have several common symptoms differing from toxin to toxin. The most toxic group is aflatoxins. Aflatoxins are known to be carcinogenic substances causing necrosis, cirrhosis and carcinomas in humans and animals. *Aspergillus, Penicillum, Fusarium, Alternaria* and *Claviceps* are the major mould genus in foods with most species causing spoilage and producing mycotoxins. *Aspergillus* species produce aflatoxins (*Aspergillus flavus* and *Aspergillus parasiticus*), ochratoxin A and citrinin both causes kidney diseases. *Penicillum* is a large genus produces nine different mycotoxins (i.e. ochratoxin A) that affect liver, kidney and neurotoxic. *Fusarium* toxins are another group of mycotoxins. Corn, wheat, barley and their final products are frequently contaminated with *Fusarium* toxins. *F. graminearum*

(produces DON, ZEA and Nivalenol) and *F. moniliforme* (produce fumanisin) are the major species.

Toxigenic moulds and mycotoxins problem is not specific to any geographical region and commodity type in the world. Generally tropic and sub tropic climates are favourable for mycotoxin formation. Grains, oil seeds, nuts, and some dried fruits are risky products for mould contamination and mycotoxin formation. Mould contamination and mycotoxin formation can occur any stage of food production chain under favourable conditions. There are different environmental conditions affecting occurrence of toxigenic moulds and formation of mycotoxins. Generally, temperature and relative humidity (RH) of the environment, moisture content /water activity (a_w) of the food as substrate are the major factors. However, the min requirements for moulds species for germination and their mycotoxin production capacities show differences according to mould species and mycotoxin types. Nonetheless, it has been reported that 70% RH (or 0.7 a_w of the substrate) is the absolute value for prevention of toxigenic moulds. Decreasing the temperature below 25 °C will reduce the mould development.

Grains, nut and dried fruits can be contaminated with toxigenic moulds and mycotoxins in the field or orchard at pre harvest stages. High temperature and RH%, drought stress, damage from insects and birds, high crop density are some factors that favour mould development on the crops in the field. Good Agricultural Practices (GAP) (proper use of fertilisers, pesticides and fungicides, irrigation) can help to minimise the mould contamination. Cleaning away the residual crops will also improve the hygienic conditions in the fields. After harvest, the most critical factor is drying the crops as soon as possible (time factor) to the safe moisture content (i.e. 15% in corn). Delay in drying, especially during the rainy seasons will make the crop susceptible to mould infection. Storage is another important stage during post harvest applications. The crops, dried to safe moisture content should be stored in adequate storage conditions. Again, temperature, RH% are critical factors to be controlled during storage. Ventilation by air circulation in the storehouses and use of fungicides and insecticides are effective to prevent toxigenic moulds during storage. Mycotoxin free storage time of the crops is dependent on the initial moisture level, type of the crop and storage conditions. In case of grains, i.e. corn, if corn is not dried to adequate moisture level, the mould will grow and contaminate

healthy grain during storage. Mould development can occur during transportation and distribution channels of the foods if the conditions are favourable i.e. high temperature and RH%, using no hygienic equipment, re-wetting, etc. Cross contamination may also occur if healthy products are in contact with contaminated lots. Concerning the nuts, such as pistachios, Brazil nuts and hazelnuts, drying is the most critical stage for mycotoxin formation. The most frequently found mycotoxin in nuts is aflatoxins. It has been determined that formation of aflatoxin, most frequently formed mycotoxin in this crop, may starts in the orchard when the fruit is fresh, and further develops during the extended and inadequate drying conditions. Ochratoxin A is the major contaminant in raisins.

Since mycotoxins are major component of food safety issue, many countries have established regulations for the maximum levels of mycotoxins to protect consumer health. The maximum levels are generally at ppb levels and different levels have been set for different toxins and food groups. The foods containing high levels of mycotoxins than the permitted limits are not allowed to be imported or marketed in the country. Moulds can be destroyed anyway during food processing technologies i.e. heat treatment, but their metabolites, mycotoxins, are very stable chemical compounds and show resistance to comparable high temperatures. However, food processing technologies e.g. heat treatment (i.e. roasting, boiling and baking), sorting, refining found to be effective in destroying mycotoxins at different ratios. Decontamination (physical sorting out the damaged kernels) and detoxification (treatment with chemicals) are also subjected to various research studies for their efficiency on mycotoxins. Detoxification with chemicals is not permitted for foods to be subjected to human diet and only permitted for the grains to be used as animal feed in some countries. The best way of managing toxigenic moulds and their toxic metabolites is prevention from their occurrence. For this purpose, application of GAP in the field, GSP (Good Storage Practices) during storage and HACCP in processing plants are helpful systematic approaches through the total food chain, from the field to the table.

PROTECTIVE CULTURES AND ANTIMICROBIAL ACTIVITIES IN FOODS

Mehlika Borcakli
Tübitak, Gebze, Kocaeli, Turkey

Protective cultures are microorganisms especially selected and developed for their ability to control the growth of pathogenic and/or spoilage microorganisms in fermented foods. Lactic acid bacteria has been used traditionally to improve the aroma and texture and to prevent rapid spoilage of dairy, cereal and meat products as well as vegetables and silages. Protective effect of lactic acid bacteria cultures are two-fold. First they extend the shelflife of of foods and prevent the growth of pathogenic microorganisms. In addition, properties such as antitumour and anticholesterol activity, improvements in immunological status, and decreased gastrointestinal disorders have been attributed to the utilisation of these cultures in foods. Introduction of lactic acid bacteria (starter cultures) into dairy products in nineties was an important step for the industrialization of these cultures. Inoculants are used today for meat and dairy products, vegetable and silages. Lactic acid bacteria fermentations are characterized by the accumulation of organic acids, primarily lactic and acetic acids and the accompanying reduction in pH. Levels and proportions of fermentation end products which accumulate depend on the species of the organisms involved, the chemical composition of the culture environment and the physical conditions encountered during the fermentation process. The microorganisms associated with these LAB fermentations include species found primarily in the following genera: *Lactococcus*, *Lactobacillus, Pediococcus, Weissella* and *Leuconostoc.*

It is well known that these acidic end products tend to inhibit the growth and metabolic activities of other microorganisms which may also be present in the culture environment. These substances are produced by heterofermentation or homofermentation. Other end products can accumulate, particularly during heterofermentations, for example formic acid, acetoin, 2,3-butanediol and diacetly also can exhibit antagonistic activity. The antagonistic activities associated with other organic acids such as malic and citric acid, can vary under

certain circumstances. In this context malic acid can be decarboxylated to lactate and or lactic acid can be degradated anaerobically to either formic acid and acetic acid in the presence of citrate or to acetic acid, CO_2 and H_2. Utilisation of protective cultures in vegetables such as cabbages, olives and cuccumbers yield products more appreciated and resistant. Fresh vegetables contain high numbers of an epiphytic microflora consisting of numerous spoilage bacteria and only small numbers of LAB. The fermentation is controled by the addition of sodyum chloride and protective cultures to extend the shelflifes of the products.

In practice, bacteriocin forming micro-organisms are being specifically used in the first commercial protective cultures. Bacteriocins are substances which are formed by bacteria and have an antimicrobial effect particularly against gram-positive bacteria (e.g. bacilli, clostridia). They are (e.g. *Lactobacillus curvatus*, *Lactobacillus sakei, Leuconostoc mesenteroides*), where the optimum is a temperature of 25 °C and a pH of 5.5. The occurrence of killer phenotype in yeasts is widespread in alcoholic fermentations for beverage production such as in breweries, saké, wine and recently in sugarcane producing plants. Killer yeast contain a toxin in their cell wall structure that allows them to kill toxin sensitive foreign yeast cells. Most killer strains of *S. cerevisiae* have good fermentation kinetics and, therefore, have a greater chance of dominating the fermentation. Many of above mentioned fermentative processes use non-pasteurised medium, which can allow the predominance of wild yeast strains coming from the raw material outnumbering the starter yeast. These contaminations can bring about the fermentation slowness or blockage, acidity increase, fusel oil production, and ethanol productivity decrease. So, the killer system may be a way to avoid the effects caused by undesirable yeasts in the fermentative processes. The utilisation of killer strains with good fermentative yields can bring undoubtedly advantages to the process, once it can guarantee competitive advantages to the starter ethanol-making yeast. We aimed to isolate killer yeast strains from the fermentative process for ethanol production, to evaluate both their fermentative efficiency and killer activity expression under industrial conditions during the fermentation process, in flasks and fermenter and to assess their potential competitive advantage assaying the sensitivity of a panel of fermentative industrial yeasts to the selected killer strains.

The presence of some fungi species on a variety of food products, like cheeses or cured meat products is beneficial for the ripening of the product and for the

development of specific flavour features. The utilization of these fungi as protective cultures, which are inoculated normally as asexual spores on the food products at the beginning of the ripening process, is becoming a usual procedure in the food industry. The fungal cultures also prevent undesirable fungi or bacteria from growing on the product. *Penicillium nalgiovense* is the most frequently used starter for cured and fermented meat products.

Studies conducted to reveal the influence of fungal starter cultures on growth and secondary metabolite production of fungal contaminants associated with cheese. Isolates of the species *P. nalgiovense*, *P. camemberti, P. roqueforti* and *Geotrichum candidum* were used as fungal starters. The species *P. commune, P. caseifulvum, P. verrucosum, P. discolor, P. solitum, P. coprophilum* and *Aspergillus versicolor* were selected as contaminants. The fungal starters showed different competitive ability on laboratory media and Camembert cheese. The presence of the *Penicillium* species, especially *P. nalgiovense*, showed an inhibitory effect on the growth of the fungal contaminants on laboratory media. *G. candidum* caused a significant inhibition of the fungal contaminants on Camembert cheese. The results indicate that *G. candidum* plays an important role in competition with undesirable micro-organisms in mould fermented cheeses. Among the starters, *P. nalgiovense* caused the largest reduction in secondary metabolite production of the fungal contaminants on the laboratory medium. On Camembert cheese no significant changes in metabolite production of the fungal contaminants was observed in the presence of the starters.

Unlike starter cultures which are introduced into the product, the effect of surface cultures is concentrated on the product surface. The following effects are important: better flavour (smell, taste), greater protection against drying out, prevention of light oxidation, greater competition to unfavourable micro-organisms, and more uniform appearance of the skin. Mould of the *Penicillum* genus (main example: *P. nalgiovense, P. chrysogenum*) is primarily used in surface cultures. Aspergilla are hardly ever added because of their potential to form mycotoxins. On the other hand, interest in the use of yeasts in surface cultures seems to be on the increase. The important point to keep in mind is that utilization of protective cultures cannot substitute GMP; it, however, offers an additional processing parameter for improving the safety and assuring the quality of a given food.

BASIC PRINCIPLES OF MICROBIAL RISK ASSESSMENT

Peter Quantick
University of Lincoln, Brayford Pool, Lincoln, United Kingdom

Risk assessment is one element of risk analysis; the other two, as described by the WHO, being risk assessment and risk communication and risk analysis is accomplished through the effective integration of these separate elements. Over recent years, Microbial Risk Assessment (MRA) has given way to Quantitative Microbial Risk Assessment (QMRA) as an important approach in dealing with food safety problems. Whereas MRA is used to evaluate the likelihood of undesirable health effects towards humans occurring after their exposure to pathogens, in QMRA, the likelihood or risk is expressed in quantitative, probabilistic terms. The main stages in risk assessment include hazard identification, exposure assessment, hazard characterisation (dose-response assessment) and risk characterisation. Some authors would include "statement of the problem" as the first stage whereas others would omit this stage but add risk management as the last stage. Challenges to the further development and use of QMRA routinely include data acquisition (data which is fit for purpose), suitable modelling tools which can be used to include dose-response data, processing data including thermal activation for specific pathogens, better education of researchers, scientists and food industry stakeholders so that future efforts are targeted to meet the development requirements of risk assessment models.

THE HYGRAM®-SYSTEM – A PRACTICAL TOOL FOR RISK ASSESSMENT IN THE FOOD INDUSTRY

Laura Raaska
VTT Technical Research Centre of Finland, Espoo, Finland

'Risk' in food safety is defined as the probability and severity of biological, chemical or physical agent in, or condition of, food with the potential to cause an adverse health effect. Risk assessment in the food industry consists of the following steps: Hazard identification (The identification of known or potential health effects associated with a particular agent.), Hazard characterisation (The qualitative and/or quantitative evaluation of the nature of the adverse effects associated with biological, chemical, and physical agents which may be present in food.), Exposure assessment (The qualitative and/or quantitative evaluation of the degree of intake likely to occur.), Risk characterisation (Integration of hazard identification, hazard characterization and exposure assessment into an estimation, i.e. a risk estimate, of the adverse effects likely to occur in a given population, including uncertainties.). Risk assessment in the food industry is, together with risk management and risk communication, a part of risk analysis process. Risk assessment has been used mainly at national and international level, but it may also provide an improved, a more comprehensive approach in food safety management programs of food plants. In Finland, food safety management programs, i.e. so called own-checking programs, consist of Hazard Analysis Critical Control Point (HACCP) -based system together with prerequisite programs, including training on hygiene and own-checking. Risk assessment can be qualitative or quantitative. Traditionally, a more qualitative, descriptive approach has been used in the food industry. Quantitative risk assessment provides, however, means of estimating the risk to consumer health caused by the product instead of the safety of the product. This approach is challenging, and better tools and information on performing the assessment are needed for food processors (Aarnisalo *et al*., 2008). Furthermore, information needed for performing the analysis, e.g. for estimating the dose-response is often lacking. Several computer programs have been launched for estimating bacterial growth and inactivation in different products (e.g. Pathogen Modeling Program,

PMP, (http://www.arserrc.gov/mfs/pathogen.html), Combase Predictor (http://www.combase.cc/predictor.html), and Seafood Spoilage and Safety Predictor (http://www.difres.dk/micro/sssp/)), and for helping in development of HACCP-systems or in performing quantitative risk assessment. A summary of these methods has recently been published by McMeekin *et al*. (2006).

CRAN Tools In the Nordic "Company Risk Assessment Network" (CRAN) project (2004–2007) a calculation software for assessment of number of bacteria along the processing line has been developed. The Calculation tool has an interface in Excel and the simulations are performed in Matlab. As a result change in bacterial number along the process chain, distributions of bacterial number after each process step and percentage of contaminated packages can be obtained. The project concentrated on dairy projects and by now the tool can be used for three specific processes: *Listeria monocytogenes* in soft cheese, *Bacillus cereus* in pasteurized milk and *Enterobacter sakazakii* in milk powder. Both the demonstration of the Calculation Tool and a PowerPoint based Decision Tool for dairy industry (work co-ordinated by Matforsk) are available at the projects internet-pages: http://www.sik.se/cran/.

Hygram®-2.0 Hygram®-2.0 is a semiquantitative model for assessment of most important hygienic hazards in companies (Hielm *et al*., 2006). This practical tool has been developed in cooperation between the Finnish Food Safety Authority Evira and VTT Technical Research Centre of Finland and has been finalized in 2007. Hygram®-2.0 is based on the previous Hygram® -version developed by Evira, VTT and Department of food and environmental hygiene at Helsinki University. A scientific article has been published about the model in 2003 (Tuominen *et al*., 2003). The model construction includes parts of microbiological risk assessment as modules, and it familiarizes the user to basics of risk assessment. The program consists of the following modules: Background module, 14 Hygiene modules and 14 Hazard modules, HACCP-module, Databank and Results module. Hazard modules contain modules for 11 pathogenic bacteria, moulds, viruses (norovirus) and a module for assessing physical and chemical risks. The user has also a possibility to create own hazards modules. The modules where hygienic practices, microbiological or other hazards are assessed, mainly account for the exposure assessment part of risk assessment. Risk estimates are presented as tables and illustrative figures. In HACCP-module it is possible to build and evaluate a HACCP-system. The model is freely available

for own use in the internet http://hygram.vtt.fi). Hygram®-2.0 can be used in the food plants, at retail shops and institutional kitchens for identification and assessment of risks and for assessment and development of own-checking plans and HACCP-systems. Additionally it can be used in training, documentation of audits and inspections and in collecting information for scientific research purposes. In developing this new version, special attention has been paid to user friendliness.

Further reading to this extended abstract can be found in the publications:

Aarnisalo, K., Vihavainen, E., Rantala, L., Maijala, R., Suihko, M.-L., Hielm, S., Tuominen, P., Ranta, J. & Raaska, L. 2008. Use of results of microbiological analyses for risk-based control of Listeria monocytogenes *in marinated broiler legs. Int. J. Food Microbiol., in press.*

Hielm, S., Tuominen, P., Aarnisalo, K., Raaska, L. & Maijala, R. 2006. Attitudes towards own-checking and HACCP plans among Finnish food industry employees. Food control, 17, 402–407.

McMeekin, T.A., Baranyi, J., Bowman, J., Dalgaard, P., Kirk, M., Ross, T., Schmid, S. & Zwietering, M.H. 2006. Information systems in food safety management. Int. J. Food Microbiol., 112, 181–194.

Tuominen, P., Hielm, S., Aarnisalo, K., Raaska, L. & Maijala, R. 2003. Trapping the food safety performance of a small or medium-sized food company using a risk-based model. The HYGRAM® system. Food Control, 14, 573–578.

PAST, PRESENT AND FUTURE OF HACCP SYSTEMS

Peter Quantick
University of Lincoln, Brayford Pool, Lincoln, United Kingdom

HACCP is a systematic method that serves as the foundation for assuring food safety in the modern world. HACCP is designed to control foodborne hazards form production, through manufacturing, storage and distribution of food products. HACCP originated in the late 1950's when NASA required food at the highest safety level for manned space flight and the system was developed in order to achieve this objective by the Pillsbury Company. The first public showing of an early form of HACCP took place in 1971 during the National Conference of Food Protection. Since that time, when there were only 3 principles, HACCP has developed and it is now defined as consisting of 5 preliminary steps and 7 principles. However, the basic philosophy of HACCP remains the same in that it does not rely on end product testing to ensure that the food is safe for consumer but instead builds food safety into the product through the manufacturing process. There is an increase in demand for foods to be safe by consumers and this, in recent years, relates as much to additives and allergens as it does to microbial contamination. This demand has lead food processing companies to develop food safety management systems which are based on or built around HACCP. There are numerous approaches world-wide including guidelines produced by the NACMCF, National Standards and other systems such as the BRC Global Standard and ISO 22000:2005.

VIRUSES AS A CAUSE OF FOODBORNE DISEASES

Ivan Psikal
Veterinary Research Institute, Brno, Czech Republic

Viruses cause many diseases of plants, animals and humans. They are strict intracellular parasites with cellular specificity, and their replicative cycle takes place in the host cell cytoplasm. Viral particles can be transmitted by different routes such as aerosol, subjects soiled with human or animal faeces, contact with blood of affected persons, contact with diseased animals, via contaminated food and water, sexual intercourse, or vectors such as gnats or ticks. Concerning the foodborne viral infections, people get infected orally, after ingestion of products contaminated during processing. Viruses causing foodborne disease attack living cells of the digestive tract and propagate inside them; subsequently they attack other cells of the digestive tract or enter other organs such as the liver or central nervous system and cause disease. The primary symptoms of viral foodborne disease and differences from bacterial infections are the following:

1. Only a few viral particles are necessary for the disease to develop.
2. High amounts of viral particles are further transmitted via faeces of infected people (up to 10^{11} particles per gram of faeces in members of genus *Rotavirus*).
3. Specific living cells are necessary for the replication; accordingly they cannot multiply in foods or water.
4. Foodborne viruses are relatively stabile and acid-resistant outside host cells.

Replication of viruses happens by transcription and translation of the viral genome using the equipment of the host cell. Therefore, it is not possible to commonly culture them in the environment free of living cells, and number of viral particles does not increase in food and water during production, processing, transport and storing. Sensory characteristics of products containing these pathogens and those of non-infected food are identical. Transmission of the virus does not only depend on its interaction with the host, but also on the influence of

external environment. Outside host organism, viruses appear like inert particles without their own metabolism. The longer they survive in the infectious status in the environment, the higher is the probability of transmission and spread of infection. Several groups of viruses may infect persons after ingestion and then are shed via stool. Of these, the *Rotavirus*, *Norovirus* and hepatitis A virus are currently recognised as the most important human foodborne pathogens with regard to the number of outbreaks and people affected in the Western world.

Rotaviruses belong to the family of *Reoviridae*, they are segmented bicatenary RNA viruses, which explains their genetic variability and the presence of mixed infections. According to the group and subgroup specific antigen, this genus is antigenically divided to serological groups, from A (with two to three subgroups and 11 serotypes) to E; *Rotavirus* F and *Rotavirus* G groups are provisional for the present. Groups A, B and C of human rotaviruses have been recognized. The viruses are not enveloped, and thus have a degree of robustness in the environment outside of a host. Rotaviruses can survive for weeks in potable and recreational waters and for at least four hours on human hands. The viruses are relatively resistant to commonly used hard-surface disinfectants and hygienic hand-wash agents. Their massive excretion, 10^8 to 10^{11} viral particles per gram of faeces, begins with the first day of diarrhoea. They are found in used water and can also be concentrated by shellfish; the environment thus constitutes a notable reservoir for the virus. Rotavirus is transmitted by faecal-oral contact and possibly by contaminated surfaces and hands. Numerous animal species are infected by rotaviruses which are distinct from the human ones. Human rotaviruses particularly group A are considered the main cause of viral gastroenteritis in infants and young children (from six months to three years of age) throughout the world. Antibodies against these viruses are present almost in all children under five years of age.

Noroviruses are frequent cause of sporadic cases and also of outbreaks of acute gastroenteritis in children and adults particularly in semi-closed environment such as schools, cruise ships, hospitals and residential homes. These pathogens had been viewed as exclusively human; however, viruses similar in morphological and molecular aspects have been detected in cattle and pigs. Factors that contribute to the significant impact of noroviruses include a large human reservoir, low infection dose (only 10 to 100 virions can cause the disease), and the ability to be transmitted by various routes. Water may be a

vector (in swimming pools, occasionally non-sufficiently treated potable water) and any food handled with soiled hands of an infected person. Viruses are present in faeces and vomitus of diseased people. Highly risky foods associated with noroviral infection are oysters and shellfish. These animals recruit their nourishment by filtration of ambient water and thus they can ingest small particles such as seaweeds and other micro-organisms including viruses. This virus is currently recognized as the cause of almost all outbreaks of non-bacterial gastroenteritis, particularly in Europe and Australia where there is active surveillance.

Hepatitis A virus (HAV), originally classified as enterovirus 72, belongs to the family Picornaviridae. HAV occurs as a single antigenic type; nonetheless, four human genotypes, and three genotypes naturally affecting other primates (chimpanzee, non-human primates) can be discriminated. HAV differs from enteroviruses by marked tropism to liver cells, exceptional thermostability (it survives heating for 30 min to 56 °C), acid-resistance (it tolerates pH 1) or slow replication without cytopathic effect on the host cell. The virus is most commonly transmitted via the faecal-oral route, either by direct contact with an HAV-infected person or by ingestion of HAV-contaminated food or water. Foodborne or waterborne HAV outbreaks are relatively uncommon in the USA. However, food handlers with HAV are frequently identified, and evaluation of the need for immunoprophylaxis and implementation of control measures are a considerable burden on public health resources. In addition, HAV-contaminated food may be the source of HAV for an unknown proportion of persons whose source of infection is not identified. The last large outbreak of hepatitis A in the Czech Republic occurred in 1979 when 28 880 persons became ill. The outbreak was associated with frozen products made of imported strawberries watered with sewage. Despite the fact that viruses are the most common pathogens transmitted via food (it is estimated that 66.6% of food-related illnesses in the United States is caused by viruses compared to 9.7% and 14.2% for *Salmonella* and *Campylobacter* respectively) no systematic inspection and legislation exist that would set up virological criteria for food safety, regarding the presence of viruses in the food chain. Accordingly, education of food industry managers, producers, distributors and consumers about hygienic regulations and conditions of food production and processing (the use of non-infected water for watering and food processing, clean utensils etc.) and particularly their compliance are essential.

INTRODUCTION TO THE GROUP WORKS

Ivan Rychlik & Gun Wirtanen*
Veterinary Research Institute, Brno, Czech Republic
* VTT Technical Research Centre of Finland, Espoo, Finland

In this group work you are grouped into four (4) working groups depending on your process expertise i.e. meat and fish, dairy, bakery and beverage as well as miscellaneous processes. You as group are jointly asked to address a specific topic. When working in groups you should address the following questions:

- What are the methods used for monitoring the microbial contamination in your company, laboratory and country?
- What are the potential issues in the microbial detection (e.g. sampling, time etc.)?
- What are the future needs for the monitoring of microbial contamination?
- What methods are used for risk assessment (at company-, laboratory- and/or country-level)?
- What kind of preventive measures (hygienic design, GMP, process design, cleaning and disinfection procedures, protective clothing etc.) are applied in your company, laboratory and country?

We hope that you have been able to find out as much information as possible in advance. You should be able to share your opinion with the other members in your group. Here at the seminar you will have to discuss these topics and you have to finalize the group work together with the other persons in due course after the seminar i.e. by end of November 2007. In the final group work report you will be asked to generate a more general group opinion.

PARTICIPANT ABSTRACTS

POULTRY, PORK AND FISH PROCESSING

MICROBIAL RISK ASSESSMENT IN CYPRIOT FOOD INDUSTRIES USING THE HYGRAM® PROGRAMME COMBINED WITH PRACTICAL IN PLACE HYGIENE INVESTIGATIONS

Savvas Gennaris
EPTA Laboratories and Veterinary Diagnostics, Nicosia, Cyprus
(visiting scientist at VTT Technical Research Centre of Finland, Espoo, Finland)

The hygiene in food industries is an issue of utmost importance since high hygienic standards assure the safety and quality of end products and therefore consumer's health. This study investigates the microbiological status of different food enterprises in Cyprus using various sampling and detection methods for environmental sampling within each industry and proceeds with conducting semi-quantitative risk assessment using the HYGRAM® model in order to show possible correlation between virtual and experimental approaches. The parameters examined in this study are sampling place topography e.g. contact and non-contact food surface and other process parameters such as temperature, humidity and pH. Each sampling place was examined for different types of spoilage micro-organism such as total bacteria, *Eneterobacteriaceae*, coliforms, *E. coli*, *Salmonella* spp., *Listeria* spp., *Bacillus cereus* as well as yeast and moulds. The following four sampling techniques where used: RIDA® COUNT (R-Biopharm AG), contact agars (Oxoid), transport swabs with charcoal (Labema), and non woven clothes. At the same time water, packaging material and final product samples were taken from each enterprise and analysed. The outcome will be a general overview of the hygienic status involving some main food pathogens of interest and the estimation of the risk due to the presence of microbiological hazards in different food stuffs. The different sampling techniques used in this study will be evaluated in order to indicate the most suitable for each circumstance, the efficacy of each method will also be reported. The results will be used to further evaluate the hygienic status of food enterprises and planned future research needs in that area. At the same the outcomes of these pilot studies can be used for risk management and educational purposes both from the enterprises themselves but also by competent authorities.

ANTIMICROBIAL RESISTANCE IN *CAMPYLOBACTER JEJUNI* ISOLATED FROM BROILER CHICKENS IN ESTONIA IN 2002–2003 AND 2005–2006

Mati Roasto, Kadrin Juhkam, Terje Tamme, Kristi Praakle*, Ari Hörman & Marja-Liisa Hänninen*
Estonian University of Life Sciences, Tartu, Estonia
* University of Helsinki, Helsinki, Finland

Our study was conducted in 2002–2003 and 2005–2006 to isolate campylobacters from a poultry production chain and determine the prevalence of antimicrobial resistance. All together we studied 167 *Campylobacter jejuni* isolates from broiler chicken of Estonian origin. The resistance to ciprofloxacin, nalidixic acid, tetracycline, ampicillin, and erythromycin occurred in 44.4%, 44.4%, 22.2%, 19.4%, and 16.6% among the 36 *C. jejuni* isolates in 2002 and 2003 using a disc diffusion method (Oxoid, UK) against ampicillin (25 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (30 µg), and tetracycline (10 µg) and E-test (AB Biodisk, Sweden) against ampicillin, ciprofloxacin, erythromycin and tetracycline. We found no simultaneous resistance, of isolated strains, to three or more unrelated antimicrobial agents. Resistance to one or more antimicrobials was detected in 24 isolates (66.7%). None of the chicken isolates were resistant to gentamicin. Over a 13-month period in 2005 and 2006 a total of 131 *C. jejuni* isolates were collected and their minimal inhibitory concentrations (MIC) were determined by a broth microdilution method (National Veterinary Institute, Uppsala, Sweden) against ampicillin, enrofloxacin, erythromycin, gentamicin, nalidixic acid and oxytetracycline. Resistance to one or more antimicrobials was detected in 104 isolates (79.4%). A high proportion of the isolates were resistant to enrofloxacin (73.3%) and nalidixic acid (75.6%). Multidrug resistance (to three or more unrelated antimicrobials) was detected in 36 isolates (27.5%), all of which were resistant to enrofloxacin. Our results showed that multidrug resistance was significantly associated with enrofloxacin resistance ($p<0.01$). The use of enrofloxacin may select multiresistant strains.

PREVALENCE OF *CAMPYLOBACTER* IN RAW CHICKEN MEAT OF ESTONIAN ORIGIN

Mati Roasto, Kristi Praakle*, Hannu Korkeala*, Priit Elias & Marja-Liisa Hänninen*
Estonian University of Life Sciences, Tartu, Estonia
* University of Helsinki, Helsinki, Finland

Thermophilic *Campylobacter* spp. contamination of raw chicken meat of Estonian origin produced in both a small-scale and a large-scale company was studied during 2000 and 2002. Altogether, 279 samples (90 originated from small-scale and 189 from large-scale company) were analysed. The isolation and identification of *Campylobacter* spp. carried out using the NCFA method, which includes the enrichment in Preston broth. 25 g of minced meat or skin and muscle taken from multiple point on the products was placed in sterile plastic bags and 250 ml Preston enrichment broth (Oxoid, UK) was added. The sample was stomached for 60 s using a Lab-Blender 400 stomacher. The enrichment broth was incubated in sealed jars for 24 h at 42±0.5ºC in microaerobic conditions. Preston enrichment media was plated out on selective modified Charcoal Cefoperazone Deoxy-cholate agar (mCCDA; Oxoid). The plates were incubated microaerobically at 42±0.5ºC and examined for growth after 48 h. Four typical colonies (if presented) were streaked on *Brucella* agar (Pronadisa, Madrid, Spain), and were identified using Gram stain, phase contrast microscopy for motility, oxidase and catalase test. One isolate from each positive sample was identified to the species level by using hippurate hydrolysis, and susceptibility to nalidixic acid (30 µl/ml). Agar plates were incubated under microaerobic conditions produced by the Campy-Gen™ atmosphere generation system (Oxoid). At least one colony from each sample was stored in glycerol broth (15% [vol/vol] glycerol in 1% [wt/vol] proteose peptone) at –70°C. *Campylobacter* spp. was found in 15.8% of the raw chicken products (breasts, carcasses, thighs and wings) tested. The prevalence of *Campylobacter* in the products of the small-scale company (35.6%) were significantly higher than in those originated from the large-scale company (6.3%) ($P < 0.001$). The chicken carcasses and wings (28% and 31.3%) had significantly higher contamination level than breasts and thighs (0% and 0%) ($P < 0.001$). Of 44 *Campylobacter*

strains, 75% were identified as *C. jejuni* and 25% as *C. coli*. In this study, Estonian raw chicken meat products of the small-scale company did show a higher prevalence of *Campylobacter* than similar products of the large-scale company.

SUSCEPTIBILITY IN *CAMPYLOBACTER* SPP. ISOLATED FROM ESTONIAN BROILER CHICKENS IN 2005 AND 2006

Terje Tamme, Mati Roasto, Kadrin Juhkam & Marja-Liisa Hänninen*
Estonian University of Life Sciences, Tartu, Estonia
* University of Helsinki, Helsinki, Finland

Resistance to one or more antibiotics was detected in 104 isolates (79.4%). Twenty isolates (15.3%) were resistant to three unrelated antimicrobials, thirteen isolates (10%) to four unrelated antimicrobials and three isolates (2.3%) to all tested antimicrobials. Enrofloxacin and nalidixic acid were regarded as one group of antimicrobials. Resistance of isolates to three unrelated antimicrobials was mainly to a combination of enrofloxacin/nalidixic acid, erythromycin and oxytetracycline (4.6%). Resistance of isolates to four unrelated antimicrobials was mainly to a combination of enrofloxacin/nalidixic acid, erythromycin, gentamicin and oxytetracycline (8.4%). Three isolates were resistant to five unrelated antimicrobials, comprising a combination of ampicillin, enrofloxacin/nalidixic acid, erythromycin, gentamicin, and oxytetracycline (2.3%). The highest frequency of resistance was to nalidixic acid and enrofloxacin (75.6% and 73.3%, respectively), followed by oxytetracycline (32.1%), erythromycin (19.8%), gentamicin (19.1%) and ampicillin (7.6%). Multidrug resistance (to three or more unrelated antimicrobials) was significantly ($p<0.01$) associated with enrofloxacin and nalidixic acid resistance. The level of antimicrobial resistance was higher for nalidixic acid in multiresistant *C. jejuni* strains than in non-multiresistant strains (Mann-Whitney test, $p=0.026$), while resistance for other antimicrobials was not statistically different ($p>0.05$) between multi- and non-multiresistant strains. The statistical analysis was performed with the Statistical Package for Social Sciences 13.0 for Windows (SPSS Inc.; Chicago, IL, USA) of individual results recorded using MS Excel 2003 software (Microsoft Corporation; Redmond, WA, USA). Non-parametric Spearman's rank order correlation coefficients with two-tailed p-values and odds ratios (ORs) were calculated for bivariate cross-correlations between resistances to the six antimicrobials analysed as well as between antimicrobials and multiresistance,

which was defined as resistance to three or more unrelated antimicrobials simultaneously. Furthermore, a non-parametric Mann-Whitney independent samples test was conducted to compare the level of antimicrobial resistance between multiresistant and non-multiresistant strains. All 131 *C. jejuni* isolates were tested for minimal inhibitory concentration (MIC) by a broth microdilution method obtained from the National Veterinary Institute in Uppsala (Sweden) against ampicillin, enrofloxacin, erythromycin, gentamicin, nalidixic acid and oxytetracycline. The *Campylobacter* isolates were first cultured on *Brucella* blood agar (Oxoid, Basingstoke, Hampshire, England) and incubated at 37 °C for 48 h. A loopful (1 µl) of bacterial growth was transferred to 10 ml of cation-adjusted Mueller-Hinton (CAMHB) broth (Oxoid) and incubated at 37 °C for 24 h to achieve a level of around 10^8 CFU/ml. The bacterial suspension was diluted to 10^6 CFU/ml. Bacterial suspension in portions of 100 µl was inoculated into the microtitre plate wells and the plates were incubated at 37 °C for 40 h at microaerobic conditions. The MIC was read as the lowest concentration completely inhibiting visible growth of *Campylobacter* spp. in accordance with the instructions given by the test manufacturer. Control of the purity of the bacterial suspension was carried out by plating 10 µl of bacterial suspension on *Brucella* agar. The density of the bacterial suspension was controlled according to the guidelines of the Estonian Veterinary and Food Laboratory, and colony counts from 50 to 250 per plate were accepted. *C. jejuni* ATCC 33560 was used as a control strain in the antimicrobial susceptibility testing. The following MIC breakpoints for resistance were applied: ampicillin 32 µg/ml, enrofloxacin 1 µg/ml, erythromycin 16 µg/ml, gentamicin 8 µg/ml, nalidixic acid 32 µg/ml and oxytetracycline 4 µg/ml.

MECHANISMS OF ANTIBIOTIC RESISTANCE OF *CAMPYLOBACTER* SP. FOOD-RELATED ISOLATES

Ana Kramar, Marija Kurinčič, Blaž Medja, Nika Janež, Tina Zorman, Katja Zelenik[1], Mojca Lušicky[1], Lieve Herman[2] & Sonja Smole Možina
University of Ljubljana, Ljubljana, Slovenia
[1] Institute of Public Health Maribor, Maribor, Slovenia
[2] Ministry of the Flemish Community, Institute for Agricultural Research, Melle, Belgium

The increasing antimicrobial resistance rates of micro-organisms especially in multi-drug resistance phenotypes, which are dispersed also among food-related bacteria, is a prompt world-wide problem. Resistant campylobacters are usually transmitted in the food chain by contaminated poultry meat and water. In this study, the resistance against ciprofloxacin, erythromycin and tetracycline, antibiotics of choice in human and veterinary medicine, was studied in sixty meat and water *Campylobacter* sp. isolates, which were collected from retail poultry and pork meat or from surface water in different Slovenian regions according to the ISO standard isolation procedure. For comparison, also some human clinical isolates were included in the study. Antibiotic resistance phenotypes were studied both by the reference agar dilution and by the broth microdilution method with CellTitre-Blue® reagent and automated fluorescence signal detection. The involvement of efflux pumps was evaluated by measurements with/without the efflux pump inhibitor phenylalanine-arginine ß-naphthylamide (PAßN). Beside classical phenotyping methods for species identification and resistotyping also restriction fragment length polymorphisms of specific amplicons (PCR-RFLP) and/or mismatch amplification mutation assay (MAMA-PCR) procedures were used for species identification and strain resistance characterization on genetic level (mutations in *23S rRNA, gyrA* and *TetO* genes). Good correlation was found between phenotypic and genetic results of phenotyping. Highly resistant strains were found among water and meat isolates, including multidrug resistant isolates. Efflux pumps activity as well as target mutations were confirmed as responsible mechanisms in resistant strains, especially in case of erythromycin resistant isolates.

POSSIBILITIES OF OZONE TREATMENT OF FOOD IN O3 FRESH INSTRUMENT

Jaroslav Šimek
Institute of Public Health, Ostrava, Czech Republic

O3 FRESH is an instrument intended for decontamination of food mainly fruits and vegetables in household. Decontamination effect is based on ozone treatment. Ozone is generated from water or from air. There are four programs according to intensity, three programs use water and one is without added water. The aim of this study was to evaluate decontamination effect of ozone treatment using O3 FRESH. For testing were chosen fruits e.g. apples and plums, vegetables e.g. tomatoes and food of animal origin e.g. chicken meat, bacon and cheese. The decontamination efficiency was evaluated according to the total microbial count and presence of pathogens (*Salmonella* spp. and *Listeria monocytogenes*). Apples, plums and tomatoes were treated using the two programs HEAVY with water and INTENSE without water and chicken meat, bacon and cheese only using the program INTENSE. Chicken meat, bacon and cheese were inoculated by *Salmonella enterica* CCM 4420 and *L. monocytogenes* CCM 5576. All the samples of apples, plums, tomatoes and chicken meat were analysed before and after the treatment for total microbes and *L. monocytogenes* and chicken meat, bacon and cheese for presence of *Salmonella* spp. using the ISO method: ISO 4833 for total count of microorganisms, ISO 6579 for *Salmonella* spp. and ISO 11290 for *L. monocytogenes*. Average number of microorganisms was in un-treated apples $8x10^1$, plums $2.8x10^5$, tomatoes $5.3x10^3$ cfu/g. No significant reducing of total count of microorganisms after treatment was not found. Differences between programs were not found either. Almost the same situation was in total count of microorganisms in chicken meat. Number of microorganisms before treatment was $1.4x10^6$ and after treatment $9.4x10^5$ cfu/g. Decrease of number of *L. monocytogenes* was evident in all food of animal origin. Average number of *L. monocytogenes* in un-treated food was $1.5x10^2$ cfu/g, after treatment this value was $<1x10^1$ cfu/g. *Salmonella* spp. was detected in un-treated and treated food. The effect of ozone treatment in O3 FRESH was evident on *L. monocytogenes* only. There was no influence of

treatment on reducing of total count of microorganisms and *Salmonella* spp. This study was carried out in cooperation with an import company. The testing will be continued.

DECONTAMINATION OF SALMON FILLETS BY USING NOVEL PROCESSING TECHNOLOGIES

Nil Pembe Özer & Ali Demirci*
Kocaeli University, Izmit, Kocaeli, Turkey
* Pennsylvania State University, PA, USA

Pulsed ultraviolet (UV) light and electrolyzed oxidizing (EO) water as novel processing technologies have been evaluated for inactivation of *E. coli* 0157:H7 and *L. monocytogenes* Scott A on salmon fillets. Salmon fillets inoculated with *Escherichia coli* 0157:H7 and *L. monocytogenes* Scott A were placed in the treatment chamber at 3 different distances (3, 5, and 8 cm) from the UV strobe for the pulsed UV-light treatment. The pulsed UV-light treatment was performed at each distance for 15, 30, 45, and 60 s. The inoculated salmon fillets were treated with acidic EO water at 22 °C and 35 °C in the EO water treatment using sodium hypochlorite solution (90 ppm free chlorine) as control at 22 °C for 2, 4, 8, 16, 32, and 64 min, respectively. Also, a response surface model was developed for alkaline EO water treatment followed by acidic EO water treatment to predict effective times in the range of 5–30 min and temperatures in the range of 22–35 °C for both alkaline and acidic water treatments. The experiments will be continued.

QUALITY CONTROL AT A SLOVENE MARINE AQUACULTURE PLANT

Irena Fonda
FONDA.SI d.o.o., Portorož, Slovenia

The family company is a marine aquaculture plant, with the capability for production of approx. 50 tons of best quality fresh fish and approx. 60 tons of fresh shellfish (*Mytilus*) per year. The aquaculture plant is situated in the environmentally unique costal area in the Gulf of Piran, Adriatic Sea, Slovenia, where it offers possibilities for economic development of the local community. Since 2003, when the farm was bought commercialisation of high quality products has started but it has not yet reached full production capacity. The company has gained governmental concessions for shellfish and fish production and use of the sea for a period of 20 years. Furthermore, the company has acquired a new plant as a centre for depuration and distribution of molluscs, which is built and equipped in accordance with the newest European requirements and regulations. Both plant facilities are under constant veterinary control of the Slovenian Veterinary Adminstration to ensure food safety. Risk assessment of microbial problems and preventive actions are crucial in our production lines. In the near future i.e. in 2 years time a doubling of the production of shellfish and fish and further modernisation and optimisation of cost effectiveness and competitiveness of the whole aquaculture plant is planned. Furthermore, efforts will be laid down to minimize the impact of the plant on the surrounding environment. A new distribution and production centre is also planned.

PARTICIPANT ABSTRACTS

DAIRY PROCESSING

HYGIENE SURVEY IN ESTONIAN DAIRIES WITH SEQUENTIAL PATHOGEN ANALYSIS

Helen Ehavald
AS Fazer Bakeries Estonia, Tallinn, Estonia

Milk contains low numbers of bacteria when it is milked from healthy cows. Drinking and fodder places in the cow shed, milking machines, utensils and equipment at farms, during transportation and in processing may add contaminants to the milk. Biofilm growing on surfaces are problematic in the food industry, because they are protective barrier for micro-organisms against sanitizers, and due to their formation cleaning can be more complicated. A hygiene survey was performed in three Estonian dairies, which were visited in spring 2004, to find out the hygiene level in the process environment. The production lines under observation were milk packaging lines, yoghurt lines and quark and rice based desert lines. Samples were collected from process environment, equipment, packaging material. Liquid and solid samples were taken from raw material, product from the process, final product, wastewater and water. Samples for detecting *Bacillus cereus*, *Listeria monocytogenes*, *Mycobacterium* spp., total bacteria count, *Enterobactriaceae*, coliforms, β-glucuronidase positive bacteria, yeast and mould, were collected from all dairies and analyzed. Gauze test, Rida Count and Hygicult tests were used for detecting bacteria from surfaces. Traditional cultivation techniques and DryCult test were used for liquid samples. Results were compared with each other. Equipment samples were both surfaces in direct and indirect contact with food. The result showed that food contact samples were less contaminated than the surfaces in indirect contact with food. Dairies should, therefore, pay more attention to cleaning equipment surfaces in indirect contact with food as contamination from non-contacted places (environmental and equipment) may be carried to food contact places and from there to the process line. Carriers can be for example employees' hands. Two *L. monocytogenes* positive samples were found in two dairies, both were from raw milk samples and one *L. seeligeri/ivanovii* was found from food contact equipment sample and 13 mycobacteria were found from all dairies, two of them were identified with

DNA sequence method. One was either *M. porcinum* or *M. fortuitum* (both risk group 2) and it was found from wastewater. The other was *M. phlei* and it was found from a final product. One final product was “very contaminated” with aerobic bacteria and two material samples were “contaminated” according to Finnish regulation.

ENHANCED MONITORING OF MICROBES ON PROCESS SURFACES

Marite Mets, Liina Kutsar, Tiina Veskus, Marjaana Rättö*, Satu Salo* &
Gun Wirtanen*
Tallinn University of Technology, Tallinn, Estonia
* VTT Technical Research Centre of Finland, Espoo, Finland

The objective of this study was to determine the hygiene of Estonian dairies and also monitor the adherence of microbes on surfaces. The aim was to identify aerobic count bacteria, yeasts and moulds, *Enterobacteriaceae*, coliforms, *Listeria* spp. and pathogenes *E. coli*, *L. monocytogenes* and *Bacillus cereus* on several surfaces in dairies. Direct food contact surfaces, indirect food contact surfaces, environmental surfaces were under observation. Surface samples were taken using direct contact methods – three different types of contact plates (Chromogenic *E.coli*/Coliform, Chromogenic *Bacillus cereus* and Chromogenic *Listeria* spp. contact plates) and petrifilms (Aerobic Count 3M™, *Enterobacteriaceae* 3M™ and Yeast and Mould 3M™), non-woven cloths and special *Listeria* Isolation Transport swabs. Additionally, water, raw material and product samples from dairies were investigated. The microbes in water samples were analysed by using filtering and petrifilm methods. Raw materials and products were analysed for aerobic bacteria (ISO standard 4833), yeast and mould, *Enterobacteriaceae*, *E. coli* and for *Bacillus cereus* (ISO standard 7932) using cultivation technique. *L. monocytogenes* was detected in surface samples, water, raw material and product samples using *L. monocytogenes* traditional method (ISO standard 11290) and PCR detection according to the instructions on iQ-Check™ *Listeria monocytogenes* II Kit. The quality of air in factories is also a significant hygiene factor in food processing. For this reason, aerobic bacteria and yeasts and moulds were determined in air samples. Samples were collected by using Microbiological Air Sampler MAS and Airscanner MD8. Dilution and cultivation technique was carried out to analyse the samples. The results of this study show that dairies kept the hygiene of direct food contact surfaces but it was noticed that the hygiene operations on indirect food contact surfaces in the dairy plants are inappropriate. Generally, the amount of aerobic

bacteria, *Enterobacteriaceae* and yeast and moulds was lower on direct food contact surfaces than indirect contact surfaces. These results also showed that the hygiene of hands and clothing of the personnel is at a very low level with several cases showing very high levels of microbes. On a whole, the pathogen risk in equipment, which are difficult to clean, was elevated. The results of the environmental samples indicated problems, because in several factories noticeable growth of microbes was detected on hand washing sink, control switches and drains. In addition, the pathogen risk in such places was quite high, e.g. *L. monocytogenes* was found from drains in several cases. The dairies must pay more attention to the quality of air in the factory, because the survey shows that the amounts of aerobic bacteria and yeast and moulds are high in the air. In conclusion, the hygiene in Estonian dairies express fairly auspicious level both on product contact surfaces and in products, but there is also need for improvements to achive better hygiene in the whole food production environment. The survey and results of this study are useful for EU projects to compare the hygiene condition in different EU countries.

VALIDATION AND DEVELOPMENT OF CLEANING SYSTEMS IN DAIRIES

Liina Kutsar, Marite Mets, Tiina Veskus, Marjaana Rätto*, Satu Salo* & Gun Wirtanen*
Tallinn University of Technology, Tallinn, Estonia
* VTT Technical Research Centre of Finland, Espoo, Finland

Hygiene has an important role in food industry to produce healthy and high quality products. Therefore, effective cleaning systems are irreplaceable to provide hygiene design in dairies. The aim of this study was to investigate hygiene situation before and after improvement in the hygiene management in four Estonian dairies. To make improvement dairies had used ultrasound equipment and personnel had practical hand hygiene course. The literature part of this study gives overview about microbes in focus (*Enterobacteriaceae, Escherichia coli* and *Listeria monocytogenes*). Secondly detection and identification of pathogens from dairies industry and removing of biofilms are described. In addition to basic cleaning methods also advanced cleaning systems like ultrasound, ozone and UV cleaning methods are introduced. On the last point, this study presents information about common hygiene problems, cleaning procedures for equipment and quality control systems in dairies. In this study following microbes were identified in dairies: aerobic count bacteria, yeast and mould, *Enterobacteriaceae*, coliform, *E. coli*, *Listeria* spp., *L. monocytogenes* and *Bacillus cereus*. Microbiological samples were taken aseptically from surfaces with 3 various contact plates (Nunc™ with Oxoid chromogenic agars for detection of *E. coli*/coliform, *Listeria* spp. and *B. cereus*) and 3 various Petrifilms (3M Microbiology Products; Aerobic Count 3M™ Petrifilm, *Enterobacteriaceae* 3M™ Petrifilm and Yeast & Mould 3M™ Petrifilm), non woven cloths (Tendra) and *Listeria* Isolation Transport swabs (Medical Wire & Equipment). The following microbes were determined from water samples: aerobic bacteria, yeast and mould, *Enterobacteriaceae*, *E. coli, B. cereus* and *Listeria* spp. Air, raw material and product samples were also analysed for the same type of microbes. Contact plates and Petrifilms from the surfaces were incubated and typical colonies were counted. Water samples were analysed by

filtering water sample through filters (pore size 0.45 μm) which were placed in different agar plates and incubated. In addition water samples were analysed by cultivating on Petrifilms. Results based on counting typical colonies and calculated to cfu/ml. Results of surface samples were divided into three groups: indirect product contact surface, direct product contact surface and environmental surface. Direct product contact surfaces showed cleaner results than indirect product contact surfaces. Most problematic results were detected from environmental surfaces. The water quality was a serious problem only in one dairy. The majority of raw material and product samples indicated good hygienic condition. Result of this study showed in generally better results of aerobic bacteria and *Enterobacteriaceae* after improvement. On the other hand there was noticeable increase of pathogens; especially *L. monocytogenes* was detected in many cases after the improvement, which most probably is due to the fact that *L. monocytogenes* has better growth conditions when there are less *Enterobacteriaceae* present. In conclusion hygiene, including cleaning systems in Estonian dairies was in good condition, but improvement could be made especially in the production environment.

DETECTION OF *ESCHERICHIA COLI* O157: H7 IN FOODS BY IMMUNOMAGNETIC SEPARATION AND POLYMERASE CHAIN REACTION TECHNIQUES

Elżbieta Maćkiw & Piotr Bartosz
National Food and Nutrition Institute, Warszawa, Poland

This study was carried out to investigate the detection levels of *Escherichia coli* O157: H7 in foods by immunomagnetic separation (IMS) and polymerase chain reaction (PCR) techniques. The methodology was set up to determine the detection levels of the *E. coli* O157: H7 in foods. Raw ground beef was inoculated with 10^1, 10^2, 10^3, 10^4 and 10^5 organisms of *E. coli* O157, which were cultured in mTSB with novobiocin (Fluka) according to the standard method EN ISO 16654 'Microbiology of food and animal feeding stuffs-Horizontal method for detection of *E. coli* O157', in 10 g to test the sensitivity of the method. IMS was performed in duplicate using immunomagnetic beads coated with an antibody against *E. coli* O157 (Dynabeads anti *E. coli* O157, Dynal). The first sample from IMS was spread onto SMAC-CT (Fluka, Merck) and HiCrome™ Mac Conkey-Sorbitol Agar. Presumptive *E. coli* O157 isolates were confirmed by latex test (Lateks *E. coli* O157, Biomex). The second sample from IMS was used for the PCR reaction. Bacterial DNA was isolated by chelex. Analysis by PCR with primers AE 22 and AE 20-2 was used to detect the presence of gene eae (397 bp). The PCR samples were subjected to 94 °C for 2 min and then 35 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 60 s, and extension at 72 °C for 60 s. Following the 35 cycles, a final extension for 10 min at 72 °C was carried out. PCR products of the required size (397 bp) indicated a presence of the target gene eae. *E. coli* O157 of serotype O157:H7 is a dangerous pathogen with a very low infectious dose. Several foodborne outbreaks of *E. coli* O157:H7 have been linked epidemiologically to the consumption of ground beef, raw milk, poultry products and drinking water. Although several methods have been used for the detection or enumeration of *E. coli* O157 cells in water and foods, the time and accuracy limitations of these methods suggest a need of rapid, specific methods. The results were obtained faster using IMS combined with PCR than with IMS with SMAC-CT and HiCrome™. The detection based on IMS-PCR has a low limit (1–10 cfu/10 g) of *E. coli* O157: H7 in raw ground beef.

NEEDS FOR KNOWLEDGE IN MICROBIAL RISK ASSESSMENT AND RISK MANAGEMENT IN DAIRY PROCESSING

Mateja Juvančič
Ljubljanske mlekarne, Ljubljana, Slovenia

In a dairy industry, we are every day challenged by micro-organisms trying to spoil the products produced. In the work we are thus confronted to find the infection sources. The EU Regulation on microbiological criteria for foodstuffs published in late 2005 deals with numerous new requested analyses. The new hygiene standards that are due to big crises i.e. BSE and chloramphenicol are a challenge in producing fresh milk products. Just to focus on ice cream the requested analysis on enterobacteriacea has resulted in big challenges in our ice cream department, because the microbiological criteria are very stringent for old equipment. Therefore we need to obtain relevant information in risk assessment and management.

NEEDS FOR KNOWLEDGE IN MICROBIAL RISK ASSESSMENT AT COMPANY LEVEL

Karmen Dugar
Pomurske mlekarne, Murska Sobota, Slovenia

The company in which I am working is producing hard, semi-hard and cottage cheese and melted cheese, UHT treated products e.g. milk, cream and deserts, as well as butter, fermented products, milk powder and whey concentrate. In our company we need knowledge and experience with process related problem micro-organisms e.g. *Clostridium* spp., *Bacillus* spp., mould and yeast as well as methods for fast detection and techniques to destroy these micro-organisms. Furthermore, basic principles in microbial risk assessment would help in finding the places in the production lines in which problems can be expected and how we efficiently can solve these problems through our control. Knowledge on viruses as foodborne disease causing agents is great importance in our quality control system. From this seminar I expect to obtain new knowledge which I be used in renewing the own-checking system in our production.

BEHAVIOUR AND CONTROL OF *LISTERIA INNOCUA* DURING MANUFACTURING AND STORAGE OF TURKISH WHITE CHEESE

Şebnem Öztürkoğlu, G. Candan Gurakan & Hami Alpas
Middle East Technical University, Ankara, Turkey

There is a need to assess the risk of their survival and growth of *Listeria* spp., because of the difficulty in preventing contamination of foods that are not treated to destroy listerial organisms just before consumption. In this respect, the effect of HACCP and risk assessment to reduce the level of *Listeria* contamination in Turkish White Cheese was investigated. The physico-chemical changes occurring in Turkish White Cheese and the survival of *L. innocua*, total aerobic count (TAC) and lactic acid bacteria (LAB) were investigated in artificially inoculated Turkish White Cheese during manufacturing and storage for 45 d. Turkish White Cheese was manufactured in a short-set, pilot-plant-sized procedure in vats in a commercial dairy plant. Pasteurized cow milk was inoculated with *L. innocua* to a final level of 3.84 (lowdose) and 7.12 (high dose) log10 CFU/ml. The bacterial load of inoculated milk, whey, post-ripened curd and post-salted cheese was determined during processing at 23 °C. Cheeses were stored in 16% saline solution at 4 °C up to 45 d. Samples were taken from each treatment and analyzed at 5-day intervals. Total reduction in viable cell numbers of *L. innocua* in Turkish White Cheese with each inoculum dose was approximately 2 log10 cycles during the storage period with a first-orderrate of inactivation at 4 °C. Variations in initial number of LAB in *L. innocua* seeded cheeses after overnight pressing were observed as 3.84, 4.24, 5.85 log CFU/ml at conditions of high-dose, low-dose inoculation of *L. innocua* and without inoculation as control sample, respectively. The salt concentration, pH, starter activity and storage time were determined to be the main causes of this reduction in LAB. The results had shown that *L. innocua* was able to survive during the manufacture and storage due to inadequate pasteurization or post-process contamination. The storage period has been defined as a critical control point (CCP) for consumption of Turkish White Cheese and calculated to be at least 90 and 178 d at refrigeration temperature, in low and high inoculum doses of *L. innocua*, respectively, for a

safe consumption. This study, which gives the result of behavior of low and high dose of *L. monocytogenes* during storage as CCP, can even guide the future risk assessment studies on Listeria in Turkish White Cheese. **Methods:** A loopful of *L. innocua* cells were added into 10 ml of BHIB and incubated at 37 °C for 24 h to give an initial load of 10.93 log10 colony forming unit (CFU)/ml. Pasteurized cow's milk was inoculated with *L. innocua* to obtain a final level of approximately 3–4 (low dose) and 6–7 log10 CFU/ml (high dose). Three batches of cheeses were produced. For the first batch, 2 l of pasteurized cow milk was inoculated with 1 ml of *L. innocua* to a final microbial load of around 4 log10 CFU/ml. For the other two batches, 2 l milk was inoculated with 1 ml of *L. innocua* to a final microbial load of around 7 log10 CFU/ml. The remaining 2 l milk was left to make cheese to be used as control and was not inoculated. Samples of 10 ml milk or 10 g cheese was taken from pasteurized milk, inoculated milk, whey, post-ripened curd, post-salted cheese and homogenized in 90 ml peptone water. Three replicates were carried out and experiments were repeated twice. **Results:** Three replicate samples of two independent trials from control (no addition of *L. innocua*), low- and high-dose *L. innocua* inoculated cheese samples were tested during the experiments. Counts from replicate plates were averaged and converted to log10 CFU/ml for regression analyses. Curves were fitted by linear regression program of Microsoft Excel. The inactivation time of *L. innocua* over weeks of storage in each inoculum dose was assessed by calculating the decimal reduction time (*D*10) values, which is defined as the negative reciprocal of the slope. Regression equations, coefficients and slopes were defined for all regression lines. **Conclusion:** In this study, the survival of *L. innocua* as an indicator of *L. monocytogenes* in Turkish White Cheese was studied. It was concluded that pH drop due to starter activity caused slight decrease in the number of *Listeria* cells after the addition of starter culture. However, cells decrease more effectively following the salt treatment. As a consequence, the number of *Listeria* can be reduced drastically by both salt concentration and starter culture activity with the effectn of ripening (storage) at refrigeration temperature. The presence of *Listeria* species in the production line indicates that post-processing contamination can occur. It is evident that development and use of the Good Manufacturing Practices (GMP) and hygienic rules as well as HACCP during handling and cheese processing are a must for all processing plants. This study, which gives the result of behavior of low and high dose of *L. monocytogenes* during storage as CCP, can even guide the future risk assessment studies on *Listeria* in Turkish White Cheese.

RESEARCHING THE ANTOGONISTIC EFFECT OF MOULD GROWTH ON THE CHEESE MICROFLORA DURING SURK CHEESE PRODUCTION (A TRADITIONAL TURKISH DAIRY PRODUCT)

Emine Aksan
University of Mustafa Kemal, Alahan, Antakya-Hatay, Turkey

In recent years, there has been growing interest in the use of protective cultures as natural biocontrol agents. The antagonism of the protective cultures refers to the inhibition of other microorganisms through competition for nutrients and/or by the production of antimicrobially active metabolites e.g. organic acids, carbon dioxide, hydrogen peroxide, diacetyl, ethanol, bacteriocins and reuterin. Several studies on the effectiveness of bacteria as protective cultures have been reported. The bacteria involved are mainly strains of *Lactobacillus plantarum*, *L. rhamnosus*, *L. sakei*, *L. paracasei* and *Propionibacterium freundenreichii subsp. shermanii*. However, antagonistic effects of yeasts against other yeasts are well known. It has also been reported that *Candida* sp. and *Kluyveromyces* repressed growth of *L. monocytogenes*. Nowadays, several authors have reported a protective effect of some of moulds against some unwanted micro-organisms. In several studies, *Penicillium* sp. mould growth on the surface of sausages has been used as protective cultures for production of raw dry sausages. Cultured moulds are also used in the production of certain foods e.g. cheese (*Penicillium* spp.), tempeh (*Rhizopus oligosporus*), quorn (*Fusarium venenatum*) and the black tea puerh. Surk, a mould surface ripened cheese from the Hatay region, manufactured from skim milk cheese, is made from diluted yoghurt through boiling with subsequent pressurization for 5–6 h to release water through a bag of cotton. Various spices including peppermint, thyme, cumin, black pepper, cinnamon, ginger (0.1%–0.3% each), chili pepper (2%) and salt (5%) are added to the skim milk cheese. Garlic (1%) can also be used in seasoning. The mixture is formed in a pear-like shape after kneading. The aim of this study was to determine the mould flora growing on the surface of the Surk cheese during ripening and the antagonistic effect of mould growth surface of Surk against other microflora in cheese. The results showed that *Penicillum* sp. was the most

frequently isolated mould from surface of Surk cheese. No antagonistic effect of the mould was determined against the aerobic mesophilic bacteria and yeasts on the Surk cheese, whereas the mould growth on the Surk surface showed antagonistic activity towards pathogenic bacteria.

PARTICIPANT ABSTRACTS

BAKERY AND BEVERAGE PROCESSING

ALICYCLOBACILLUS SPOILAGE OF SOFT DRINKS

Gregor Perne
Pivovarna Union d.d., Ljubljana, Slovenia

In our factory, beside beer, we also produce soft drinks and bottled drinking water. We are performing microbiological analysis of samples for detecting (1) beer spoiling bacteria and yeasts e.g. *Lactobacillus, Pediococcus, Pectinatus*; (2) soft drinks spoiling microorganisms e.g. moulds, yeasts, acidotolerant bacteria; (3) pathogenic microorganisms in water e.g. *Escherichia, Enterococcus, Pseudomonas.* We are achieving good results whit methods currently used, but we would like to compare our methods with others used in diferrent labs, especially comparison with quicker less labour demanding methods. I am also taking part in HACCP team, which assure us to produce as safe pruducts as possible. In future I would like to make more effort in validation of microbiological methods and possibly also acreditation of some methods. At the moment our biggest problem is spoilage of some soft drinks as a result of bacterium *Alicyclobacillus*. This are thermophilic acidotolerant bacteria, which spores survive comercial pasteurization and growth can be observed in final product. As a result product become hazy and/or an offensive chemical odour guaiacol is produced. Not all strains of genus *Alicyclobacillus* produce guaiacol. Until today only three such strains were identified, *A. acidoterrestris, A. acidiphilus* and *A. herbarius.* Other strains like *A. acidocaldarius* and *A. pomorum* apparently pose little to no risk of causing beverage spoilage. Because of that, we have lately improved our method for detecting bacteria in samples, and now we also use an enzymatic method for detecting if the strain is guaiacol positive or negative. We have confirmed that we get bacteria with raw material (shipments of sugar), but unfortunately until now, we could not find any supplier of sugar, that can guarantee us *Alicyclobacillus* free sugar. We have also found bacteria in our water for production of soft drinks. Because of that, we have just now decided, that we will install sterile filtration of final product. I think, we have acquired a lot of knowledge about *Alicyclobacillus* in past few months that we can share with others, that might face this problem in future. However, we are still very interested for any additional information considering this problem.

FOOD SAFETY AND HYGIENE ISSUES IN MANUFACTURING FROZEN BAKERY PRODUCTS AND DRY PASTA

Sebastjan Filip
Pekarna Pečjak d.o.o., Trzin, Slovenia

As food technologist and production manager in Pekarna Pečjak the daily work means confrontation with food safety and hygiene issues. Pekarna Pečjak is a private company manufacturing frozen bakery products and dry pasta and it is one of the largest food enterprises in Slovenia. The wide assortments of product comprise a good deal of raw materials of both animal and vegetable origin. It seems that the wide assortment of products is one of the main factors causing microbial hazard in our company. In spite of all knowledge about good manufacturing practice and good hygiene practice we must realize that established basic knowledge is not enough, e.g. *Salmonella* is not the only concern for consumers' health. A recent report published by EFSA reveals that in 2005 *Campylobacter* overtook *Salmonella* as the main cause of food poisoning in Europe. Therefore, new concepts in solving the problems are needed. Furthermore, the potential risk of mycotoxins must never be underestimated. Consequently new knowledge plays a vital role in safety and quality management of every food company.

ANTIMICROBIAL EFFECT OF NATURAL ANTIMICROBIAL AGENTS AGAINST FOODBORNE PATHOGENS

Erdal Eroglu, Hasan Ture, Ferda Soyer & Banu Ozen
Izmir Institute of Technology, Urla, Izmir, Turkey

Besides that foodborne infections and intoxications cause great danger for human health they have also adverse impact on the economics. Alternative food preservation techniques to produce safer foods e.g. systems containing natural antimicrobial agents have recently gained popularity. The consumers prefer foods with no chemical additives without shortened shelf-life. In this sense, natural antimicrobials have become promising alternatives for hurdle technology. However, most of the natural antimicrobials have limited spectrum of activity. Therefore, more efficient systems could be developed by using these antimicrobials in combinations. Activated lactoferrin (ALF) and rosemary extract, which are the agents used in this study have already been reported to be antimicrobially active against some selected microorganisms. ALF is an immobilized form of lactoferrin, and is present in almost all mammalian secretions e.g. milk, tears and saliva. Rosemary is a plant that has been used since ancient times as a spice and remedy for many illnesses. The aim of this study was to determine the individual and combined antimicrobial activities of these natural antimicrobials agents against *Salmonella enteritidis* and *Escherichia coli*. Minimum inhibitory concentration (MIC) value for each antimicrobial agent was determined by using 96 well-plate method. To obtain the inhibition profiles, a wide range of concentrations of each agent were tested on the bacteria at a concentration of $1x10^4$ cfu/ml. Data was collected every 2 h for a period of 24 h by turbidimetric measurements. ALF reduced the microbial load of *S. enteriditis* and *E. coli* at and above 2% and 1.5%, respectively. Rosemary extract showed no inhibitory effect against tested pathogens. However, these two natural antimicrobials acted synergistically when they were tested together and rosemary extract enhanced the activity of ALF at least four times. Result of this study indicates that use of ALF in combination with rosemary extract could be very effective in preventing food spoilage.

SHORT WAVE ULTRAVIOLET LIGHT (UVC) DISINFECTION OF SURFACE – INHIBITION OF *ALICYCLOBACILLUS ACIDOTERRESTRIS* SPORES ON AGAR MEDIUM

Ayşe Handan Dinçer Baysal & Sevcan Ünlütürk
Izmir Institute of Technology, Urla, Izmir, Turkey

Alicyclobacillus species are thermoacidophilic spoilage spore-formers found in soil. The spores can survive the normal hot fill processes that are carried out on commercial fruit juices. It seems likely that fruit in contact with soil are susceptible to contamination by *Alicyclobacillus.* Beverage ingredients e.g. liquid sugar are also a potential contamination sources. *Alicyclobacillus* has also been isolated from citrus processing lines. Ultraviolet irradiation is a potential alternative for chemical disinfection. Short-wave ultraviolet light irradiation (UVC) has been reported to be effective in inactivating bacteria that contaminate water and material surfaces. The effects of 254-nm UVC were investigated on spores of *A. acidoterrestris* DSM 3922 on agar plates. Agar plates inoculated with *A. acidoterrestris* spores in levels of 10^3–10^5 cfu, which were recovered from inoculated slants by washing the culture surface with sterile water, were subjected to UV light. The effects of UVC on the growth response of *A. acidoterrestris* spores were determined after different exposure times individually. The inoculation was made on medium containing glucose, yeast extract, KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, $FeSO_4.7H_2O$, $MgCl_2.4H_2O$, $ZnSO_4.7H_2O$, trace element and deionised water. The inoculated (10^3–10^5 CFU or spores/ml) plates were placed under the UV-lamp (30 W, 254.3 nm), and exposed to UVC treatment in intervals of 10 s to 5 min. Two plates were UVC treated, while one plate served as control. The UV intensity at the surface of the sample was measured using a radiometer with UVX-25 sensor (UVX, UVP Inc., CA, USA) calibrated by reference to a National Institute of Standards and Technology. The UV lamp was switched on for about 30 min prior to UV treatment of inoculated agar plates in order to minimise fluctuations in intensity. The UV intensity was kept constant at 1315 $\mu W/cm^2$. The UV irradiation of samples was conducted using a collimated beam apparatus

consisted of a UV-lamp (UVP XX-15, UVP Inc., CA, USA). The UV radiation was collimated with a flat black painted tube. The controls and UVC treated plates were incubated 43ºC up to 120 h and observed for colony formation. This experiment was repeated twice. UVC light treatment of inoculated plates revealed almost complete elimination (99.9%) of spores of *A. acidoterrestris* DSM 3922 at 1315 μW/cm^2. The results of this study indicate that UVC light inactivation can induce a 5-log reduction of spores of *A. acidoterrestris* on agar plates. In conclusion, UVC light can be used in disinfection of surfaces and fruit juice processing lines. Our results encourage further studies to elucidate the effect of UVC as surface disinfectant and to demonstrate inactivation of a wide spectrum of microbes using different UV-doses.

PROBLEMS WITH *AEROMONAS* IN BOTTLED WATER IN ISTANBUL

Fatma Köksal, Nalan Oğuzkurt* & Mustafa Samasti
Istanbul University, Istanbul, Turkey
* Istanbul Metropolitan Municipality, Istanbul, Turkey

The primary concern with consumption of drinking water in the world is still acute gastrointestinal disease. In this study, we evaluated the performance of water treatment works and drinking water samples in terms of indicator and pathogenic bacteria in Istanbul. Between October 2002 and April 2006, we collected a total of 2237 water samples derived from 134 raw water samples, 446 mains distribution samples from Omerli, Emirli, Elmali, Kagithane, Ikitelli and Bcekmece purification plants and 1657 network water samples. In these samples, faecal *Escherichia coli* and coliform bacteria and, additionally, *enteropathogenic E. coli* (EPEC) *Salmonella* sp., *Shigella* sp., *Vibrio cholerae*, *Vibrio* sp., *Yersinia enterocolytica, Campylobacter* sp., *Aeromonas* sp., *Pseudomonas* sp. and *Plesiomonas* sp. were investigated. The results were evaluated according to the Turkish standard (TS 266 – Regulation Concerning Water Intended for Human Consumption). It was found that 100% of the raw water samples contained coliform bacteria and 46% faecal *E. coli*. Additionally, *Aeromonas* sp., *Pseudomonas* sp., *Vibrio* sp., *Plesiomonas* sp., *Yersinia* sp. and *Shigella* sp. were positive in 97%, 67%, 8%, 5%, 1% and 1% of these water samples, respectively. In the sample from the main distributions from purification plants and networks water, it was found that 4% and 5% contained coliform bacteria, 1% and 2% faecal *E. coli,* 16% and 23% *Aeromonas*, 10% and 14% *Pseudomonas* strains, respectively. EPEC, *Salmonella, Shigella, Yersinia, Campylobacter, Vibrio* and *Plesiomonas* sp. were not detected in these samples. In conclusion, all of the raw water samples in Istanbul were found to be contaminated. On the contrary, it was observed that 95% of network water samples are safe according to TS 266. We determined that the water treatment work in purification plants is usually effective in terms of bacteriologic, but it is not sufficiently effective in removing *Aeromonas*, which might be a potential risk for public health.

BACTERIOLOGICAL EVALUATION OF BOTTLED WATER IN ISTANBUL

Fatma Köksal, Nalan Oğuzkurt* & Mustafa Samasti
Istanbul University, Istanbul, Turkey
* Istanbul Metropolitan Municipality, Istanbul, Turkey

The contamination of waterborne pathogens in drinking water is a serious public health concern. Thus, it is important to check the microbiological quality of drinking water. Our aim in this study was to investigate the samples of bottled (polycarbonate carboy) water used as alternative drinking water in Istanbul in terms of pathogenic bacteria. Between May 2005 and May 2006, we collected a total of 92 polycarbonate carboy (19L) water samples from companies bottling water in different districts. The samples were examined by membrane filtration method, and the results were evaluated according to the Turkish standard (TS 266 – Regulation Concerning Water Intended for Human Consumption). It was found that 54% contained coliform and 3% faecal *E. coli* of these samples. Additionally, 23% contained *Aeromonas* sp. and 38% *Pseudomonas* sp. Pathogenic bacteria e.g. enteropathogenic *E. coli* (EPEC), *Salmonella* sp., *Shigella* sp., *Yersinia* sp., *Campylobacter* sp., *Vibrio* sp. and *Plesiomonas* sp. were not detected. In conclusion, it was observed that 54% of polycarbonate carboy water samples are not safe according to TS 266. Additionally, *Aeromonas* sp. and *Pseudomonas* sp. were found in big amounts. Therefore, polycarbonate carboy water is a major risk in terms of public health.

MICROBIAL QUALITY CONTROL OF BAKERY PRODUCTS WITH FILLINGS

Martina Weiss
Žito Group, Ljubljana, Slovenia

In our microbiological laboratory on daily bases we control four major products. In bakery products we are focusing on moulds, especially on breads with longer freshness (for example: toast). Because we do not produce toast in sterile conditions and we do not add preservatives, but we use microwave pasteurization. Occasionally according to time of the year, we have problems with moulds. With thorough hygiene practice in the process of cooling and slicing of the toast and closer monitoring of the process of pasteurization we managed to reduce the number of reclamation of mouldy toast before the expiry date to less than 0.5% of our production. At the moment, we are in the middle of the study, how to prevent the ropiness in spoiled bread and which ingredient is the most critical and how to lower the number of *Bacillus subtilis* in those ingredients. Frozen dough based products do not represent a big microbiological hazard, because they must be cooked or baked before use. The fillings made from pasteurized curd, which is guaranteed by our supplier. Anyhow, precautions are always welcomed. Two of our dough products include fillings and one of the fillings is of animal origin. Most of the sweet fillings can be allergenic and can from that point of view represent a medical hazard. Meat is very hazardous ingredients, but we have solved the problem with using pasteurized meat filling. In production of fresh cakes-patisserie hygiene plays a very, very important role. The cleaning plan for all parts of the production is very thorough, because the food poisoning can easily become from sweets, candies, cakes, etc. In production of sandwiches the hygiene is also very important even though the shelf is only 3 days. The fresh cakes – patisserie and the sandwiches are the two types of products where the *Listeria monocytogenes* can cause a lot of problems. So within two mounts we are starting our own diagnostic of this micro-organism, following the ISO standard. The important task of my work is also to check the hygienic status of the production and to check if the cleaning plan is fulfilled. According to the plan, made at the

beginning of the year, I visit all our production facilities. At that time, we took some swabs, control the atmosphere and check if all cleaning and temperature evidence are fulfilled according to the plan. In our production the cleaning plan is dual. In some part of the industry we have dry cleaning (in bakery production and in some part of the production of frozen foods – preparing the dough) and in some part we have wet cleaning (in production of frozen food specially in the part, where filling comes into products and in some part of production of sweets and candies and in production of sandwiches). The mixture of those two ways of cleaning is sometimes difficult and demanding. It demands the coordination and good hygiene practice to fulfil all criteria for good and hygiene production.

PARTICIPANT ABSTRACTS

MISCELLANEOUS FOOD PROCESSING

SERVICE QUALITY OF THE PRIMARY SCHOOL CANTEENS IN ÇANKAYA DISTRICT OF ANKARA – ENCOUNTERED PROBLEMS AND SOLUTION SUGGESTIONS

M. Fatih Uyar
Hacettepe University, School of Health Technology, Ankara, Turkey

This research was planned and carried out in order to examine hygiene scores, service quality, physical conditions and personnel in primary school canteens as well as quality of the food and beverage served for pupils in these canteens in the Çankaya District of Ankara within the programme of Institutional Food Service Systems. Forty school canteens of total 104 state primary schools were included in this study. It was determined that physical conditions in the canteens were inadequate, and the personnel of these canteens do not perfectly take care of the personal, environmental and food hygiene. It was seen that only 17.5% of the employees use cap and bonnet properly, 55% of them do not know how to wash their hands properly. None of the school canteens use probe thermometers for measuring the internal temperature of potential hazardous foods at all critical control points in the intuitional food service (purchasing, delivery, storage etc.). Furthermore, twenty five percent of the personnel are not examined within appropriate intervals for necessary health tests. There is no possibility for having a bath (necessary for personnel hygiene) before and after working hours at all school canteens. It was also determined that the personnel do not have enough information about the importance of service quality of intuitional food service establishments, personal, environmental and food hygiene.

NITRIC ACID AS CLEANING SOLUTION IN INDUSTRIAL EQUIPMENT FOR PRODUCING BAKER'S YEAST

Roxana Ursu
Rompak, Pascani, Romania

In food processing industry, cleaning and sanitation are of prime necessity for routine work. In baker's yeast industry the cleaning in place (CIP) is performed before the production of each batch is stared in order to remove fermentation residues and dead yeast cells and thus to avoid bacterial contamination of the batch. CIP is done using a dilute caustic soda solution and hot water. The tank and pipes are first rinsed with hot water in a recirculation system with consequent cleaning for 50 min using 5–6% caustic soda solution. In the end, all equipment is rinsed with hot water for about 30 min. Regularly, once on every four batches the washing procedure also includes a 45 min cleaning with 1.7% nitric acid solution. The microbiological quality of 37 cream yeast batches, using two different cleaning methods for the industrial equipment before each batch was produced. Method A for 25 bathes was a CIP-procedure with 30 min rinsing, 50 min recirculation of caustic soda solution with a final water-rinsing for 30 min. Method B for 12 batches was as Method A extended with a 45 min recirculation of 1.7% nitric acid solution and a final 5 min water rinse. Microbiological samples for determination of total bacteria and coliform counts were taken aseptically after the complete fermentation program, which is approximately 15 h long. In the analysis we used Nutrient agar for determination of total bacteria and Endo agar for coliforms. Statistical methods were applied for interpretation of the microbiological results. Method A gave an average result of total bacteria as 179 cfu/ml and coliforms 10 cfu/ml whereas Method B gave the total bacterial count 70 cfu/ml and coliform count 1 cfu/ml. As conclusions we notice that method B (using HNO3 1.7%, after caustic soda solution) reduced the contamination of both total bacteria and coliforms. The efficiency of nitric acid is obvious. The cleaning operation must thus include a nitric acid phase every time to decrease the contamination level.

ANTIOXIDANT PROPERTIES OF NON-POLAR EXTRACTS FROM SELECTED CRETAN LAMIACEAE SPECIES

Vasiliki Lagouri, Angeliki Mpantouna & Prokopios Stathopoulos
Technological Educational Institute of Thessaloniki, Thessaloniki, Greece

This study was designed to explore the antioxidant properties of some endemic species e.g. members of the genera *Origanum*, *Thymus* and *Rosmarinus* that grown in Crete. The obtained information is of particular interest for small countries, such as Greece, because they may be further used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits. The non-polar fractions of the species *O. vulgare* ssp. *hirtum*, *O. dictamnus*, *T. vulgaris* and *R. officinalis* were extracted using hexane in a Soxhlet extractor for 6 h. The antioxidant activities were screened by measuring a) bleaching of β-carotene at 470 nm in the coupled oxidation of β-carotene and linoleic acid and b) peroxide values in a stability test using corn oil at 63 °C. In the case of β-carotene/linoleic acid emulsion system, oxidation of linoleic acid was inhibited by all the hexane extracts of the examined plant species, which is an important issue in food processing and preservation. The antioxidant concentration influenced the antioxidant power of the extracts (at the concentration 1 g/l the inhibition was 25–58% and at the concentration 4 g/l the inhibition was 45–87%). The antioxidant activity of plant extracts in comparison with those of synthetic and natural antioxidants BHT and α-tocopherol at 4 g/l decreased in the order: BHT ≈ *R. officinalis* ≈ *O. vulgare* › *T. vulgaris* ≈ α-tocopherol › *O. dictamnus*. Furthermore, the results from the stability test showed that addition of the plant extracts delayed effectively the oxidation of corn oil during storage at 63 °C and their antioxidant activity at 500 ppm was comparable to that of BHT at 200 ppm. Total phenol contents were determined using a spectrophotometric technique, based on the Folin-Ciocalteau reagent and calculated as gallic acid equivalents (mg GA/g extract). The phenol content of the plant extracts ranged from 7.3 to 39.5 mg gallic acid/g extract and may contribute to the overall antioxidant activity.

ANTIMICROBIAL PROPERTIES OF EDIBLE FILMS CONTAINING NATAMYCIN AND ROSEMARY EXTRACT

Hasan Ture, Erdal Eroglu, Banu Ozen & Ferda Soyer
Izmir Institute of Technology, Urla, Izmir, Turkey

Food quality and safety are major concerns for the food industry. In particular, fungal contamination occurring on the food surfaces during handling and storage is a major cause of spoilage for dairy products. Edible films have been utilized as packaging materials due to their favourable barrier properties against moisture, oxygen and aroma. Incorporating natural antimicrobial compounds into the edible films is an innovative method to extend the shelf-life of the product and to maintain the food safety and quality. Natamycin (NA) is a well known natural antimycotic agent used in food industry to retard the mould growth. Rosemary (*Rosmarinus officinalis*) extract (RE) is also known as a natural antimicrobial and antioxidant agent. The objective of this study was to asses the inhibitory activities of NA and/or RE added to edible methyl cellulose (MC) and wheat gluten (WG) films against *Aspergillus niger*. NA and RE were incorporated into MC and WG film forming solutions at various levels to add antimicrobial function to the resulting films. NA and RE were added to these films at concentrations of 0.2–40 mg/10 g film solution and 1.5–3 ml/10 g film solution, respectively. The antimicrobial activities of edible films were tested using agar diffusion method. Our data revealed that minimum inhibitory concentration of both films containing NA against *A. niger* was 2 mg/10 g film solution after 72 h incubation. The films containing RE did not show any inhibitory effect. However, synergistic effect was determined when the NA was used together with RE at the concentration of 1.5 ml/10 g film solution. Our findings indicate that MC and WG film containing NA and RE can be used to prevent mould spoilage of the dairy products.

EVIDENCE OF RE-OCCURANCE OF *LISTERIA MONOCYTOGENES* IN A FROZEN PEPPER FACTORY

Solmaz Lee
Food Research and Control Institute, Hurriyet, Bursa, Turkey

Pepper (200) and swab (50) samples from the production line were analysed for *Listeria monocytogenes* in a frozen pepper factory during the production season between June and October 2002. The findings reviled that, there were no *L. monocytogenes* in 2002 although, an exporting country detected *L. monocytogenes* from one of the exported frozen pepper product party earlier. The same plant was re-evaluated in 2003 and *L. monocytogenes* were detected from 7 of the 94 pepper and environmental samples in 2003. *L. monocytogenes* was again detected in 2007 at the same production plant when 100 product and 50 environmental samples were analysed; *L. monocytogenes* was detected from 12 samples; finished product as well as production line after the thermal processing step and from the plastic basket which was used mobile in the factory for carrying the diced paper. This is the evidence about re-occurrence of *L. monocytogenes* in the frozen pepper plant and it might be carried from one place to another by the equipment and tools used in the process.

DETERMINATION OF OCHRATOXIN A BY ELISA AND ISOLATION OF MOULDS FROM GRAPES AND RAISINS SOLD IN MARKETS AND BAZAAR

Işıl Var & Deniz Yalçındağ
University of Çukurova, Adana, Turkey

Recently, okratoxin A (OTA), which also is known as a mould toxin, was found in the important export products raisins and grapes. This is known as a great economic burden because many export products were returned due to detected OTA in the products. It has also become a health problem because OTA has carcinogenic and genotoxic effects. In grapes, climate, ripening, pesticides, harvest and storage conditions, food processing effect the amount of OTA from *Aspergillus* and *Penicillium* spp. In this research the quantity of OTA was determined and moulds were isolated from grapes grown and sold in Cukurova region and raisins sold in markets and bazaars. The results show that the amount of OTA in 22 raisin and 22 grape samples were 5.2–18.2 μg kg^{-1} and 0.2–0.7 μg kg^{-1}, respectively. OTA was thus found in excessive quantities above the Turkish legal limit which is 10 μg kg^{-1} in most of the raisin samples. The moulds *A. niger*, *P. citrinum* and *Moniliella acetoabutens* were isolated from the grape and raisin samples analysed.

THE MICROBIOLOGICAL QUALITY OF BLACK AND HERBAL TEA FROM TURKISH MARKETS

Neşe Asli Kisikkaya & Ceyda Pembeci Kodolbaş
Tübitak, Gebze, Kocaeli, Turkey

The increase in the consumption of tea has made their use a public health problem due to the possibility of the potential microbial contamination. For this purpose, a total of 230 samples were collected from Turkish markets and tea producing companies randomly within a period of 64 weeks and these samples analyzed for Total viable count (TVC), coliform bacteria, *Escherichia coli* and moulds.

One hundred thirty three samples of black tea (bagged tea – loose tea) include 19 different brands and 97 samples of herbal tea (sage tea, green tea, lime tea, rosehip tea, mint tea, fennel and mixed tea) which include 7 different brands were surveyed for microbial quality. In this survey, TVC, mould, coliform bacteria and *E. coli* were found to vary in the range of <10–1,10x10^9 CFU/g, <10–3.00x10^5 CFU/g, 0->1100 MPN/g and 0–1100 MPN/g respectively. Within all samples the most high levels of microbiological level were found for rosehip tea (TVC 1.10x10^9 CFU/g), for sage tea (mould 3.00x10^5 CFU/g), for loose tea (*E. coli* 1100 MPN/g). The load of the coliforms which is bigger than 1100 MPN/g, have been examined in brands of loose black and bagged tea, sage tea, green tea and fennel tea. When black loose (96) and bagged tea (37) samples were compared, no *E. coli* was detected in bagged tea. On the other hand, loose tea found to be contaminated with *E. coli* by 5%. Furthermore, from all loose black tea samples, 36% of TVC, 56% of mould, 49% of coliforms and 5% of *E. coli* microbial loads were found to be over the limits of Turkish Food Codex. Within all bagged tea brand this ratio determined as TVC (43%), mould (78%), coliforms (57%). Other all samples of herbal tea; TVC, mould, coliforms and *E. coli* having average percentage of %76, %40, 49% and 5% respectively and these ratios were found to be over the limits.

It is still an on-going debate that what should constitute the criteria's for the assessment of microbiological load of tea products in general. Most of the countries evaluate tea products as a high risk category for the reason of product

characteristic and way of process for consuming of tea. In Turkey, concerns about the microbial quality of loose black tea have been determined but there is still none microbial limits for herbal teas. Results from this study showed that there are insufficient hygienic parameters for tea products and also risks of mycotoxin presence due to existing mould load. Herbal tea which use as alternative of drugs and loose black tea should be free of microbial hazards in order to reduce the risks for consumers' health. For this reason, starting from the raw material until the consumption of the tea necessary precautions has to be applied to reduce the microbiological risks to an acceptable level.

GROUP WORKS BASED ON THE LECTURES, PARTICIPANT ABSTRACTS & PREPARATION BEFORE AND AFTER THE SEMINAR

MICROBIAL DETECTION WITH SPECIAL REFERENCE TO CURRENT ISSUES AND FUTURE CONSIDERATIONS IN THE FOOD INDUSTRY

Savvas Gennaris[1], Mati Roasto[2], Ivan Rychlik[3], Ayşegül Eyigör[4], Sonja Smole Možina[5], Cilka Vučak[6], Nil Pembe Özer[7], Kadrin Juhkam[2], Terje Tamme[2], Ana Kramar[5], Jaroslav Šimek[8] & Ziya Gökalp Ceylan[9]

[1] EPTA Laboratories and Veterinary Diagnostics, Nicosia, Cyprus
(visiting scientist at VTT Technical Research Centre of Finland, Espoo, Finland)

[2] Estonian University of Life Sciences, Tartu, Estonia

[3] Veterinary Research Institute, Brno, Czech Republic

[4] Uludağ University, Bursa, Turkey

[5] University of Ljubljana, Ljubljana, Slovenia

[6] Perutnina Ptuj d.d., Ptuj, Slovenia

[7] Kocaeli University, Izmit, Kocaeli, Turkey

[8] Institute of Public Health in Ostrava, Ostrava, Czech Republic

[9] Atatürk University, Erzurum, Turkey

INTRODUCTION

Harmful microbes may enter the manufacturing process and reach the end product in several ways such as through raw materials, through air in the manufacturing area, through chemicals used, through process surfaces and/or through factory personnel. Thus the environmental monitoring programs are getting more and more important in the food industry today. A good environmental monitoring program will include testing to verify that cleaning and disinfection procedures are keeping indicator organisms and any organisms of particular concern under control. This kind of monitoring is either done as a part of the company's internal control or by an external laboratory or by a competent authority as part of hygiene investigation or national surveillance program. Unfortunately it can not be considered as the most efficient and accurate method since only a small amount of the microorganisms present in the industrial environment can be sampled with the currently available methods and even a smaller amount can be isolated and identified with the standard usual

cultivation techniques. Therefore, the need of improving sampling and detection methods or even replacing them seems to be a critical future issue. Generally, the methods used for monitoring microbial contamination vary depending on whether we refer to company, laboratory or national level. In daily practice companies prefer simple and fast methods that can be easily performed during a production day. External laboratories or laboratories of food industries can use a greater variety of methods depending on microorganisms investigated and other needs. On national level the methods used for these purposes can also vary from simple to more sophisticated ones depending on the investigation. The five group work questions will be dealt with below:

1. *What are the methods used for monitoring the microbial contamination in your company laboratory and country?*

The methods used for monitoring the microbial contamination of processing environment can be divided into two main categories:

- → Cultivation methods e.g. swab, contact method, Petrifilm® and RODAC®
- → Rapid methods e.g. ATP bioluminescence and protein detection

The primary aims of monitoring of the food processing environment can be:

- → to measure the total bacterial population present in a production environment or in a sample,
- → to identify indicator microorganisms or
- → to look for specific pathogens.

The secondary aims of the monitoring are to:

- → ensure that the end-product meets customer specifications,
- → validate and verify HACCP programs,
- → determine the effects of any changes in the processing operation and
- → control the effectiveness of the cleaning and disinfection routines.

The following approaches may be used to verify the microbiological acceptability of the food processing equipment and environments (ICMSF, 1988):

- → sampling and testing equipment,

- → measuring microbial loads in food products after all steps in the processing, packaging, and handling are completed,
- → collecting and testing in-process samples,
- → collecting and testing samples from the food processing environment.

Environmental sampling and testing can be an early warning system to detect and eliminate niches of undesirable microorganisms before the risk of product contamination increases significantly. The environmental monitoring program should be designed to measure the occurrence and numbers of the normal spoilage flora and the pathogens that present the greatest risk to the product (Evancho *et al*., 2001). The Total Bacterial Count (TBC) or Aerobic Plate Count (APC) is a count of viable bacteria based on counting of colonies grown in various nutrient agar plates e.g. Nutrient agar and Plate Count agar. These are commonly employed to monitor sanitation efficacy, product self-life and hygiene in the process environment.

The term 'indicator organism' refers to the selected surrogate markers. The main objective of using bacteria as indicators is to reflect the hygienic quality of food. *E. coli* is commonly used as surrogate indicator as well as *Enterobacteriaceae*, *Enterococcus*, coliforms and fecal coliforms. The presence in food generally indicates unhygienic production or storage conditions. Substantial number of *E. coli* in food suggests a general lack of cleanliness in handling and/or improper storage. Specific pathogens on the other hand refer to bacteria that may cause foodborne illnesses. Mechanisms involved may be production of toxins in food or intestinal infections. The symptoms of food poisoning vary from nausea and vomiting caused e.g. by *Staphylococcus aureus*, through diarrhea and dehydration caused e.g. by *Salmonella* spp. and *Campylobacter* spp. to paralysis and death for severe infections caused especially *Clostridium botulinum* is involved. The infectious doses vary from less than 10 to more than 10^6 organisms depending on the microorganism involved.

The most frequently used monitoring methods of the food processing environment at company level as reported by all of our group members (SAFOODNET seminar, Istanbul, October 22–23) were agar contact plates techniques such as Hygicult® and contact plates in general filled in with the required amount of agar so that the agar surface is slightly projecting above the

edge of the dish. Moreover swabs either simple ones or with color indicators (*Listeria* Transwab®) were also often employed for this purpose. Regarding the detection and isolation of microorganisms, companies that have their own analytical laboratories perform the analyses according to ISO standard methods. Moreover, the ATP test was reported as an express method for monitoring microbial contamination in industry level. Application of ATP bioluminescence for monitoring sanitation and hygiene has gained some acceptance in the food industry and may offer some evidence of surface cleanliness and hygiene as well as providing a means to quickly validate that effective cleaning and sanitation have occurred. Protein detection tests were not used in represented countries. The main aim of using of most of previously mentioned techniques is to examine the hygienic status of surfaces either those in direct or indirect contact with food and to identify the areas where corrective actions are need to be taken.

Analytical laboratories performing the tests for sanitation effectiveness or environmental surface sampling are using mostly conventional swab techniques but may use Hygicult®, ATP bioluminescence and other express methods as well. In addition, laboratories might use variety of sample materials such as raw materials and final products, air/water/ice samples, animal tissues, egg shells, chicken dust, animal feces and blood, packaging materials, and even carcasses surface swabs in order to assess the hygienic level of foods and identify possible contamination routes. Generally, laboratories depending on the customer's needs or the investigation purposes may use apart from up to date ISO cultivation methods for conventional microbiology, serological tests and molecular techniques based on polymerase chain reaction (PCR) and real-time PCR, as well as more sensitive detection, including enzyme-linked immunosorbent assay (ELISA) or PCR-ELISA.

Both for scientific purposes and for tracing the sources and understanding epidemiology of *Campylobacter* molecular typing is increasingly being used (de Boer *et al*., 2000; Wassenaar & Newell, 2000; Nielsen & Nielsen, 1999). Various molecular sub-typing methods have been developed including pulsed-field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD) analysis (Hilton *et al*., 1997). Additionally, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the flagella (*flaA* and *flaB*) genes and amplified fragment length polymorphism (AFLP) are useful for epidemiological studies (Newell *et al*., 2000). In conclusion, methods

particularly useful for epidemiological studies are: PFGE, MLST, PCR-RFLP of the flagella (*flaA* and *flaB*) genes and AFLP (Hänninen *et al*., 2003; Dingle *et al*., 2001; Hänninen *et al*., 2000; Newell *et al*., 2000).

At country level all the above mentioned methods can also be applied. The method of identification/detection of choice depends on whether we need to identify the isolate to the genus or to more specific level, the proportion of negative samples expected, the number of species required to be detected, the cost in terms of staff-time, materials and equipment available. Furthermore, an important factor affecting the method of choice is whether pure cultures of strains are required for further examination, such as typing for epidemiological studies or examination for antimicrobial resistance. Generally, monitoring is planned in accordance with national and EU legislation and is further based on national needs and requirements. Examples of available ISO methods:

- → ISO 6579:2003 – Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.
- → ISO 11290:1:2000 – Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection methods.
- → ISO 11290:2:2000 – Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method.
- → ISO 7937:2004 – Microbiology of food and animal feeding stuffs – Horizontal method for enumeration of *Clostridium perfringens* – Colony count technique.
- → ISO 10272-1:2006 – Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method.
- → ISO 10273:1994 – Microbiology – General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica*.

2. *What are the potential (developed in the future) issues in the microbial detection (e.g. sampling, time etc.)?*

Generally when dealing with microbial detection we refer to two different entities which are sampling and isolation/identification. These entities should be addressed together in order to have the best applicable outcome. Therefore, a potential issue in microbial detection can be the improvement of the sampling methods. It is reported that only 20% of microorganisms can be loosened from the surfaces using conventional techniques and depending on the microbial flora even less might grow on agar provided. Therefore, these results represent only an indication of the hygiene level. There is a need for the development of more efficient isolation and identification methods with high throughput capacity. Furthermore, words such as speed, cost-efficiency and reliability should characterize the future detection methods. There are not many available techniques for detection of enteric parasites, viruses, or toxins, and those available are complicated to perform and expensive. Both sampling and isolation techniques should be characterized by some basic properties such as accuracy, precision and sensitivity which are thoroughly described below. In order to perform a proper monitoring it is important to start for a properly designed and standardized sampling plan. Moreover the accessibility to samples, the transportation (labeling, packaging, temperature, time), the handling of samples until they are analyzed and properly tested (time and materials) are issues that can be further developed and improved in the future. Furthermore, you must answer the 5 Ws and 3 Hs questions, which are given in the Table 1, in order to build up how representative samples are taken.

Table 1. Questions to be answered when building up sampling protocols.

1. Where to sample?	1. How long to sample?
2. What to sample?	2. How to sample?
3. Who is going to sample?	3. How many samples to take?
4. Why are you sampling?	
5. When to sample (day/night, month/season)?	

An issue of great importance is also the experience and training of the technical personnel who will perform the analysis. Adequately trained, well paid, loyal

personnel, working under good conditions is probably on of the main factor that will determine the success in detection and identification of microorganisms.

3 What are the future needs for the monitoring of microbial contamination?

A uniform and accredited international detection method scheme will also aid the international trade of products and goods. The development of cost-effective, reliable, fast, standardized monitoring methods is a future need to be addressed. As already mentioned the term of microbial contamination comprises of two main fields i.e. sampling and detection/isolation/confirmation. In order to have proper monitoring all methods used for this purpose should be characterized to find out if they suit the aims of the study:

- → Accuracy, which refers to the agreement between the analytical results and the "true value".
- → Precision, which is an agreement between independent analytical results.
- → Repeatability and reproducibility, which describe the minimum and the maximum variability in results. Repeatability is the variability of the measurements obtained by one person while measuring the same item repeatedly. This is also known as the inherent precision of the measurement equipment. On the other hand reproducibility is the variability of the measurement system caused by differences in operator behavior.
- → Specificity, which refers to the capacity of the method to identify the specific compound under study (poor specificity = false positives results).
- → Sensitivity, which refers to the ability to detect the lowest concentration of the compound under study (poor sensitivity = false negatives results).
- → Detection level, which refers to the lowest measurable concentration is another important characteristic that could be further improved.

4. Which methods are used for risk assessment in your company, laboratory and country?

Risk assessment for toxic and hazardous substances consists of four basic stages:

1. Hazard identification. During this stage it is found out which effects (if at all) the substance under question can elicit.

2. Dose-response relationship demonstration. Evaluation of the causal relationship between the exposure to a hazardous substance and the occurrence of adverse effects either on the individual or population level.
3. Exposure assessment. Determination of the level, incidence and duration of human exposure to the hazardous substance.
4. Risk characterization. Estimation of the incidence of adverse effects at various conditions of human exposure.

The risk of microbiological hazards is of immediate and serious concern to human health. Microbiological Risk Analysis is a process consisting of Risk Assessment, Risk Management and Risk Communication and it has the overall objective to ensure public health protection (WHO/FAO, 2006). The first of the three components, Risk Assessment, is the key element in assuring that scientific approach is used to establish standards, guidelines and other recommendations for food safety to enhance consumer protection and facilitate international trade. Only by using the principles of Risk Assessment the reaching to risk estimation and finally, of most importance, practical risk reduction will be achieved. It should always be kept in mind that risk analysis is not an one way procedure, various interactions occur between different levels and often revaluation might be necessary since new scientific data may become available, consumption patterns may change or new emerging hazards be identified. Although, risk assessment is an important process and can be considered as a subsequent step of microbiological monitoring, it is not generally widespread and not very well understood as a concept. The importance of risk assessment as a continuation of microbial monitoring can be understood from the questions that come up after monitoring, which are:

→ What do the results mean?
→ What is the risk of presence of a certain microorganism?
→ How much is too much?
→ What differentiates safe from unsafe, acceptable form unacceptable?
→ Which microorganisms are the potentially hazardous ones?
→ Which consumers are under greater risk than others?

These questions can only be answered by conducting a proper Risk Assessment. After performing proper Hazard Identification, one way to do that is by monitoring the microbial contamination, the next step is to proceed to Exposure

Assessment. This step indicates the extent of actual and anticipated exposure. From that point and on qualitative or quantitative description of the severity and duration of the adverse effects that may result from the ingestion of a microorganism or its toxin in food, the so called Hazard Characterization will finally lead to the Risk Characterization. It represents the integration of Hazard Identification, Hazard Characterization and Exposure Assessment determinations to obtain a risk estimate and provide a qualitative or quantitative estimate of the likelihood and severity of the adverse effects which could occur in a given population, including a description of the uncertainties associated with these estimates. Unfortunately since Risk Assessment is a procedure that requires the interactions from different scientific fields, it is not always easy to perform on national base or even company level. Therefore often the methods used for risk assessment within a company/laboratory or country include:

- → HACCP principles
- → ISO 22000/2005, BRC standards, national standards (TSI, TSE, ESA, ELOT etc.)
- → Company standards
- → Legislation – EU level and national level (EU directives and regulations; Directive 2003/99/EU, Food law, Regulation 2073/2005, Turkish Food Codex, etc.)
- → National monitoring data
- → Results from national laboratories and surveillance programmes
- → Recommendation and guidelines from WHO/FAO, CODEX, ICMSF and EFSA
- → Risk assessment studies conducted by WHO/FAO.

5. *What kind of preventive measures (hygienic design, GMP, process design, cleaning and disinfection procedures, protective clothing etc.) are applied in your company, laboratory and country?*

In order to minimize the risks and avoid those factors that weaken the safety of food different preventative measures can be applied. Generally, methods such as zoning (separation of clean and dirty areas, high and low risk areas), hygienic design of processing lines, use of proper protective clothing, appropriate

sanitation procedures, GMP (monitoring the quality of incoming and final products) and GLP (Good Laboratory Practices) are a few of applicable measures mentioned from the majority of the SAFOODNET seminar participating countries. It was noticed that less concern is given to the important regular training of personnel and control of visitors, which are issues that need to be addressed in order to reduce the risks.

References:

1. de Boer, P., Duim, B., Rigter, A., van Der Plas, J., Jacobs-Reitsma, W.F. & Wagenaar, J.A. 2000. Computer-assisted analysis and epidemiological value of genotyping methods for *Campylobacter jejuni* and *Campylobacter coli*. J. Clin. Microbiol., 38, 1940–1946.

2. Dingle, K.E., Colles, F.M., Wareing, D.R.A., Ure, R., Fox, A.J., Bolton, F.E., Bootsma, H.J., Willems, R.J.L., Urwin, R. & Maiden, M.C.J. 2001. Multilocus sequence typing system for *Campylobacter jejuni*. J. Clin. Microbiol., 39, 14–23.

3. Evancho, G.M., Sveum, W.H., Moberg, L.J. & Frank, J.F. 2001. Microbiological Monitoring of the Food Processing Environment. *In:* Pouch Downes, F. & Ito, K. (Eds.) Microbiological Examination of Foods. 4th ed. Washington, DC: American Public Health Association. Pp. 25–35.

4. Hilton, A.C., Mortiboy, D., Banks, A.G. & Penn, C.W. 1997. RAPD analysis of environmental, food and clinical isolates of *Campylobacter* spp. FEMS Immunol. Med. Microbiol., 18, 119–124.

5. Hänninen, M.-L., Perko-Mäkelä, P., Pitkälä, A. & Rautelin, H. 2000. A three-year study of *Campylobacter jejuni* genotypes in humans with domestically acquired infections and in chicken samples from the Helsinki area. J. Clin. Microbiol., 38, 1998–2000.

6. Hänninen, M.-L., Haajanen, H., Pummi, T., Wermundsen, K., Katila, M.-L., Sarkkinen, H., Miettinen, I. & Rautelin, H. 2003. Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organisms in three waterborne outbreaks in Finland. Appl. Environ. Microbiol., 69, 1391–1396.

7. ICMSF. 1988. ICMSF, Microorganisms in foods 4: Application of the hazard analysis critical control point (HACCP) system to ensure microbiological safety and quality. Palo Alto: Blackwell Scientific Publications. Pp. 93–116.

8. ISO 10273:1994 – Microbiology – General guidance for the detection of presumptive pathogenic *Yersinia enterocolitica*.

9. ISO 11290:1:2000 – Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1: Detection methods.

10. ISO 11290:2:2000 – Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 2: Enumeration method.

11. ISO 6579:2003 – Microbiology of food and animal feeding stuffs – Horizontal method for the detection of *Salmonella* spp.

12. ISO 7937:2004 – Microbiology of food and animal feeding stuffs – Horizontal method for enumeration of *Clostridium perfringens* – Colony count technique.

13. ISO 10272-1:2006 – Microbiology of food and animal feeding stuffs – Horizontal method for detection and enumeration of *Campylobacter* spp. – Part 1: Detection method.

14. Newell, D.G., Frost, J.A., Duim, B., Wagenaar, J.A., Madden, R.H., van der Plas, J. & On, S.L.W. 2000. New developments in the subtyping of *Campylobacter* species. In: Nachamkin, I. & Blaser, M.J. (Eds.) *Campylobacter.* 2nd ed. Washington DC: ASM Press. Pp. 27–44.

15. Nielsen, E.M. & Nielsen, N.L. 1999. Serotypes and typability of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry products. Int. J. Food Microbiol., 46, 199–205.

16. Wassenaar, T.M. & Newell, D.G. 2000. Genotyping of *Campylobacter* spp. Appl. Environ. Microbiol., 66, 1–9.

17. WHO/FAO. 2006. Food safety risk analysis. A guide for national food safety authorities. FAO Food and Nutrition Paper 87.

RISK ASSESSMENT OF MICROBIAL PROBLEMS AND PREVENTIVE ACTIONS IN DAIRY INDUSTRY

Esra Agel[1], Karmen Dugar[2], Mateja Juvančič[3], Liina Kutsar[4], Elżbieta Maćkiw[5], Marite Mets[4], Şebnem Öztürkoğlu[1], Işil Var[6], Emine Aksan[7], Tarik Öztürk[1], Mehlika Borcakli[1] & Gun Wirtanen[8]

[1] Tübitak, Gebze, Kocaeli, Turkey

[2] Pomurske mlekarne, Murska Sobota, Slovenia

[3] Ljubljanske mlekarne d.d., Ljubljana, Slovenia

[4] Tallinn University of Technology, Tallinn, Estonia

[5] National Food and Nutrition Institute, Warszawa, Poland

[6] School of Agriculture, University of Çukurova, Adana, Turkey

[7] University of Mustafa Kemal, Alahan, Antakya-Hatay, Turkey

[8] VTT Technical Research Centre of Finland, Espoo, Finland

INTRODUCTION

Safety of the food is a major issue for food producers. Affairs linked to food safety, to mention just acryl amide and BSE, have persuaded consumers that not everything the industry does is good. Producers, on the other hand, had or still have a difficult task to fulfil – they have to do their best to regain consumers' confidence. Few years ago, with a change of legislation, producers became fully responsible for their end products looking from quality and health point of view. Previously they were controlled from veterinarians on a regular basis and now they are only controlled periodically. For end consumer nothing has changed, he expects to buy nothing less than good and safe products. Producers have had to adapt to new rules quickly and efficiently and the HACCP procedures became a necessity and an obligation. The dairy producers are very well aware of the fact, that milk and milk products are extremely sensitive to microbial contamination since milk is almost an ideal nutrient medium for a wide range of micro-organisms. This is the reason, why hygiene standards in dairy plants are on a very high level. By following articles published it enhances the understanding of why microbial risk assessment and the preventive measures taken in a dairy are of great importance for consumers.

PATHOGENS IN MILK

The raw milk can be potential source of human pathogens. The most common human pathogens in milk are *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Listeria monocytogenes* and in some cases *Salmonella* spp. In the following chapters these pathogens are describes more thoroughly.

B. cereus is a Gram-positive, aerobic, spore-forming bacterium that is widely spread in the environment. *B. cereus* cells are large and motile. It is a potential food poisoning organism that can produce several enterotoxins and an emetic toxin causing diarrhoea and vomiting. *B. cereus* is a common contaminant in raw milk. *Bacillus* food poisoning usually occurs because heat-resistant endospores survive cooking or pasteurization and then germinate and multiply when the food is inadequately refrigerated. *Bacillus* sp. have also been found to be involved in biofilm formation in different dairy processes. Biofilms are serious concern to the food industry because of their strong adherence and high resistance to cleaning and disinfection procedures. Moreover, adhering bacteria may detach and cause further cross-contamination of products during processing. Contamination can occur along the whole processing line. *Bacillus* spores, even of mesophilic strains, are highly resistant and therefore they are of high importance in the milk industry where they can survive most of the heat treatment processes. The spores survive pasteurization and psychrotrophic strains of *B. cereus* limit the keeping quality of milk stored above 6 °C. *B. cereus* contamination of milk by has been shown to occur in the pasteurizer and in the filling machine. *Bacillus* species have frequently been isolated from various heat-treated milk products – pasteurized milk, milk powder, cheese and yoghurt. Different *Bacillus* species, and among them *B. cereus*, have been found on liquid packaging boards and blanks and these could thus be an additional source of contamination.

S. aureus is a spherical Gram-positive bacterium that on microscopic examination appears in pairs, short chains, or bunched in grape-like clusters. Some strains are capable of producing a highly heat-stable protein toxin, which is capable of causing illness in humans. Other salient characteristics are non-motile and asporogenous and may be present in young cultures. *Staphylococcus* species are aerobes or facultative anaerobes and have both respiratory and fermentative metabolism. *S. aureus* causes a variety of infections and toxinoses in humans.

S. aureus causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of superantigens into the blood stream. It is one of the most frequent bacteria isolated from raw milk. Human handlers, milking equipment, the environment, and the udder and teat skin of dairy animals are possible sources of bulk milk contamination. Staphylococcal strains may vary considerably in virulence and epidemiological potential. To control the spread of staphylococcal infections, sources of contamination and mechanisms of transmission must be identified. In processed foods in which *S. aureus* is destroyed by processing, its presence usually indicates contamination from the skin, mouth, or nose of food handlers. This contamination may be introduced directly into foods by process line workers with hand or arm lesions caused by *S. aureus* coming into contact with the food or by coughing and sneezing, which is common during respiratory infections. Contamination of processed foods may also occur when deposits of contaminated food collect on or adjacent to processing surfaces to which food products are exposed. When large numbers of *S. aureus* are encountered in processed food, it may be inferred that sanitation, temperature control, or both were inadequate. Raw milk and non-pasteurized dairy products may contain large numbers of *S. aureus*.

E. coli is described as a Gram-negative, non-sporforming rod, which is often motile, with peritrichate flagella. It is easy to cultivate on ordinary laboratory media, aerobic and facultative anaerobic. All species ferment glucose with the formation of acid or of acid and gas, both aerobically and anaerobically. All reduce nitrates to nitrites and are oxidase negative and catalase positive. Typically, they are intestinal parasites of humans and animals. Depending on the virulence genes acquired, different types of pathogenicity are conferred to certain strains of *E. coli.* These strains are classified as enteropathogenic *E. coli* (EPEC), entero-toxigenic *E. coli* (ETEC), entero-invasive *E. coli* (EIEC), entero-haemorrhagic *E. coli* (EHEC) and entero-aggregative *E. coli* (EAEC). The optimum temperature of *E. coli* is 35–40 °C. The optimal pH is 4.4–9.0. The pathogenic *E. coli* has an a_w of 0.95, it grows vigorously in 2.5% NaCl, slowly in 6.5% NaCl and not at all in 8.5% NaCl. Some reports indicate the growth of *E. coli* O157 in raw milk at temperatures as low as 7 °C (Heuvelink *et al.*, 1998) although only a 1.5 log increase occurred over a 144 h period. The milk should be stored under effective refrigeration (<8 °C) and the process equipment should be efficiently cleaned and disinfected to inhibit spread to further batches. Raw milk should be subject to routine monitoring for indicators of contamination

including *E. coli* or coliform bacteria, because microbiological surveys of raw milk frequently show high incidences of faecal contamination e.g. *E. coli*, but pathogenic strains are rarely found. It is principally exposed to contamination with faecal pathogens from the faeces of the cow, which contaminate the udder and teats of the cow and pass into the milk during the milking stage.

Although *E. coli* is a common and harmless member of the normal commensal microflora of the distal part of the intestinal tract of humans and other warm-blooded animals, some strains are responsible for causing severe illness, sometimes resulting in death. Production of cytotoxins, also referred to as Shiga toxins, is a common feature of VTEC, which may also be referred to as Shiga toxin-producing *E. coli* (STEC) and entero-haemorrhagic *E. coli* (EHEC). The very low infective dose of some VTEC, particularly VTEC O157: H7, underlines the importance of ensuring that the highest possible standards are maintained in agricultural practice and that food processors consistently operate well-designed and effective hygienic food production processes based on Hazard Analysis Critical Control Point (HACCP) assessments of each food process. In addition to attention to the detail of cleaning and hygiene procedures, the treatment and formulation of food products are important for controlling any residual *E. coli* and preventing their potential to cause harm to consumers. In the food industry, *E. coli* is commonly included in buying specifications relating to raw materials and finished food products as an indicator of the hygienic status of the food.

L. monocytogenes is widely distributed in the environment and occurs in almost all food raw materials from time to time. It is recognised that its presence in raw foods cannot be completely eliminated, but it is possible to reduce its incidence and levels in food products through the application of effective hygienic measures. *L. monocytogenes* grows in temperatures –0.4 to 45 °C, in pH range 4.4 to 9.4 and water activity (a_w) 0.92. The capacity to grow at low temperatures also means that refrigerated storage, while useful in slowing the growth of the organism cannot prevent it from increasing in number completely. Furthermore, it is essential to ensure that cleaning and disinfection are carried out properly. Currently, six clearly distinguishable species of *Listeria* are recognised: *L. monocytogenes*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, *L. ivanovii* and *L. grayi*. The most commonly occurring species in food are *L. innocua* and *L. monocytogenes.* *L. monocytogenes* is the main human pathogen of the *Listeria* genus. Individuals,

principally at risk from listeriosis, are pregnant women, elderly people, patients with cancer, organ transplant patients, patients with HIV and AIDS. *L. monocytogenes* can also cause a variety of infections. *L. monocytogenes* is transmitted via three main routes: contact with animals, cross-infection of new-born babies in hospital and foodborne infection.

L. monocytogenes is a relatively frequent contaminant of raw milk. *Listeria* spp. most commonly gain access to the milk from the cows' udder during milking. *L. monocytogenes* can be found in the faeces of animals. As with enteric contaminants, *Listeria* spp. can gain entry to the milk from faecal contamination of the udder. Hygienic milking practices involving udder and teat cleaning and disinfection can help reduce contamination of the milk. It is clear that eliminating *L. monocytogenes* from most foods is both impractical and impossible but it is possible to reduce and control this hazard in foods, thereby minimising the risk presented to public health. In order to ensure the safety of food products in respect of potential foodborne bacterial pathogens, growing, harvesting, handling, storage, processing and associated food supply systems must be managed by food producers and processors in such a way as to reliably control the growth of *L. monocytogenes* which must be prevented from multiplying to potentially harmful levels. It should be noted, however, that *L. monocytogenes* is known to grow at the temperatures used for refrigeration, although only slowly. The food industry always reacts to positive findings of the species, not specific serotypes of the species, and many manufacturers use the presence of *Listeria* spp. as a general indicator for the presence of *L. monocytogenes*.

METHODS FOR DETECTION OF FOODBORNE PATHOGENS

The detection of pathogenic bacteria is a key to the prevention and identification of problems related to health and safety. Currently in microbiology, beside classical or traditional methods, also new rapid methods are being applied. Traditional methods to detect foodborne bacteria often rely on growth in culture media in which the culture may require several stages to allow for the recovery of stressed cells and confirmation may require additional growth steps, followed by isolation, biochemical and sometimes serological identification. Overall these methods are labour intensive and time consuming. The methods, used for identification and characterization of micro-organisms (pathogens) are mostly

standardized (ISO methods). However, in the microbiological practice appear also the problems with these methods:

- → methods are time-consuming; traditional detection may take up to 7 or 8 d,
- → they are insensitive for detecting some organisms,
- → they fail to detect infections caused by uncultivable (e.g. novel) organisms,
- → visual appearance of micro-organisms is non-specific.

The increasing need for microbiological analyze in the food industry has not only served to expose further the limitations of the traditional techniques of food microbiology. It has also prompted the development of several novel and rapid methods for the enumeration and identification of micro-organisms in foods. The rapid detection of pathogens and other microbiological contaminants in food is critical for ensuring the safety of consumers. These methods can give results in 24–30 h. Recent advances in technology make detection and identification not only faster, but more sensitive and more specific than traditional method. The AOAC status of rapid tests is indicated for those methods that have been validated or evaluated by AOAC and have been adopted as AOAC Official methods. However, these methods continue to be modified or adapted, so that published information may not be the most current. Rapid methods are generally used as screening techniques, with negative results accepted as is, but positive results requiring confirmation by the appropriate official method, which, in many instances, is based on culturing. In many other instances, the rapid method has not been validated; therefore, the listing of a method or kit in this chapter in no way constitutes FDA recommendation or approval.

Experts, who in 1981 were asked about future developments in methods used for food microbiology, accurately predicted the widespread use of miniaturized biochemical kits for the identification of pure cultures of bacteria isolated from food. Most consist of a disposable device containing 15–30 media or substrates specifically designed to identify a bacterial group or species. With the exception of a few kits where results can be read in 4 h, most require 18–24 h incubation. In general, miniaturized biochemical tests are very similar in format and performance, showing 90–99% accuracy in comparison to conventional methods. However, kits that have been in use longer may have a more extensive identification database than newer tests. Most miniaturized kits are designed for enteric bacteria, but kits for the identification of non-*Enterobacteriaceae* are also

available, including for *Campylobacter*, *Listeria*, anaerobes, non-fermenting Gram-negative bacteria and for Gram-positive bacteria. Advances in instrumentation have enabled automation of the miniaturized biochemical identification tests. These instruments can incubate the reactions and automatically monitor biochemical changes to generate a phenotypic profile, which is then compared with the provided database stored in the computer to provide identification. Other automated systems identify bacteria based on compositional or metabolic properties, such as fatty acid profiles, carbon oxidation profiles or other traits. The potential applications of immunological and genetic techniques in food microbiology were not forecasted in the 1981 survey. During the 1980s, major advances in basic research were transferred rapidly to applied areas, as 'biotechnology' companies emerged and sought markets in the diagnostic field. DNA and antibody-based assays for numerous microbes or their toxins are now available commercially.

There are many DNA-based assay formats, but only PCR and bacteriophage probes have been developed commercially for detecting foodborne pathogens. Probe assays generally target ribosomal RNA (rRNA), taking advantage of the fact, that the higher copy number of bacterial rRNA provides a naturally amplified target and affords greater assay sensitivity. The basic principle of DNA hybridization is also being utilized in other technologies, such as the polymerase chain reaction (PCR) assay, where short fragments of DNA (probes) or primers are hybridized to a specific sequence or template, which is then enzymatically amplified by *Taq* polymerase using a thermocycler. Theoretically, PCR can amplify a single copy of DNA by a million fold in less than 2 h; hence its potential to eliminate, or greatly reduce the need for cultural enrichment. However, the presence of inhibitors in foods and in many culture media can prevent primer binding and diminish amplification efficiency, so that the extreme sensitivity achievable by PCR with pure cultures is often reduced when testing foods. Therefore, some cultural enrichment is still required prior to analysis. The highly specific interaction of phage with its bacterial host has also been used to develop assays for foodborne pathogens. One example is an assay for *Salmonella*, in which a specific bacteriophage was engineered to carry a detectable marker (ice nucleation gene). In the presence of *Salmonella*, the phage confers the marker to the host, which then expresses the phenotype to allow detection. The highly specific binding of antibody to antigen, especially monoclonal antibody, plus the simplicity and versatility of this reaction, has

facilitated the design of a variety of antibody assays and formats, and they comprise the largest group of rapid methods being used in food testing. There are 5 basic formats of antibody assays, the simplest of which is latex agglutination (LA), in which antibody-coated coloured latex beads or colloidal gold particles are used for quick serological identification or typing of pure culture isolates of bacteria from foods. A modification of LA, known as reverse passive latex agglutination (RPLA), tests for soluble antigens and is used mostly in testing for toxins in food extracts or for toxin production by pure cultures.

In the immunodiffusion test format, an enrichment sample is placed in a gel matrix with the antibody; if the specific antigen is present, a visible line of precipitation is formed. The enzyme-linked immunosorbent assay (ELISA) is the most prevalent antibody assay format used for pathogen detection in foods. Usually designed as a 'sandwich' assay, an antibody bound to a solid matrix is used to capture the antigen from enrichment cultures and a second antibody conjugated to an enzyme is used for detection. The walls of wells in microtiter plates are the most commonly used solid support; but ELISA tests have also been designed using dipsticks, paddles, membranes, pipette tips or other solid matrices. Antibodies coupled to magnetic particles or beads are also used in immunomagnetic separation (IMS) technology to capture pathogens from pre-enrichment media. IMS is analogous to selective enrichment, but instead of using antibiotics or harsh reagents that can cause stress-injury, an antibody is used to capture the antigen, which is a much milder alternative. Captured antigens can be plated or further tested using other assays.

Immunoprecipitation or immunochromatography, still another antibody assay format, is based on the technology developed for home pregnancy tests. It is also a 'sandwich' procedure but, instead of enzyme conjugates, the detection antibody is coupled to coloured latex beads or to colloidal gold. Using only a 0.1 ml aliquot, the enrichment sample is wicked across a series of chambers to obtain results. These assays are extremely simple, require no washing or manipulation and are completed within 10 min after cultural enrichment. The last mentioned 'category' of rapid methods includes a large variety of assays, ranging from specialized media to simple modifications of conventional assays, which result in saving labour, time, and materials. Some, for instance, use disposable cardboards containing dehydrated media, which eliminates the need for agar plates, constituting savings in storage, incubation and disposal procedures.

Others incorporate specialized chromogenic and fluorogenic substrates in media to rapidly detect trait enzymatic activity. There are also tests that measure bacterial adenosine triphosphate (ATP), which can be used to rapidly enumerate the presence of total bacteria.

APPLICATIONS AND LIMITATIONS OF RAPID METHODS

Almost all rapid methods are designed to detect a single target, which makes them ideal for use in quality control programs to quickly screen large numbers of food samples for the presence of a particular pathogen or toxin. A positive result by a rapid method however, is only regarded as presumptive and must be confirmed by standard methods. Although confirmation may extend analysis by several days, this may not be an imposing limitation, as negative results are most often encountered in food analysis. Most rapid methods can be performed in a few minutes up to a few hours, so they are more rapid than traditional methods. But, in food analysis, rapid methods still lack sufficient sensitivity and specificity for direct testing; hence, foods still need to be culture-enriched before analysis. Although enrichment is a limitation in terms of assay speed, it provides essential benefits, such as diluting the effects of inhibitors, allowing the differentiation of viable from non-viable cells and allowing for repair of cell stress or injury that may have resulted during food processing.

Evaluations of rapid methods show that some perform better in some foods than others. This can be attributed mostly to interference by food components, some of which can be especially troublesome for the technologies used in rapid methods. For example, an ingredient can inhibit DNA hybridization or *Taq* polymerase, but has no effect on antigen-antibody interactions and the converse situation may also occur. Since method efficiencies may be food dependent, it is advisable to perform comparative studies to ensure that a particular assay will be effective in the analysis of that food type. The specificity of DNA based assays is dictated by short probes; hence, a positive result, for instance with a probe or primers specific for a toxin gene, only indicates that bacteria with those gene sequences are present and that they have the potential to be toxic. But, it does not indicate that the gene is actually expressed and that the toxin is made. Likewise, in clostridial and staphylococcal intoxication, DNA probes and PCR can detect only the presence of cells, but are of limited use in detecting the presence of preformed toxins. Currently, there are at least 30 assays each for

testing for *E. coli* O157:H7 and for *Salmonella*. Such a large number of options can be confusing and overwhelming to the user, but, more importantly, has limited the effective evaluation of these methods. As a result, only few methods have been officially validated for use in food testing.

HYGIENIC DESIGN

Public concern about food safety and quality has increased dramatically in recent years. Taking into account the large variation of products and low consumer loyalty, products spoilt by microbiological growth can have a large impact on existing established products as well as on new products introduced into the market. In the past, hygiene in the food industry was largely a matter of experience, whereby a liberal use of cleaning agents and disinfectants often served to create a comfortable illusion of safety. Today, however the war on dirt and germs is a highly coordinated campaign, waged with weapons of science. Poor hygiene in food production can be down to negligence or design faults, so the correct choice machinery and methods of utilising it is essential. Also important is some understanding of the complex international rules, regulations, and standards governing the various hygiene issues. Planning and design can improve food hygiene by: reducing cross-contamination, reducing the likelihood of infestation and increasing the efficiency of equipment for hot and cold holding of foods.

Planning and design includes:

- → plan for linear workflow as far as possible,
- → plan work-centres and amenities,
- → planning of traffic between work-centres and stores,
- → systematically design, considering site, construction details, and services,
- → waste disposal areas, staff rest rooms and lavatories in the total design,
- → food hygiene aspects in total design,
- → analyze and control interactions between work centres,
- → separate ‘clean’ and ‘dirty’ areas and
- → economy of movement.

Equipment should be designed and constructed so that cleaning, maintenance, and inspection are facilitated. Hygienically designed equipment means: the

production which will remain clean during operation or which can be restored to the desired degree of cleanliness with the minimum cleaning effort. Machine designers should aim to reduce guarding but, where necessary, it should not interfere with cleaning. Far too often guards are hygiene hazardous, being made of close-fitting mesh which acts as a dirt and dust trap; guards must be designed with good hygiene. Different authors and advisory bodies list many desirable features for equipment but there is general agreement on key points. The seven basic principles for hygienic design by the Joint Technical Committee of the Food Manufacturers Federation (FMF) and Food Machinery Association (FMA) are:

- → All surfaces in contact with food must be inert to the food under the conditions of use and must not migrate to or be absorbed by the food.
- → All surfaces in contact with food must be smooth and non-porous so that tiny particles of food, bacteria, or insect eggs are not caught in microscopic surface crevices and become difficult to disledge, thus becoming a potential source of contamination.
- → All surfaces in contact with food must be visible for inspection, or the equipment must be easily disassembled for inspection, or it must be demonstrated that the routine cleaning procedures eliminate possibility of contamination from bacteria or insects.
- → All surfaces in contact with food must be readily accessible for manual cleaning, or if not readily accessible then readily disassembled for manual cleaning, or if clean-in-place techniques are used, it must be demonstrated that the results achieved without disassembly are the equivalent of those obtained with disassembly and manual cleaning.
- → All interior surfaces in contact with food must be so arranged that the equipment is self emptying or self draining.
- → Equipment must be so designed as to protect the contents from external contamination.
- → The exterior or non-product contact surfaces should be arranged to prevent harbouring of soils, bacteria, or pests in and on the equipment itself as well as in its contact with other equipment, floors, walls or hanging supports.

Hygienic and safety design of equipment includes:

- → suitable equipment and construction materials,
- → surface treatments, surface structures and internal angels,
- → dead ends in pipelines and dead areas in processing equipment,
- → ease of dismantling and re-assembly of equipment,
- → accessibility to process surfaces and components of food processing machinery,
- → accessibility to surfaces in indirect contact with food products and external surfaces,
- → possibilities for process control and cleaning operations,
- → suitable air conditioning system,
- → appropriate sewerage and waste management system and last but not least
- → design features for individual items of equipments:
  - o tanks and vats,
  - o pumps,
  - o gaskets, joints and seals,
  - o valves,
  - o fasteners,
  - o bearings
  - o motors,
  - o mixers and mixing paddles,
  - o mechanical separators,
  - o heat exchangers,
  - o product, water, steam and air pipelines,
  - o equipment transferring solid foods,
  - o size reduction equipment,
  - o control panels and switches,
  - o instrumentation and
  - o assembly of equipments.

PATHOGENS IN MILK

Milk is the most rigorously regulated foodstuff. Raw milk can be contaminated which can cause an illness with flu-like symptoms. Most of pathogen bacteria are destroyed when milk is pasteurised by heating and processing equipment is continuously cleaned and sterilised including devices and pressure sensors. These instruments must meet hygienic design requirements according to Good Manufacturing Practices (GMP), Good Hygienic Practices (GHP) and other Standards. Recent studies showed that the filling machine is the main source of recontamination. Some studies show that even a low initial number of bacteria adapted to growth in refrigerated milk can cause spoilage. In fact, contamination at levels < 10 cfu/ml of Gram-negative psychrotrophic bacteria (GNP) spoil the milk after 7–11 d of refrigerated storage, reaching about 10^7 cfu/ml when the generation time is 4–5 h. Furthermore, it was shown that the milk-spoiling GNP bacteria were present both in the air inside the filling machine and from water samples from different surfaces inside the machine. Bacterial samples from these locations were identified from the species of *Pseudomonas*, *Enterobacteriacae* and *Aeromonas*, which matched in RAPD typing with incubated samples of pasteurized milk. Bacterial aerosols may derive from sink and floor drains, water spraying and air conditioning systems. Furthermore, aerosols condensing when in contact with a cold surface may transfer the contamination within the filling machine and further into the package. As a consequence, it is regarded as best practice not to leave a water-hose on the floor or into a drain. Although after testing the bacteria it was not found bacteria from contaminated milk packages as coming from water hoses, there was a connection between bacteria in the waste water at the bottom of the filling machine and in water from other surfaces inside the machine. Of particular concern is the filler nozzles as these also have been identified in another study as the main recontamination source.

The potential contribution from the packaging material is mainly restricted to Gram-positive bacteria with little potential of growth at refrigerated temperature (6 °C).One major finding in the study by Eneroth *et al*. (1998) showed that the same bacterial type could be present in the filling machine over longer periods. In particular, the water at the bottom of the machine is of concern as the stationary flora it may support may derive by aerosols from elsewhere in the plant. However, Gram-positive spore formers may in addition follow another route of contamination due to their heat resistance. This route involves the

processing line with dead ends, pockets, corners, crevices, cracks and joints; and the hydrophobic properties of the spores of *Bacillus cereus* make them attach easily to surfaces of steel, glass and rubber. The demand for hygienic filling equipment in the dairy industry is increasing, and it is the responsibility of the supplier to ensure adequate hygienic design of their equipment. European legislation requires that handling, preparation, processing and packaging of food are done hygienically, with hygienic machinery in hygienic premises (see EC Directives 98/37/EC and EC Regulation 852/2004). It is, however, left to the industry to decide how to comply with these requirements. The European Hygienic Equipment Design Group (EHEDG) is a consortium of equipment manufacturers, food industries, research institutes and public health authorities, with the aim of promoting hygiene during the processing and packing of food products. This organization issues documents on hygienic design that are used as guidelines for standards produced by the European Federation of Standardization Institutes (CEN). EHEDG actively promotes global harmonization of guidelines and standards. The US-based organizations NSF and 3-A have agreed to cooperate in the development of EHEDG Guidelines and in turn, EHEDG cooperates in the development of 3-A and NSF standards. The EHEDG has issued the document 'Challenge tests for the hygienic characteristics of packing machines' (www.ehedg.org). This document gives proven methods for testing the performance of the various functions of packing machines, and thus provides the industry with independent criteria and challenge test methods to compare machinery from different suppliers. In order for a processor to evaluate different packaging systems, the nomenclature itself is not critical, but both hygienic challenge tests and criteria must be evaluated. The EHEDG has not defined the various filling machine classifications, and there is no common recommendation or legislation. It is therefore important that the supplier states the performance level based on a set of accepted challenge tests, and that the processor can independently compare different systems based on comparable test results. Upon delivery, a packing machine needs to be verified by a commissioning procedure to be agreed in advance between the food processor and the supplier of the machine. Commissioning may include physical as well as microbiological tests. Additional tests are specified for commissioning of machines for aseptic packing. The German Engineering Federation (VDMA) has joined forces with the Industrial Organisation for Food Technology and Packaging (IVLV) as well as other partners to collate the scientific knowledge available and make it accessible to the industry as a whole. The idea is to provide assistance and

advice on relevant laws and standards and how to implement them. Also, some devices are designed for detection and dry run protection highly polished for use in sensitive foodstuffs like milk.

PREVENTIVE MEASURES IN FOOD INDUSTRY

Food products are sensitive to micro-organism contamination. After contamination, foods provide an excellent environment for growth of bacteria. Bacterial contamination and growth is a problem because it may result in foodborne illness. To improve product safety, the food industries are adopting a process control system known as Hazard Analysis of Critical Control Points (HACCP). The HACCP system improves product safety by anticipating and preventing health hazards before they occur. But prior to HACCP system implementation in food manufacturing companies, there are some important issues and applications for supporting this system. Shortly we can say prerequisite programs (PRPs) are needed.

PREVENTIVE MEASURE 1 – PRPs

PRPs are procedures, including GMPs, Good Agricultural Practises (GAPs), Euro-Retailer Produce Working Group (EUREPGAPs) and Sanitation Standard Operating Procedures (SSOP) etc. that address operational conditions providing the foundation for the HACCP system. Infrastructure and maintenance programs are used to address basic requirements of food hygiene and accepted good (manufacturing, agricultural, hygienic etc.) practices of a more permanent nature; whereas operational PRPs are also used to control or reduce the impact of identified food safety hazards in the product or the processing environment. The HACCP plan is used to manage the CCP(s) identified to eliminate, prevent or reduce specified food safety hazards from the product, as determined during hazard analysis. During hazard analysis the organization determines the strategy to be used to ensure hazard control by combining the PRPs and the HACCP plan. This international standard requires from the organizations to identify, monitor, control and routinely update both the PRPs and the HACCP plan.

PREVENTIVE MEASURE 2 – HACCP & CCP DECISION TREE

HACCP is a system of extensive evaluation and control over an entire food production process for the sole purpose of reducing potential food-related health risks to consumers. HACCP program maintains safety and wholesomeness because potential hazards that may occur during processing are anticipated, evaluated, controlled and prevented. A hazard is defined as any biological, physical or chemical property that could cause a product to be unsafe for consumption. Processing plants are required to have a HACCP plan for each product. Once HACCP team has been identified, the team should use the basic principles of HACCP to design a plan. The basic principles of HACCP are listed in Table 1 and are briefly described below. The next and most important step is to determine the critical control points (CCPs) in the process, or the points at which a loss of control could result in a biological, chemical or physical hazard. In some cases, it may be difficult to decide if a processing step is a CCP. As a result, many companies introduce too many CCPs. A CCP decision tree, such as the one shown in Figure 1, can be used to identify CCPs. CCPs should be noted on the flow chart as part of the HACCP documentation.

Table 1. Principles of HACCP as defined by the National Advisory Committee on Microbiological Criteria for Foods.

Principle	HACCP Steps
1	Conduct an analysis of the hazards in your plant. Make a list of all processing steps where a hazard might occur.
2	Identify all critical control points (CCPs) in the process. CCPs are critical to the safety of the product.
3	Establish a critical limit for each of the identified CCPs.
4	Establish CCP monitoring requirements.
5	Establish corrective action to be taken if the CCP deviates from the critical limit.
6	Establish effective record-keeping procedures to document the HACCP program.
7	Establish a procedure to verify that your HACCP program is working.

PREVENTIVE MEASURE 3 – RISK ANALYSIS & RISK ASSESSMENT

Risk analysis is broadly defined to include risk assessment, risk characterization, risk communication, risk management, and policy relating to risk. In the estimation of the risks, three or more steps are involved, requiring the inputs of different disciplines. The first step, Hazard Identification, aims to determine the qualitative nature of the potential adverse consequences of the contaminant (pest, waste etc.) and the strength of the evidence it can have. The second step for Chemical Risk Assessment is determining the relationship between dose and the probability or the incidence of effect (dose-response assessment). The complexity of this step in many contexts derives mainly from the need to extrapolate results from experimental animals (e.g. mouse, rat) to humans, and/or from high to lower doses. In addition, the differences between individuals, due to genetics or other factors, mean that the hazard may be higher for particular groups, called susceptible populations. An alternative to dose-response estimation is to determine an effect unlikely to yield observable effects. In developing such a dose, to account for the largely unknown effects of animal to human extrapolations, increased variability in humans, or missing data, a prudent approach is often adopted by including safety factors in the estimate of the 'safe' dose, typically a factor of 10 for each unknown step. The third step, Exposure Quantification, aims to determine the amount of a contaminant (dose) that individuals and populations will receive. This is done by examining the results of the discipline of exposure assessment. As different location, lifestyles and other factors likely influence the amount of contaminant that is received, a range or distribution of possible values is generated in this step. Particular care is taken to determine the exposure of the susceptible population(s). The results of the three steps above are then combined to produce an estimate of risk. Because of the different susceptibilities and exposures, this risk will vary within a population. The decisions based on the application of risk assessment are sometimes based on a standard of protecting those most at risk.

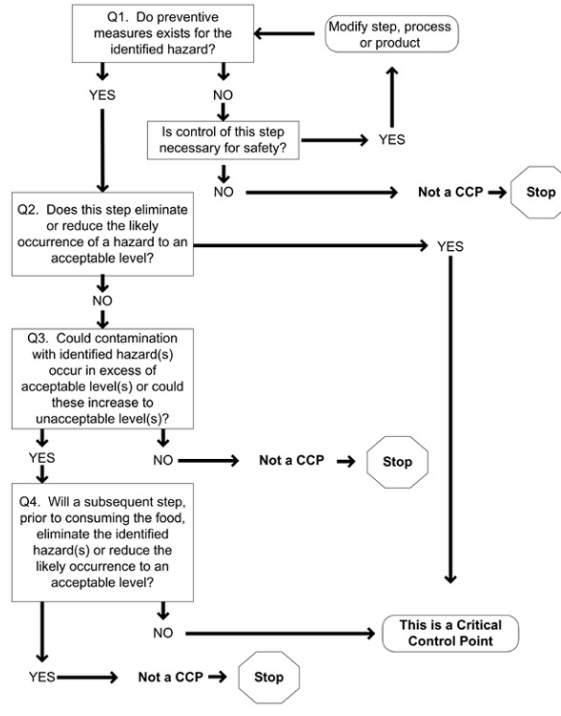


Figure 1. CCP decision tree.

PREVENTIVE MEASURE 4 – RAPID ALERT SYSTEM FOR FOOD AND FEED

The Rapid Alert System For Food and Feed (RASFF) is a system adopted within the European Union whereby Member States are alerted when a residue of potential concern has been detected in food of domestic or imported origin. The purpose of RASFF is to provide control authorities with an effective tool for exchange of information on measures taken to ensure food safety. To assist the members of the network, information is classified under two different headings:

1. Alert notifications, which are sent when the food or feed presenting the risk is on the market and when immediate action is required.
2. Information notifications concern a food or feed for which a risk has been identified, but for which the other members of the network do not have to take immediate action, because the product has not reached their market.

The possible working procedures in implementing measures for RASFF are:

- → Requirements for contact points of the network
- → Confidentiality of the RASFF system
- → Criteria for notification to the RASFF
- → Elaboration of a RASFF notification
- → Assessment of a RASFF notification
- → Transmission of a RASFF notification, including performance indicators
- → Follow-up to a RASFF notification
- → Reporting and statistics on notifications and information relating to the RASFF

PREVENTIVE MEASURE 5 – STANDARDIZATION AND IMPROVEMENT OF EQUIPMENT

Standardization means reducing unnecessary variation in a process. It is required to reduce variability for accurate and precise results. It is considered at operator work process level. There are two types of standards; indirect system standards, e.g. for skills, organization, information and communication; and direct standard operating procedures (SOPs).

CASE OF HACCP PLAN DEVELOPMENT IN TURKISH WHITE CHEESE

An example of setting CCPs in a production of Turkish White Cheese is shown in Figure 2. First CCP in a process is the transportation of raw milk. Raw milk is critical because if exposed to relatively high temperature and temperature variation during transportation, the growth of pathogens and the production of heat resistant metabolites such as toxins and enzymes are possible. For this reason, transportation should be done at around 5 °C to prevent the growth of micro-organisms in raw milk. Moreover, controls of time and temperature in this stage should be established and systematically monitored to prevent development of a possible hazard. Milking practices should be done hygienically to prevent contamination of milk. After the reception of milk to the dairy, the most important application is to change the filters frequently as they can be covered with sediments, which can act as milk contaminants. Control measures for raw milk include milk acidity (pH 6.2–7.5) and TAC count ($<10^6$ CFU/ml in TSA at 30 °C for 24 h).

In this study, pH of raw milk was 6.71 and TAC count was around 6.45 $\log_{10}$ CFU/ml. There was approximately 3 $\log_{10}$ CFU/ml TAC after the pasteurization of raw milk, which is in accordance with Turkish Standards Institution (TSI) regulation. Most probably these present bacteria are *Lactobacillus* spp., thermoduric and other bacteria with spores. Especially, *Clostridium* and *Bacillus* spp. are so resistant to heat, that corrective actions during pasteurization should be implemented according to the records of pasteurization temperature and possible deviations. The holding time of the pasteurizer in AOC, dairy plant was 5 min as the raw milk contains *Listeria* spp. including phagocytes, which is so heat resistant that pasteurization time is higher as compared to the general procedure of 15 s at 72 °C.

Another CCP is the addition of the starter culture and its amount. During starter culture addition and acidification, monitoring the temperature of milk and controlling the development of acidity is very important. Therefore it would be very wise to include strains of lactococci which are able to produce nisin, a bacteriosin which is active against *Listeria*. Under normal conditions of pasteurization, although *Listeria* is expected to be totally inactivated, problems may arise from post-pasteurization contamination. Bacteria can enter cheese at many stages during processing due to environmental diversity of dairy processing plants and a possibility of migration of pathogens that are present in raw milk.

Ripening period of curd in cheese vat is also a CCP as the curd remains in the vat dipped in whey at 32 °C which is suitable for the multiplication of the TAC and other hazardous bacteria that could not be inactivated by pasteurization during this period. The starter culture continues to reduce the pH of the coagulum and ripening of the curd is completed overnight when pH reaches to 5.1–5.2. The possible contamination from the processing environment is prevented by covering the vat during the experiments. The end of curd ripening should be checked by experienced personnel. In addition, potential cross-contamination of the curd from personnel should be prevented. Storage is determined to be the last CCP, as reduced temperature inhibits the growth and survival of *L. innocua* and *L. monocytogenes* for a certain time. During storage the product temperature must be maintained at 5 °C or less in order to ensure the microbial safety of the product. Moreover, if milk and/or cheese are contaminated during the process, the product should be stored at refrigeration temperature depending on the contamination level of the product.

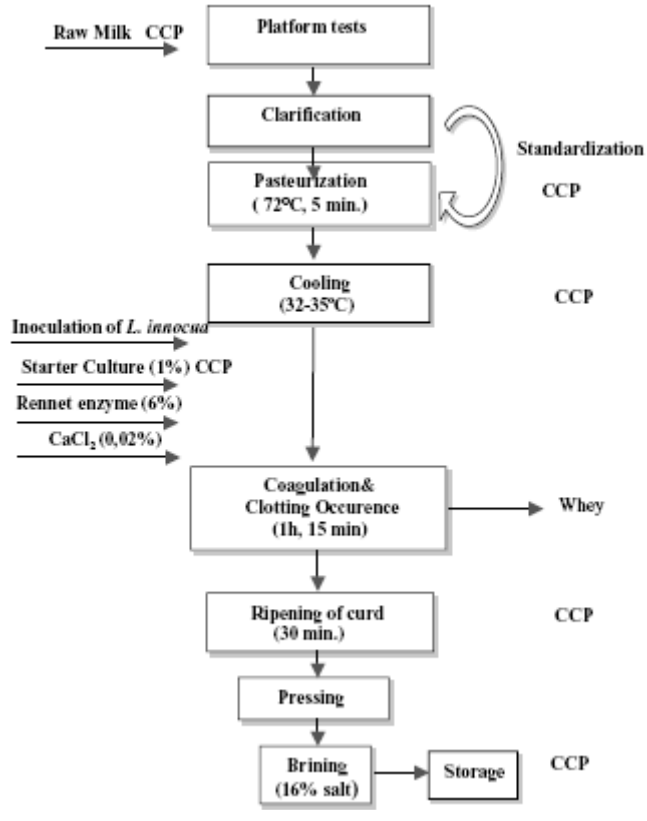


Figure 2. Implementation of HACCP in the production of Turkish White Cheese.

HOW TO PREVENT *LISTERIA* CONTAMINATION IN DAIRY PLANT?

L. monocytogenes is widespread in the environment and has been isolated from water, soil, dust, plants, animal feed, feces and sewage and has been associated with mammals, birds and possibly fish. Many animals, including dairy cows, can carry the bacterium in their intestinal tract without becoming sick. Raw foods from which *L. monocytogenes* has been isolated include non-pasteurized raw milk or foods made from non-pasteurized milk, red meats, poultry, seafood, vegetables, and fruits. *Listeria* can be a common contaminant in the dairy environment, both on the farm and in the processing plant. On the farm important sources include improperly fermented silage and manure. Though rare, the bacterium is also a cause of mastitis. In the dairy plant, *Listeria* has been isolated from a variety of sites though it is most often found in moist environments or areas with condensed or standing water or milk including

drains, floors, coolers, conveyors and case washing areas. Pasteurization of milk is considered to be effective in destroying *L. monocytogenes*. However, post-pasteurization contamination can occur within the processing plant. In addition, *Listeria monocytogenes* is capable of growing at refrigeration temperatures. Therefore, even very low numbers of *L. monocytogenes* in processed dairy products can multiply to dangerous levels, despite proper refrigeration. The dairy industry's trend toward production of refrigerated products with longer shelf lives further exacerbates this problem. Though many of the requirements of the Pasteurized Milk Ordinance are designed to assure the safety and quality of dairy products, dairy plants may still be at risk. Dairy processors take steps to prevent *Listeria* contamination in the processing environment at minimum with *Listeria* prevention program:

1. Proper pasteurization

- → time/temperature
- → controls/records
- → trained personnel

2. Elimination of post pasteurization contamination

- → clean, sanitize and inspect equipment
- → use proper chemicals, time, concentrations, temperatures
- → protection of water cooling systems
- → segregate raw milk handling areas and equipment (i.e. brushes, gaskets, fittings, piping, tanks) from areas and equipment used for pasteurized product
- → no cross connections of raw and pasteurized lines (hoses, CIP)
- → restrict unauthorized persons from the processing area, including truck drivers, receivers and raw product handlers. Milk haulers and others who have been on the farm should not be allowed in the processing area as they are likely to carry contaminants on their boots and clothing
- → ensure that separators/clarifiers are properly cleaned, maintained and that they desludge directly to a drain. Somatic cells concentrated in separator/clarifier sludge can contain large numbers of *Listeria*
- → develop an environmental cleaning and sanitizing program that includes plant and cooler, floors, drains, milk case handling areas and equipment and piping exteriors

- → prevent water or milk from becoming stagnant on plant floors, all floors slope to nearby drains, all drains are free flowing and cleaned and sanitized on a routine basis
- → avoid hand contact with milk contact surfaces that have been cleaned and sanitized; if it is necessary to disassemble equipment during processing it should be cleaned and resanitized before reassembling

3. Airborne contamination

- → avoid creation of aerosols during processing, especially in the milk storage and packaging
- → areas for aerosols can carry harmful bacteria that can contaminate pasteurized product
- → clean heating/air conditioning and vent systems regularly
- → filter outside air and clean filters regularly
- → eliminate aerosols such as high pressure hoses, pumps

4. Plant environment

- → clean and sanitize floors, walls, ceilings, drains and exterior of all equipment on a regular basis
- → eliminate pool water, cracked floors, poor drainage and holes that can harbour bacteria
- → validate daily that cleaning, sanitizing and maintenance of milk processing, storage and packaging equipment is effective and performed in a manner that prevents post-processing contamination

5. Plant traffic

- → restrict access to plant for milk haulers and raw milk receiving personnel
- → use a sanitizer footbath for entry in to pasteurized production areas

6. Personnel cleanliness and training

- → provide adequate training and guidance for all workers; cleaning and sanitizing procedures are important activities that are not be delegated to any employee without proper training

7. **Recall**

- → develop a HACCP based program integrating prevention, validation and an action plan for possible product recalls

8. Environment testing and sampling

- → testing of finished dairy products – yearly program
- → testing of environmental samples (method of collecting swab) – yearly program

→ testing of finished dairy products is initiated with validation of a new process
→ *Listeria* testing is done by an outside laboratory

Further Reading:

1. André, M.C.D.P.B., Campos, M.R.H., Borges, L.J., Kipnis, A., Pimenta, F.C. & Serafini, Á.B. 2008. Comparison of *Staphylococcus aureus* isolates from food handlers, raw bovine milk and Minas Frescal cheese by antibiogram and pulsed-field gel electrophoresis following SmaI digestion. Food Control, 19, 200–207.
2. Andrews, W.H. 1996. AOAC International's three validation programs for methods used in the microbiological analysis of foods. Trend Food Sci. Technol., 7, 147–151.
3. Barrett, T., Feng, P. & Swaminathan, B. 1997. Amplification methods for detection of foodborne pathogens. In: Lee, H.H., Morse, S.A. & Olsvik, O. (Eds.). Nucleic acid amplification techniques: application to disease diagnosis. Boston: Eaton Publishing. Pp. 171–181.
4. Candish, A.A.G. 1991. Immunological methods in food microbiology. Food Microbiol., 8, 1–14.
5. Chain, V.S. & Fung, D.Y.C. 1991. Comparison of Redigel, Petrifilm, spiral plate system, Isogrid, and aerobic plate count for determining the numbers of aerobic bacteria in selected foods. J. Food Prot., 54, 208–211.
6. Cox, N.A., Fung, D.Y.C., Bailey, J.S., Hartman, P.A. & Vasavada, P.C. 1987. Miniaturized kits, immunoassays and DNA hybridization for recognition and identification of foodborne bacteria. Dairy Food Sanit., 7, 628–631.
7. Curiale, M.S., Klatt, M.J. & Mozola, M.A. 1990. Colorimetric deoxyribonucleic acid hybridization assay for rapid screening of *Salmonella* in foods: Collaborative study. J. Assoc. Off. Anal. Chem., 73, 248–256.
8. D'Aoust, J.-Y., Sewell, A.M. & Greco, P. 1991. Commercial latex agglutination kits for the detection of foodborne *Salmonella*. J. Food Prot., 54, 725–730.
9. Dziezak, J.D. 1987. Rapid methods for microbiological analysis of foods. Food Technol., 41, 7:56–73.

10. Eneroth, Å., Christiansson, A., Brendehaug, J. & Molin, G. 1998. Critical contamination sites in the production line of pasteurised milk, with reference to the psychrotrophic spoilage flora. Int. Dairy J., Vol. 8, pp. 829–834.
11. Faille, C., Fontaine, F. & Bénézech, T. 2001. Potential occurrence of adhering living *Bacillus* spores in milk product processing lines. J. Appl. Microbiol., 90, 892–900.
12. Feldsine, P.T., Forgey, R.L., Falbo-Nelson, M.T. & Brunelle, S. 1997. *Escherichia coli* O157:H7 visual immunoprecipitation assay: a comparative validation study. J. AOAC, 80, 43–48.
13. Feng, P. 1992. Commercial assay systems for detecting foodborne *Salmonella*: a review. J. Food Prot., 55, 927–934.
14. Feng, P. 1996. Emergence of rapid methods for identifying microbial pathogens in foods. J. Assoc. Off. Anal. Chem. Int., 79, 809–812.
15. Feng, P. 1997. Impact of molecular biology on the detection of foodborne pathogens. Mol. Biotech., 7, 267–278.
16. Feng, P.C.S. & Hartman, P.A. 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol., 43, 1320–1329.
17. Feng, P., Lampel, K.A. & Hill, W.E. 1996. Developments in food technology: applications and economic and regulatory considerations. In: Dangler, C.A. & Osburn, B. (Eds.). Nucleic acid analysis: principles and bioapplications. New York: Wiley & Sons. Pp. 203–229.
18. Fung, D.Y.C. 1991. Rapid methods and automation for food microbiology. In: Fung, D.Y.C. & Matthews, R.F. (Eds.). Instrumental methods for quality assurance in foods. New York: Marcel Dekker. Pp. 1–38.
19. Fung, D.Y.C., Cox, N.A. & Bailey, J.S. 1988. Rapid methods and automation in the microbiological examination of food. Dairy Food Sanit., 8, 292–296.
20. Gaillot, O., Camillo, P.D., Berche, P., Courcol, R. & Savage, C. 1999. Comparison of CHROMagar Salmonella medium and Hektoen Enteric agar for isolation of salmonellae from stool samples. J. Clin. Microbiol., 37, 762–765.
21. Goldschmidt, M. 1999. Biosensors – scope in microbiological analysis. In: Robinson, R.K., Batt, C.A. & Patel, P. (Eds.). Encyclopedia of food microbiology. London: Academic Press. Pp. 268–278.

22. Gutteridge, C.S. & Arnott, M.L. 1989. Rapid methods: an over the horizon view. In: Adams, M.R. & Hope, C.F.A. (Eds.). Rapid methods in food microbiology: Progress in industrial microbiology. New York: Elsevier. Pp. 297–319.

23. Hartman, P.A. 1989. The MUG (glucuronidase) test for *Escherichia coli* in food and water. In: Balows, A., Tilton, R.C. & Turano, A. (Eds.). Rapid methods and automation in microbiology and immunology. Brescia: Brixia Academic Press. Pp. 290–308.

24. Hartman, P.A., Swaminathan, B., Curiale, M.S., Firstenberg-Eden, R., Sharpe, A.N., Cox, N.A., Fung, D.Y.C. & Goldschmidt, M.C. 1992. Rapid methods and automation. In: Vanderzant, C. & Splittstoesser, D.F. (Eds.). Compendium of methods for the microbiological examination of foods. 3rd ed. Washington DC: American Public Health Association. Pp. 665–746.

25. Hill, W.E. 1996. The polymerase chain reaction: application for the detection of foodborne pathogens. CRC Crit. Rev. Food Sci. Nutrit., 36, 123–173.

26. Holmes, B. 1989. Comparative evaluation of the Roche Cobas IDA and Enterotube II systems for identifying members of the family *Enterobacteriaceae*. J. Clin. Microbiol., 27, 1027–1030.

27. Hornstra, L.M., de Leeuw, P.L.A., Moezelaar, R., Wolbert, E.J., de Vries, Y.P., de Vos, W.M. & Abee, T. 2007. Novel method for the proteomic investigation of dairy-associated Bacillus cereus biofilm. Int. J. Food Microbiol., 116, 367–371.

28. Ibrahim, G.F. 1986. A review of immunoassays and their application to salmonellae detection in foods. J. Food Prot., 49, 299–310.

29. Kalamaki, M., Prioce, R.J. & Fung, D.Y.C. 1997. Rapid methods for identifying seafood microbial pathogens and toxins. J. Rapid Meth. Automat. Microbiol., 5, 87–137.

30. Lampel, K.A., Feng, P. & Hill, W.E. 1992. Gene probes used in food microbiology. In: Bhatnagar, D. & Cleveland, T.E. (Eds.). Molecular approaches to improving food safety. New York: Van Nostrand Reinhold. Pp. 151–188.

31. Lantz, P.-G., Hahn-Hagerdal, B. & Rådstrom, P. 1994. Sample preparation methods in PCR-based detection of food pathogens. Trends Food Sci. Technol., 5, 384–389.

32. Manafi, M., Kneifel, W. & Bascomb, S. 1991. Flurogenic and chromogenic substrates used in bacterial diagnostics. Microbiol. Rev., 55, 335–348.

33. Miller, J.M. & Rhoden, D.L. 1991. Preliminary evaluation of Biolog, a carbon source utilization method for bacterial identification. J. Clin. Microbiol., 29, 1143–1147.

34. Moberg, L.J., Wagner, M.K. & Kellen, L.A. 1988. Fluorogenic assay for rapid detection of *Escherichia coli* in chilled and frozen foods: collaborative study. J. Assoc. Off. Anal. Chem., 71, 589–602.

35. Oggel, J.J., Nundy, D.C. & Randall, C.J. 1990. Modified 1-2 test system as a rapid screening method for detection of *Salmonella* in foods and feeds. J. Food Prot., 53, 656–658.

36. Olsvik, O., Popovic, T., Skjerve, E., Cudjoe, K.S., Hornes, E., Ugelstad, J. & Uhlen, M. 1994. Magnetic separation techniques in diagnostic microbiology. Clin. Microbiol. Rev., 7, 43–54.

37. Ramsay, G. 1998. DNA chips: state-of-the art. Nature Biotechnol., 16, 40–44.

38. Rose, S.A. & Stringer, M.F. 1989. Immunological methods. In: Adams, M.R. & Hope, C.F.A. (Eds.). Rapid methods in food microbiology: Progress in industrial microbiology. New York: Elsevier. Pp. 121–167.

39. Rossen, L., Norskov, P., Holmström, K. & Rasmussen, O.F. 1992. Inhibition of PCR by components of food samples, and DNA-extraction solutions. Int. J. Food Microbiol., 17, 37–45.

40. Stager, C.E. & Davis, J.R. 1992. Automated systems for identification of microorganisms. Clin. Microbiol. Rev., 5, 302–327.

41. Svensson, B., Ekelund, K., Ogura, H. & Christiansson, A. 2004. Characterization of *Bacillus cereus* isolated from milk silo tanks at eight different dairy plants. Int. Dairy J., 14, 17–27.

42. Swaminathan, B. & Feng, P. 1994. Rapid detection of foodborne pathogenic bacteria. Ann. Rev. Microbiol., 48, 401–426.

43. Tenover, F.C. 1988. Diagnostic deoxyribonucleic acid probes for infectious diseases. Clin. Microbiol. Rev., 1, 82–101.

44. Wirtanen, G. & Salo, S. 2005. Biofilm risks. In: Lelieveld, H., Mostert, T. & Holah, J. (Eds.). Handbook of hygiene control in the food industry. Cambridge: Woodhead Publishing Ltd. Pp. 46–68.

45. Wolber, P.K. & Green, R.L. 1990. New rapid method for the detection of *Salmonella* in foods. Trends Food Sci. Technol., 1, 80–82.

MICROBIOLOGICAL RISK ASSESSMENT AND PREVENTIVE ACTIONS IN BAKERY AND BEVERAGE INDUSTRIES IN ESTONIA, SLOVENIA AND TURKEY

Duygu Baş[1], Ayşe Handan Dinçer Baysal[2], Helen Ehavald[3], Erdal Eroglu[2], Sebastjan Filip[4], Fatma Köksal[1], Monika Marin[5], Gregor Perne[6], Irfan Türetgen[1], Martina Weiss[7], Günay Akdoğan[8] & & Tiina Veskus[9]

[1] Istanbul University, Istanbul, Turkey

[2] Izmir Institute of Technology, Urla, Izmir, Turkey

[3] AS Fazer Bakeries Estonia, Tallinn, Estonia

[4] Pekarna Pečjak d.o.o., Trzin, Slovenia

[5] Droga Kolinska d.d., Ljubljana, Slovenia

[6] Pivovarna Union d.d., Ljubljana, Slovenia

[7] Žito Group, Ljubljana, Slovenia

[8] Balparmak, Istanbul, Turkey

[9] Tallinn University of Technology, Tallinn, Estonia

INTRODUCTION

Risks from microbiological hazards are of immediate and serious concern to human health. Microbiological Risk Analysis (MRA) is a process consisting of three components: risk assessment (RA), risk management (RM), and risk communication (RC), which have the overall objective to ensure public health protection. The MRA process should include quantitative information to the greatest extent possible in the estimation of risk. A MRA should be conducted using a structured approach. Since MRA is a developing science, implementation of the guidelines may require a period of time and may also require specialized training in the countries that consider it necessary. This may be particularly the case for developing countries. This document deals with risk assessment, which is a key element in assuring that sound science is used to establish standards, guidelines and other recommendations for bakery and beverage safety to enhance consumer protection and facilitate international trade. This document will be of primary interest to governmental and research

organizations, companies, and other interested parties who need to prepare a MRA will find it valuable.

MICROBIAL RISK ASSESSMENT AND PREVENTIVE ACTIONS

Risk analysis in the field of food safety is a rapidly developing series of activities and during the recent years several symposiums have been addressed to the principles of the risk analysis framework (Anon., 1997b). RA is the scientific evaluation of the probability of occurrence of known or potential adverse health effects resulting from exposure to biological, chemical or physical factors in the food. There are several opened questions around the terminology and methods associated with RA and hazard analyses. The common and most important objective of the activities is to provide scientific and experimental based risk estimates in order to manage food safety (Syposs *et al*., 2005). The overall objective of RA is to provide estimates on the probability of disease occurrence using a well-structured approach according to the four steps suggested by the Codex Alimentarius Commission: hazard identification, hazard characterization (dose-response), exposure assessment and risk characterization (Anon., 1997b). The integration of quantitative risk assessment (QRA) models with the related food safety issues at international/national level, might be the driving force to improve and adopt these models by addressing purely business risk in cases where the food safety parameters are not, or likely not to deteriorate, however the level of risk to the business is still high. Based on the scope of the assessment, QRA was used as a process by which the results of the hazard analyses were used to make business decisions, which might not necessarily impact the food safety parameters of bakery and beverage products.

Effective management of microbiological hazards is enhanced through the use of tools e.g. MRA and Hazard Analysis and Critical Control Point (HACCP) systems. Sound MRA provides an understanding of the nature of the hazard, and is a tool to set priorities for interventions. HACCP is a tool for process control through the identification of critical control points. The ultimate goal is improvement of public health, and both MRA and HACCP are means to that end. In the 30 years since its conception, the HACCP system has grown to become the universally recognized and accepted method for food safety assurance. The recent and growing concern about food safety from public health authorities, food industry and consumers worldwide has been the major impetus

in the application of the HACCP system. The Codex Code on General Principles of Food Hygiene has also been revised to include recommendations for the application of the Codex HACCP Guidelines. In turn, all relevant Codes of Hygienic Practice are being revised to include HACCP Principles. The Codes Guidelines play a crucial role in the international harmonization of the application of the Codex system.

HAZARD ANALYSIS AND CRITICAL CONTROL POINTS

HACCP is a systematic preventive approach to food safety, pharmaceutical safety, etc. that addresses physical, chemical and biological hazards as part of prevention rather than finished product inspection and the HACCP system is a main feature in the new European food law laid down in the Regulation (EC) 852/2004. HACCP is used in the food industry to identify potential food hazards, so that key actions, known as Critical Control Points (CCPs) can be taken to reduce or eliminate the risk of the hazards. The system is used at all stages of food production and preparation processes. In 1994, the organization of International HACCP Alliance was established initially for the US meat and poultry industries. HACCP is obligated since 2003 in Slovenia and is now integrated in all food industry as well as in catering and stores. HACCP is a systematic approach to the identification, evaluation, and control of food safety hazards based on the following seven principles: Analyse hazards, identify critical control points, establish preventive measures with critical limits for each control point, establish procedures to monitor the critical control points, establish corrective actions to be taken when monitoring shows that a critical limit has not been met, establish procedures to verify that the system is working properly, establish effective record keeping to document the HACCP system. It is not the matter of high technology equipment or high performance analysis. Each of these principles must be confirmed by scientific knowledge: for example, published microbiological studies on time and temperature factors for controlling foodborne pathogens. In principle when the HACCP is establishing in manufacture there is a lot of paper work that should be done, but when is implemented is it very useful tool to manage the whole process. Sometimes only after HACCP is established we notice some bad habits in production process that represent unnecessary hazard that can be eliminated with low costs.

GOOD MANUFACTURING PRACTICE

Good Manufacturing Practice (GMP) is defined as the part of Quality Assurance (QA) that ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use. Howsoever, food is an area of commercial interest therefore is it important to assure enough safe and quality food. Food Safety has been discussed in various ways in various societies. In recent years because of increased commercialisation and adoption of unhygienic habits for undue commercial gains and lack of resources available to the people, food hygiene has become a major issue of international trade. Food safety has become an issue of great interest to everyone in food trade when the United States Pathogen Reduction / HACCP rule published in July 1996 combines the concepts of HACCP systems with the requirement for written Sanitation Standard Operating Procedures (SSOPs). However, HACCP and SSOPs are only part of a total food safety system. GMP provides the foundation for SSOPs and HACCP. It is important to have a clear understanding of the relationship between the GMPs, SSOPs and HACCP plans for compliance of various food safety regulations. GMPs pre-requisite programmes comprise the basic, universal steps and procedures that control operating conditions within establishments and ensure favourable conditions for the production of safe food. These differ from HACCP systems, which focus on the critical points in a manufacturing process that affects food safety. GMPs are the control factors that relate to the entire operation and are not process-specific. GMPs include programmes such as facilities/grounds, equipments/utensils, pest control, receiving and storage, process control, product recall and personnel training. GMPs are like any policy programmes those describing good habits, which leads to sufficient result, also for non-food manufactures. They require a written programme, an appropriate training programme and schedule, maintenance schedule and most importantly management commitment. Management commitment is the vital component of any programmes the company implements. Management's role takes on many forms from providing funds, guidance, and human resources, to following the rules themselves. Once management has committed to the implementation of a programme other components will fall in place. Without this no amount of investment or external assistance will deliver results. The written programme will serve as the base for other components. A good written programme includes who, what, where, when and why. It should clearly explain the scope of the programme, responsible

individuals, its importance, parameters, monitoring activities and records, corrective actions and records and also verification activities. The written programme should be written at a level that is appropriate for the educational level of employees and in a language they understand. GMP and other GPs are assuring foundation for good quality of HACCP system. Therefore effective and holistic GMP is pre condition for all other steps in Food safety process.

TRAINING OF PERSONNEL, CLEANING AND DISINFECTION, PROTECTING CLOTHING AND PERSONAL HYGIENE

Disinfectants have a highly diverse regulatory status: At the moment a common approval system for disinfectants used in food industry is being built up; now there is a approval system only for some food e.g. milk but not for others e.g. meat. Thus there is specific legislation depending of the type of production or consumption. Disinfection procedure is of good quality should be based on environmental conditions, microbial agent susceptibility, type of facility, choice of cleaning and disinfectant products, cleaning and disinfection supply need, type of surface areas to be cleaned, staff in charge of these activities, cost of these operations.

Training of personnel: Good worker health and hygiene is critical for preventing foodborne illnesses. The first step towards good worker health and hygiene practices is first hand knowledge of how foodborne illnesses and other infectious diseases spread. The second step is to know how to contain or limit the spread of foodborne diseases by practicing scientifically known intervention techniques e.g. hand washing. Here are some of the simple steps that the operators or managers can take to help prevent the spread of foodborne illnesses. Training and orientation on the basic principles of health and hygiene, hand washing techniques, and recognizing foodborne illness symptoms can help workers understand their role in disease prevention. An adequate number of hand washing units and toilet facilities should be available. Hand washing units should be fully stocked and easily accessible, and no more than a few minutes walk from where any employee is working. Instructions for proper use of them should be prominently posted. As a general rule, one facility is required for every 20 employees. Provide a clean area designated for employees to eat, drink, and use tobacco. Lockers or other suitable facilities to accommodate employee's personal belongings should also be provided. When disposable gloves are provided for

employees, the employees must be trained to use gloves properly. Even though hand sanitizers can be used, they are by no means a replacement to proper hand washing. Should a hand sanitizer be part of an operation, it needs to be one already approved by the Food and Drug Administration (FDA).

Cleaning and disinfection methods: Cleaning is the complete removal of food soil using appropriate detergent chemicals under recommended conditions. It is important that personnel involved have a working understanding of the nature of the different types of food soil and the chemistry of its removal. Cleaning frequency must be clearly defined for each process line i.e. daily, after production runs, or more often, if necessary. The type of cleaning required must also be identified. Sanitizing procedures must be evaluated for adequacy through evaluation and inspection procedures. Adherence to prescribed written procedures (inspection, swab testing, direct observation of personnel) should be continuously monitored, and records maintained to evaluate long-term compliance. Equipment can be categorized with regard to cleaning method as follows: Mechanical Cleaning is often referred to as clean in place (CIP). Require no disassembly or partial disassembly. Clean-out-of-Place (COP) can be partially disassembled and cleaned in specialized COP pressure tanks. Manual cleaning requires total disassembly for cleaning and inspection. It is important to differentiate and define certain terminology: Disinfection or sanitation refers to the reduction of micro-organisms to levels considered safe from a public health viewpoint. *Thermal Sanitization* involves the use of hot water or steam for a specified temperature and contact time. *Chemical Sanitization* i.e. disinfection involves the use of an approved chemical sanitizer at a specified concentration and contact time.

Protecting clothing and personal hygiene: When we are talking about protecting clothing we usually mean equipment that protects employees either than food from contamination. Use of protecting clothing is depending on many different aspects. First of all is necessary to know what we would like to achieve by using it. Is it only matter of satisfying the EC 852/2004 on the hygiene of foodstuff, or we would like to achieve another dimension of quality and worker's perception? All companies have on some way assured even just the presence of protecting clothing on work place. But unfortunately this is not enough to reach the goal of hygiene. Quality and serviceability of clothing cannot obviously lead us on higher level of hygiene in production. Sometimes in reality we can recognized that use of protecting clothing (like gloves and masks) could be source

of contamination while incorrect using. Employees have to be well educated and also trained how, when and why to use protecting clothing. Type of clothes depends on process e.g. high humidity and temperature and low temperature with high ventilation as well as origin of raw materials. SMS enterprises, which are the most presented in Slovenia, are common to use outsourcing in case of protecting clothing. An intact glove provides adequate protection from microbial transmission of hand-contaminating micro-organisms. However, some food-grade gloves may have existing pinhole punctures and/or can be easily ripped, torn, or punctured during use. While hand washing, on the other hand, can be very effective in removing micro-organisms, ensuring that food workers perform effective hand washes is difficult. Thus, the studies recommends donning of gloves to be preceded by an effective hand wash, ongoing employee training and education, high personal hygiene requirements, and institution of a quality control. Further, to reduce disease transmission by contaminated objects, the study suggests an effective environmental and sanitation program and restriction of tasks among workers to prevent contamination. Foreign objects e.g. glass, sand and stones can be broadly classified as food safety hazards and food non-safety hazards e.g. incorrect allergen free filling. Foreign objects that are physical hazards are referred to as hard or sharp objects and also some parts of clothing. Hard or sharp objects are further divided into metallic objects, which are divided into ferrous and non-ferrous metals, and non-metallic objects. Controls for metal inclusion can include periodic checks of metal equipment and passing the product through metal detectors or separation equipment. To achieve high level of hygiene the effective training is essential. Sometimes a language can be a barrier because of heterogeneous nationality of employees with low or none education. A picture and symbol based approach can be an affordable and effective solution. Experts can be helpful in motivating employees to comply with fundamental sanitation principles. Overall, numerous technologies are available to sanitize a plant, but they are only effective if supported by plant employees.

QUALITY STANDARDS

Food safety is linked to the presence of food-borne hazards in food at the point of consumption. Since food safety hazards can occur at any stage in the food chain it is essential that adequate control be in place. Therefore, a combined effort of all parties through the food chain is required. For this reasons many different food standards have been developed. On the other hand ISO 9001 is

standard for quality management (QM) systems for different types of production or business. The so called 'food standards' are standards for managing quality and food safety in food business or in whole food chain. **ISO 9001:2000** is maintained by the International Organization for Standardization (ISO) and is administered by accreditation and certification bodies. ISO 9001:2000 specifies requirements for a QM system where an organization needs to demonstrate its ability to consistently provide product that meets customer and applicable regulatory requirements, and aims to enhance customer satisfaction through the effective application of the system. These include processes for continual improvement of the system and the assurance of conformity to customer and applicable regulatory requirements. All requirements of International Standard are generic and are intended to be applicable to all organizations, regardless of type, size and product provided. **ISO 22000:2005** is an internationally recognized standard intended to harmonize on a global level the requirements for food safety management within the food chain. It has been designed to be compatible with other management system standards such as ISO 9001 and can be implemented within an integrated management system. The standard combines the key elements to enable management of food safety along the food chain including: integrating the principles of HACCP and application steps developed by Codex Alimentarius Commission; system management; control of food safety hazards through pre-requisite programmes and HACCP plans; interactive communication with suppliers, customers, regulators, consumers; continual improvement and updating of the management system. The **British Retail Consortium (BRC) Standard** was created to establish a standard for the supply of food products and to act as key piece of evidence for UK retailers and brand owners to demonstrate 'due diligence' (taking all reasonable precautions to prevent an unsafe or illegal product causing customer illness or injury) in the face of potential prosecution by the enforcement authorities. Certification to the BRC standard verifies technical competence and aids manufacturers, brand owners and retailers fulfilment of legal obligations. It also safeguards the consumer. This standard possesses a comprehensive scope covering all areas of product safety and legality, addresses part of the due diligence requirements of both the supplier and the retailer. It covers such critical topics as: HACCP system, QM, factory environment standard, and product and process control.

METHODS USED FOR MONITORING MICROBIAL CONTAMINATION OF BAKERY PRODUCTS AND BEVERAGES IN ESTONIA, SLOVENIA AND TURKEY

The following is the range of in that produced by beverage industries carbonated soft drinks, cordials and concentrates, energy drinks, fruit juice and fruit drinks, functional non-alcoholic drinks, iced teas and coffees, mineral, spring and packaged waters sports and isotonic drinks. Sampling is one of the most important parts, when detecting microbial contamination inside the plant. Samples should be taken throughout the production from raw materials to final products. Frequencies, where and when the sample must be taken and what kind of analysis is done, is part of HACCP plan, which is made according to general recommendations for certain type of production and also specific conditions in the plant. Only trained personnel from quality control department or production department, which are able to take the samples correctly, should perform sampling. All analysis in national labs is done according to ISO standards. National legislation prescribes them analysis for control of bottled drinking water. When analysing soft drinks and beer, national labs use guidelines issued by the National Health Institute and specifications given by the producers themselves.

METHODS USED FOR BAKERY PRODUCTS AND BEVERAGES IN ESTONIA

In Estonia the food sphere-handling of raw materials for food and feed, the Food Act and Directive of the EP and the EC 178/2002 EEC regulate self-control of food handling operator and governmental food control. As provided by the Food Act, the Veterinary and Food Board (VFB), the Estonian Consumer Protection Board and the Estonian Tax and Customs Board perform food control. The Veterinary and Food Board is to perform supervision of all the spheres of handling and materials and items, specified in Article 1 (2) of the Regulation of the European Parliament and the European Council No. 35/2004/EC. In performing the food analyses VFB uses the services of the Estonian Veterinary and Food Laboratories (VAFL). At the same time VAFL operates as a reference laboratory. The internationally certified Estonian Accreditation Centre accredits VAFL in the area of food and drinking water testing in accordance with EVS-EN ISO/IEC 17025 ON “General requirements for the competence of testing and calibration laboratories”. Performing the tests laboratories use the latest valid

editions of an international approved standards (ISO, NMKL). The most common microbiological spoilage problem in bakeries is related with moulding. Mycotoxigenic moulds can be isolated from spoiled breads, and many mycotoxins have been produced in inoculated breads, but surveys of naturally mouldy breads have yielded only aflatoxins and ochratoxin A in a few samples (Legan, 2002). Furthermore, several bakery products also hare been implicated in foodborne illnesses involving *Salmonella* spp., *Listeria monoctyogenes* and *Bacillus cereus*, while *Clostridium botulinum* is a concern in high moisture bakery products packaged under modified atmospheres (Smith *et al*., 2005). To decrease the risk coming from microbiological spoilage, all bakeries have own-checking plan (OCP) or HACCP. An effective HACCP programme requires equally competent technologies to determine and monitor each critical point (Barendsz, 1998). The exact microbiological sampling plan is set with the OCP or HACCP. One of the easiest ways to analyse microbiological spoilage from environmental samples including equipment and utensils and also from employees' hands and clothes is to use Hygicult® contact slides. There are different types of Hygicult® tests, but most common in bakeries are Hygicult® TPC for total bacterial count and Hygicult® Y&F for yeasts and moulds. Both tests are easy to use; Hygicult® slides are intended for rapid monitoring of microbiological hygiene in different types of materials, both solid and liquid. Most food productions do not analyse pathogens from their product by themselves; very often all tests for final product and water are performed in accredited laboratories. Drinking water quality is one of Estonia's priorities. The requirements of Council Directive 98/83/EU are established in Estonian law in the Public Health Act, the Water Act, and regulations passed implementation (http://www.euro.who.int/eehc/implementation/20061010_6). One of the ingredients in bakery products and beverages is water, every plant have to analyze drinking water for *Escherichia coli* (limit 0 CFU/100 ml) and enterococci (limit 0 CFU/100 ml) with frequency according to the OCP/HACCP (Anon., 2007c). Beverage productions that produce still and carbonated water have to follow microbiological limits according to Estonian law, Joogivee kvaliteedi- ja kontrollinõuded ning analüüsimeetodid (Act 82, RTL 2001, 100, 1369). Water filled to the bottles must follow microbiological limits given below: *E. coli* – 0 CFU/250 ml, enterococci – 0 CFU/250 ml, *Pseudomonas aeruginosa* – 0 CFU/250 ml, colonies at 22 °C – 100 CFU/ml, colonies at 37 °C – 20 CFU/ml (Anon., 2002). According to Estonian law ISO 9308-1 method is used for analyzing *E. coli* and coliforms, ISO 7899-2 for enterococci, EN ISO

6222 for colonies at 22 °C and 37 °C, EN 26461-2:1993 for *C. perfringens* (including endospores) and prEN ISO 12780 is used for analysing *P. aeruginosa* from drinking water (Anon., 2007b). The frequency of microbiological test for final product in bakeries is set with OCP/HACCP, but it can be 1–2 times in a year. *E. coli*, *Staphylococcus aureus*, total bacterial count, moulds and *B. cereus* can be analyzed depending on ingredients of product. ISO 16649-2 is used for analyzing *E. coli*, EVS-ISO 699-1 for *S. aureus*, NMKL nr. 86, for total bacterial count, EVS/ISO 7954 for moulds, NMKL nr. 67 for *B. cereus*. Microbiological criteria for raw material and food have set in Commission Regulation (EC) No 2073/2005 of 15 November 2005.

METHODS USED FOR BAKERY PRODUCTS AND BEVERAGES IN SLOVENIA

In bakeries the most important part of the monitoring microbial contamination in food industry is sampling. In bakeries raw materials and the end products are monitored. All sampling must be performed according to HACCP plan by trained quality control department personal. In HACCP plan frequency of sampling raw materials and end products, what kind of analysis we do on sample, which parameters must be taken in consideration and all precaution measures in case of detecting the potential pathogenic or spoilage micro-organisms in sample are established. In the process of monitoring to detect potential pathogenic micro-organisms and to detect spoilage micro-organisms are essential. Potential pathogenic micro-organisms usually cannot cause serious health problems to end consumers whereas the spoilage micro-organisms usually cannot cause health risk, but can have serious financial effect on producer. In bakery the most frequent spoilage micro-organisms on end products are yeast and moulds whereas on raw materials are the spore forming micro-organisms e.g. *B. cereus, B. subtilis* and moulds, which can survive baking. In industrial laboratory for testing our raw materials and end products we use ISO methods and also methods used in study laboratories on faculties, which are validated on ISO methods. We established the criteria for acceptable or unacceptable raw material or end product from microbiological point of view. The criteria are based on national guidelines for microbiological safety of foods for human consumption and on Croatian National legislation for microbiological standards for foods for human consumption. Our main raw material is flour and for flour is important that the number of spore forming micro-organisms is in accordance with our criteria and from that kind of raw materials we can bake the end

products of good quality. In national-accredited laboratory for testing the food samples ISO methods are used. Some methods, which they use, are not ISO methods, but all non-ISO methods are validated according to the ISO 16140:2003. Monitoring of bottled drinking water is done according to national legislation, which requires absence of *E. coli, P. aeruginosa* and faecal enterococci in 250 ml. There is no spoilage micro-organisms related to bottled water. On the other hand, by production of soft drinks and beer, spoilage micro-organisms are the main targets of detection, since pathogens are highly unlikely to be found in these products due to the processing steps e.g. pasteurisation and filtration and their characteristics e.g. low pH and carbon dioxide (CO_2) content. Main spoilage micro-organisms of cold aseptic filled soft drinks without conservation are moulds, yeasts, lactic-acid bacteria and recently also thermophilic spore forming bacteria of genus *Alicyclobacillus*. The main spoilage micro-organisms of beer are lactic-acid bacteria e.g. *Lactobacillus* spp. and *Pediococcus* spp., obligate anaerobe *Pectinatus* spp. and non-cultivable (wild) yeasts. Guidelines for beer recommend detection of yeasts in pasteurised beer and detection of *Salmonella* spp., *Enterobacteriaceae* and yeasts in unpasteurised beer. For soft drinks with pH <4.2 they recommend detection of yeasts, moulds and *Enterobacteriaceae.*

METHODS USED FOR BAKERY PRODUCTS AND BEVERAGES IN TURKEY

Evaluation of microbial contamination of indoor air, critical areas in the plant, equipment etc. is essential to ensure standard quality and safety of food. The HACCP puts strong emphasis on the importance of microbiological analysis of food products and sterility audits of manufacturing processes and facilities. In Turkey, the samples are taken by trained personnel from quality control department according to general recommendations of standard methods for certain type of productions and also specific conditions in the plant. Certain microbiological test procedures of all foods are done according to ISO standards, EC Decision 2001/471/EC including the HACCP principles and the national legislation (Turkish Food Codex Regulation) in national laboratories. A general sterility and sanitation audit includes following specific microbiological tests Heterotrophic Plate Count/Mould and Yeast: Detection/Identification (FDA/BAM: 2001), Total Coliforms/Faecal Coliforms (FDA/BAM: 2002), *E. coli/ E. coli* 0157:H7 (FDA/BAM: 2002, BAX System Q7), *Salmonella* spp. (ISO 6579: 2002, BAX System Q7), *Listeria monocytogenes* (Oxoid Listeria

Rapid Test, API Kit, BAX System Q7), *Listeria* spp. (Oxoid Listeria Rapid Test), *Clostridium perfringens* (FDA/BAM: 2001), *Staphylococcus aureus* (FDA/BAM: 2001), Mesophilic aerobic spore formers (FDA/BAM:2001), Mesophilic anaerobic spore formers (FDA/BAM: 2001), *B. cereus* (FDA/BAM: 2001), Rope spore (FDA/BAM: 2001), Mycotoxins (AOAC 999.07:2000, TS EN ISO 14501:2002) for bakery products.

The microbiological analysis of the spring and drinking water are done with membrane filtration method according to ISO standards and Turkish Standard (TS 266, Regulation Concerning Water Intended for Human Consumption) the standardization of Turkey which requires absence of coliform/faecal coliform bacteria, *E. coli, P. aeruginosa* and faecal enterococci in 250 ml, and *Salmonella spp*. in 100 ml, *C. perfringens* in 50 ml. Main spoilage micro-organisms of fermented beverages are lactic-acid bacteria and the thermophilic acidophilic spore-forming bacteria *Alicyclobacillus*. No effective control methods have yet been developed for *Alicyclobacillus.* They can grow at low pH and at moderately high temperatures such as 40 °C are known to cause spoilage of acidic beverages and produce odours. However, they do not produce gas or cause any change in the appearance of the beverage container, and therefore the spoilage is discovered only when the consumer opens and begins to consume the product. Turkish Food Codex Regulation for fermented beverages recommends the detection of mesophilic aerobic bacteria, acidophilic bacteria and mould/yeasts in fermented beverages. The Ministry of Agriculture and Rural Affairs (MARA) is responsible for the implementation of the legislative framework and carry out the food inspection in Turkey. The MARA, through its General Directorate of Protection and Control carries out the food control from farm to sales point. It also performs the food control at retailing and consumption points. Under the umbrella of the General Directorate of Protection and Control of MARA there are 81 Provincial Directorates, 39 Provincial Control Laboratories and one Food Control and Research Institute. Kalite Sistem Laboratories Group is an accredited entity that is the largest private industrial research, testing, inspection and training organization in Turkey. Kalite Sistem Authorized Food Control Laboratories have the authorization and accreditation from both the Ministry of Health and the MARA for analysis of the imported and exported foods and market inspection in the food sector. Kalite Sistem Central Laboratories, which was accredited by TSI according to ISO 17025 and affiliated to the AGES (Austrian Agency of Health and Food Safety – Österreichische Agentur für

Gesundheit und Ernährungssicherheit), performs analysis in food, feed, cosmetics, medicine, detergent and cleaning chemicals. ISO 22000, BRC, IFS, EUREPGAP, ISO 9001 certifications Turkey's basic mission is provide international recognition, food safety/quality also consumer assurance and law requirement for their brand. Laboratories performed the tests and analysis through international reference methods verified by validation studies (AOAC, AOAC, APHA, FAO and EEC). The reliability of the analysis results is regularly controlled and monitored by proficiency testing studies, ring tests, inter-laboratory comparison studies and certified reference materials.

FUTURE NEEDS

Food- and waterborne illnesses cause not only hospitalization cases which might result with death but also serious economical losses due to the hospitalization cost and product losses. In the food industry, the main goal is to produce better quality and safer products with microbial load as low as possible. In this sense, safety and quality legislations have been improved to overcome these problems. Numerous research articles have being published on new rapid and reliable microbiological techniques; however such techniques are not cheap and easy to use for on-plant applications. Conventional techniques have been still preferred in terms of cost by companies. To our common opinion, air and environmental borne microbial risk threat bakery and beverage plants. Biosensor-based sensitive techniques that produce quick results could be designed for microbial detection. Collaboration between universities or research laboratories and food companies has been limited until now. Effective and utilizable up-to-date techniques and technologies developed in research labs must be transferred to plant. In addition, companies in view of feedback tested in work place could support new researches to scale up the prototypes. Companies' demands must be determined and projects in view of these needs must be proposed to academia. Researchers must concentrate on novel projects, which produce solutions to the problems of companies. Moreover, trained personnel can be employed in critical part of operation. EU and national authorities require Microbiological Criteria for all types of food products. There is no common microbiological analysis technique covering all types of food products including functional foods. Moreover, analyses techniques among the countries and between industrial and research laboratories are not compatible. As a result, reliable, cheap, sensitive, easy and rapid microbiological analyses techniques and procedures that are

generally acceptable in all laboratories of EU countries must be revised. RA is a sensitive issue, which requires multidisciplinary teamwork. Therefore, cooperation among governments, companies and scientists is unavoidable to set up common rules. The hygiene package includes European Parliament and Council Regulations 852/2004, 853/2004, 854/2004 and 882/2004, requiring demonstration of wholesomeness of foods manufactured and distributed according to HACCP-based Good Practices, would ideally call for standardized European methods to assess compliance with respect to microbiology. For cultural reasons this goal does not seem within reach in the near future. While, admittedly different though nonetheless excellent, method collections are available, a pressing need was identified to assess whether such different methods produce roughly equivalent results with respect to accuracy, repeatability and reproducibility.

CONCLUSIONS

RA is the science-based component of risk analysis. Over the past decade, risk analysis has emerged as a structured model for improving food control systems with the objectives of producing safer food; cutting the numbers of foodborne illnesses and facilitating domestic and international trade in food. The classical RA approach is considered to carry out at the governmental level. RA should be carried out at company level. Also, during the RA process the dose-response model should be realized at the company level. On the other hand, it is well known from the relationship between the food industry, health surveillance (food safety monitoring systems) and the food inspection bodies that the barrier of RA carried out at the governmental level often is the lack of data obtained from the industry. In order to provide more precise estimates, industry, governmental agencies and scientific institutes must work together to enable the required progress of RA. Although the technology of bakery and beverage manufacturing has rapidly developed and progressed in the past decades, the ultimate goal is still to operate at low cost and implement aseptic technologies. Despite the advanced technology, spoilage of bakery or beverage products as well as detection of indicator micro-organisms in the process continues to occur. The scope of this study is to addresses microbiological RA, based on process exposure assessment versus finished-product microbiological quality control proved to be a very powerful tool. It provides added value to the bakery and

beverage industry, with a special focus on business risk reduction parallel to ensuring food safety, as the most important quality parameter.

Further Reading:

1. Aarnisalo, K., Wirtanen, G., Raaska, L., Tallavaara, K. & Maijala, R. 2006. The hygienic working practices of maintenance personnel and equipment hygiene in the Finnish food industry. Food Control, 17, 1001–1011.
2. Anon. 1992. Statement of policy: Foods derived from new plant varieties from the Department of Health and Human Services in U.S. Food and Drug Administration. Fed. Reg., 57, 22984–23005.
3. Anon. 1997a. A Guide to implementation and auditing of HACCP by the Standing Committee on agriculture and resource management council of Australia and New Zealand. Victoria: CSIRO Publishing. 26 p.
4. Anon. 1997b. Risk management and food safety. Report of a Joint FAO/WHO Expert Consultation. FAO Food and Nutrition Paper No. 65. Rome: FAO.
5. Anon. 1997c. Understanding the Codex Alimentarius. Rome: FAO/WHO.
6. Anon. 1998. Directive 98/37/EC of the European Parliament and of the Council of 22 June 1998 on the approximation of the laws of the Member states relating to Machinery.
7. Anon. 1999a. Guidebook for the preparation of HACCP plans and Generic HACCP models. Washington DC: FSIS. 71 p.
8. Anon. 1999b. Principles and guidelines for the conduct of microbiological risk assessment by WHO. CAC/GL-30.
9. Anon. 2002. Joogivee kvaliteedi- ja kontrollnõuded ning analüüsimeetodid, Sotsiaalministri määrus 82, jõustunud 01.06.2002, RTL 2001, 100, 1369.
10. Anon. 2003. Croatian National legislation for microbiological standards for foods for human consumption, Narodne novine, Klasa:011-02/03-04/29, Urboj:534-06-01-02/1-03-1, Zagreb, 25. srpanj 2003.
11. Anon. 2005. Komisjoni määrus (EÜ) nr. 2073/2005, 15. November 2005, toiduainete mikrobioloogiliste normide kohta.
12. Anon. 2007a. A quick guide to ISO 22000. http://www.bsi-global.com/en/.

13. Anon. 2007b. International Organisation for Standardization. http://www.iso.org/iso/.

14. Anon. 2007c. The British Retail Consortium (BRC) Global Standard-Food. http://www.brc.org.uk/standards/default.asp.

15. Anon. 2007d. Turkish Food Codex Regulation. http://www.kkgm.gov.tr/mev/kodeks.html.

16. Barendsz, A.W. 1998. Food safety and total quality management. Food Control, 9, 163–170.

17. Bender, A.E. & Bender, D.A. 1995. Dictionary of food and nutrition. Oxford: Oxford University Press. 152 p.

18. Bernik, J. 2002. Good manufacturing practice. In: Raspor, P. (Ed.) Priročnik za postavljanje in vodenje HACCP. Ljubljana: Biotehniška fakultera. Pp. 29–36.

19. Bryan, F.L. 1992. Hazard analysis critical control point evaluations. Geneva: World Health Organization. 72 p.

20. Chmielewski, R.A.N. & Frank, J.F. 2003. Biofilm formation and control in food processing facilities. Comp. Rev. Food Sci. Food Safety, 2, 22–32.

21. Cramer, M.M. 2003. Building the self-cleaning food plant: Six steps to effective sanitary design for the food plant. Food Safety Magazine, February/March.

22. Cramer, M.M. 2006. Food plant sanitation, design, maintenance, and good manufacturing practices. London: CRC Taylor and Francis. 283 p.

23. Grbac, V.L & Vidovic K. 2002. HACCP v velikih kuhinjah in v catering obratih. In: Raspor, P. (Ed.) Priročnik za postavljanje in vodenje HACCP. Ljubljana: Biotehniška fakultera. Pp. 391–410.

24. Gregerson, J. 2002. Third Annual Best Manufacturing Practices Survey. Food Engineering, February.

25. Herlič, M. 2003. Good hygiene practice in food distribution in the hospital. Meso in mesnine, 4, 40–43.

26. Higgins, K.T. 2002. The Culture of Clean. Dairy Foods, November.

27. Inteaz, A. 2004. Food quality assurance, principles and practices. Boca Raton: CRC Press. 151 p.

28. Keller, S.E., Merker, I.R., Taylor, K., Hsu Ling Tan, C.D., Stuart, J.C. & Miller, A.J. 2002. Efficacy of sanitation and cleaning methods in a small apple cider mill. J. Food Prot., 65, 6, 911–917.

29. Koren, I. 2002. HACCP v proizvodnji brezalkoholnih pijač. In: Raspor, P. (Ed.) Priročnik za postavljanje in vodenje HACCP. Ljubljana: Biotehniška fakultera. Pp. 459–470.

30. Legan, J.D. 2002. Mould spoilage of bread: the problem and some solutions. Int. Biodeter. Biodegrad., 32, 33–53.

31. Maris, P. 1998. Regulatory procedures for disinfectants in Europe. Int. Biodeter. Biodegrad., 18, 297–301.

32. Napper, D. 2007. Hygiene in food factories of the future. Trends Food Sci. Technol., 18, 574–578.

33. Paulson, D.S. 1996. To glove or to wash: A current controversy. Food Quality, June/July.

34. Raspor, P. 2006. How to make HACCP more efficient in practice? HACCP conference in Moravske Toplice, 3–4 November 2006. Pp. 1–3.

35. Šaddl, S.M. 2002. HACCP v gostinskem obratu. In: Raspor, P. (Ed.) Priročnik za postavljanje in vodenje HACCP. Ljubljana: Biotehniška fakultera. Pp. 375–389.

36. Smith, J.P., Daifas, D.P., El-Khoury, W., Koukoutis, J. & El-Khoury, A. 2005. Shelf life and safety concerns of bakery products-A review. Crit. Rev. Food Sci. Nutrit., 44, 19–55.

37. Syposs, Z., Reichart, O. & Mészáros, L. 2005. Microbiological risk assessment in the beverage industry. Food Control, 16, 515–521.

38. Walker, E., Pritchard, C. & Forsythe, S. 2003. Food handlers' hygiene knowledge in small food businesses. Food Control, 14, 339–343.

RISK ASSESSMENT IN MISCELLANEOUS FOOD PROCESSING INDUSTRIES INCLUDING PRODUCERS OF VEGETABLES AND SPICES

Metin Guldas[1], Roxana Ursu[2], Vasiliki Lagouri[3], N. Asli Kisikkaya[4], M. Fatih Uyar[5], Primož Mokorel[6], Hasan Ture[7], Solmaz Lee[8], Figen Korel[7] & Renata Karpíšková[9]

[1] Uludağ University, Karacabey, Bursa, Turkey

[2] Rompak Baker's Yeast Company, Pascani, Iasi, Romania

[3] Technological Educational Institute of Thessaloniki, Thessaloniki, Greece

[4] Tübitak, Gebze, Kocaeli, Turkey

[5] Hacettepe University, School of Health Technology, Ankara, Turkey

[6] ETA Food Industry, Kamnik, Slovenia

[7] Izmir Institute of Technology, Urla, Izmir, Turkey

[8] Food Research and Control Institute, Hurriyet, Bursa, Turkey

[9] National Institute of Public Health, Brno, Czech Republic

THE METHODS USED FOR MONITORING THE MICROBIAL CONTAMINATION

Testing foods for pathogenic and spoilage bacteria is the cornerstone to ensuring a safe and wholesome food supply. The methods for monitoring of microbial contamination are based on the following principles:

- → Cultivation of microbes in the presence of different energy sources,
- → Analysis of their macromolecular composition and their metabolic by-products,
- → Use of specific immunological reagents for bacterial classification and identification.

Mostly, the conventional detection methods like plate counting and rapid methods are preferred for the routine controls in order monitoring of microbial contamination. In the laboratory internationally accredited methods by U.S. Food and Drug Administration (FDA), AOAC International (AOAC), and

International Organisation for Standardization (ISO) are used in performing the microbiological analyses covering all stages in the food chain. The validation of the microbial detection method is another approach in order to improve accuracy of analysis in the governmental institutions. For the companies which do not have laboratory, the tests can be achieved by an accredited laboratory. The rapid methods as well as traditional methods are preferred in private sector laboratories. It is very important to know some aspects of microbiological safety in food technologies in every stage of processing and packaging. The traditional tests are used to detect the microbial contamination by taking the samples from critical control points in HACCP system, the process stages, finished products, working area, packing machines, trucks, air. The effectiveness of cleaning can be monitored using ATP tests. As example, the culture media (agar) used in private sector is:

→ Nutrient agar for total bacterial count

→ Endo Agar for coli form bacteria and *Escherichia coli* detection

→ Lysine agar for wild yeast determination

→ DTA (dextrose tryptone agar) for thermophilic and mesophilic bacteria such as *Bacillus subtilis*

→ YGC Agar (yeast extract – glucose – cloramphenicol agar) for moulds and yeast determination

→ Baird Parker agar for determination of staphylococci

→ *Bacillus cereus* agar

→ DCLS (Desoxycholate Citrate Lactose Sucrose) agar for *Salmonella, Shigella* and other *Enterobacteriaceae*.

In addition to these classical methods, rapid methods, e.g. electrical impedance measurement, are used for detection of micro-organisms. Membrane filtration, flow cytometry, polymerace chain reaction (PCR) based methods, pulsed-field gel electrophoresis (PFGE) and Fourier Transform Infrared (FTIR) are the other methods used for detection and enumeration of micro-organisms. On the other hand, as raw agricultural commodities, spices and herbs commonly harbour large numbers of bacteria and fungi including potential spoilage organisms. The manner and environment in which they are grown, harvested and handled, as well as the chemical nature of the spice, directly impacts its microbiological

quality. In general, roots, berries and herbs carry a greater microbiological load than the bark and seed items. Bacterial multiplication is not a concern if the products are sufficiently dried, stored and shipped under normal, dry conditions, however fungal spoilage may occur if the spices are subjected to improper storage. After harvest, various types of cleaning processes are used to progressively reduce the number and types of micro-organisms. Additional means are treatment with the ethylene oxide, high temperature steam or irradiation. Spices are subjected to different type of processes such as washing, peeling, curing, drying, fumigation, cleaning, grading and milling. Here follows a list of method to improve the product hygiene:

→ Drying is the most important step to prevent the mould growth. Dried spices undergo extensive cleaning to remove extraneous matter such as dirt, stones, stalks, leaves and metallic contamination.

→ Milling; during which a considerable reduction in the total bacterial load as a result of the increase in product temperature.

→ Ethylene oxide fumigation; vegetative cells, including coliforms, *Eschericia coli* and *Salmonella* are eliminated, with low to moderate concentrations of bacterial spores typically remaining. Many factors affect the overall reduction in microbial counts, including the initial microbial type and load, the concentration of ethylene oxide, the temperature and relative humidity in the chamber, the physical and chemical nature of the spice and its moisture content.

→ Irradiation of spices with gamma rays is a simple, safe and efficient method, allows the treatment of the products in their final packaging, which eliminates recontamination issues.

→ Treatment with high temperature steam: is another efficient and economical method for reducing microbial populations of some spices without sacrificing appearance and flavour levels.

POTENTIAL ISSUES IN THE MICROBIAL DETECTION

The most important issues in the microbial detection are sampling and time requirement for the microbial tests. Sampling and analyzing time are important issues for choosing the detection methods. Rapid methods are used when the results are needed in a short period of time. Although cost effective and

sensitive, the conventional methods are generally time consuming and require several days to obtain test results. During this period, the raw materials cannot be used for the production. It requires laboratory clearance before using in production. Therefore, microbiological evaluation procedure should be organized with enough time. Some food products such as minimally processed foods have shorter shelf-life. It may lead to restricting the use of conventional testing methods for this sort of products. From this point, rapid tests are the alternative methods for the microbial detection. There is a growing interest in having rapid systems that are faster and less time consuming in laboratory routine, but on the other hand, problems still arise with interference from the food components. During the conventional culturing methods, there is a difficulty to detect the injured cells. Most rapid methods rely on culturing methods to recover injured cells and amplify the number of target cells. However, rapid methods are needed to be verified by conventional detection methods. That's the reason why conventional testing methods are still the most common methods in most laboratories in routine. The fouling of sensors for in-line detection of microorganisms is a distinct problem associated with sensor technology. Furthermore, it is not easy to sample from big surfaces or volumes. Sampling method may create problems if the raw material is powdered e.g. powder milk and powder eggs and put in bags of 25 or 50 kg. The sample to be taken should be representing the rest of the bulk or the test area. Microbiological samples must be taken from critical spots on food processing surfaces. Taking the samples to the laboratory in proper conditions, training of staff, contamination during sampling and detection limit for the microbiological tests are other significant issues. For example, we assume that 1 ml of inoculate is used. If the contamination is lower than 1 colony forming units (CFU)/ml, it is not possible to detect it. There is an exception in detection of coliform bacteria in process water that is done by filtration and the amount of sample is 100 ml so the detection limit is 1 CFU/100 ml. In sample preparation, homogenization is an important factor and either stomachers or blenders are used for this purpose. In plants, the technological procedures and HACCP are also important in terms of microbiological evaluation. At first, pH, aw and humidity tests are done before microbiological evaluation, as indicative analysis. Swab samples are also taken from the hands of workers and the surfaces. In government labs, the samples are taken by the ministry's inspectors and tested in regional labs in Turkey. In addition, generally, 5 samples are taken from each lot for microbiological analysis.

SAMPLING AND PREPARATION OF HERBS AND SPICES FOR ANALYSIS

The choice of the sampling plan for food depends on the spoilage and health hazards associated with the micro-organisms of concern and how the food will be handled and consumed after it is sampled. A three-class attribute sampling plan with five samples taken at random from each lot of material, as described by the International Commission on Microbiological Specifications for Foods (ICMSF), is appropriate for routine microbiological examinations for aerobic plate count bacteria, yeasts, moulds, coliforms and *E. coli*. As with any type of food sampling, aseptic techniques should be employed. Most of the spices can easily be sampled with sterile three zone powder samplers, needle point samplers, scoops or spoons. The samples (200 g each) should be placed in sterile, polyethylene sample bags that are clearly labelled, submitted to the laboratory and tested. Spice samples should be stored in a cool (<20.0 °C) and dry area (<60% humidity) before testing.

Procedure: Sample preparation and the initial dilution vary according to the nature of the material being examined. Whole berries, roots, bark and large seeds should be reduced to a moderate particle size before testing. Aseptically weigh 100 g of the sample into a sterile, dry blender jar. Blend the sample at the lowest speed for 30 s or more. Take special care not to generate excessive heat during the blending step for this may injure or destroy the micro-organisms. Initial dilution: a) Ground spices, herbs, seasonings and small whole seeds: b) whole and coarsely ground leafy herbs Prepare 1:10 dilution (a) 1:20 dilution (b). Aseptically weigh 11±0.1g of the sample into a sterile filter stomacher bag, polypropylene bottle or blender jar and adding 99 ± 2ml (a) or 209 ± 2ml of 0.1% peptone water (b) and then either stomach for 30 to 60 s, shake at least 25 times or blend for 2 min depending on the type of container. Methods: It is important to note that some spices and herbs may have inhibitory action to bacteria and fungi and may produce low counts on lower dilution plates and high counts on higher dilution plates because of the transfer of the antimicrobial compounds with the inoculum. It is necessary to prepare a sufficient number of serial dilutions to overcome this natural inhibitory effect and prevent the reporting of low counts.

FUTURE NEEDS FOR THE MONITORING OF MICROBIAL CONTAMINATION

Especially, more rapid and reliable methods are needed for food manufacturing plants and for monitoring microbial contamination. For example, biosensors or fluorescence techniques could be utilized since they offer high sensitivity, short collection times and capability of monitoring large areas/volume. In Turkey, SMEs should establish a laboratory consisting of at least minimum instruments for microbial detection such as coliform, total aerobic bacteria, etc. in their plant. The most important and target micro-organisms responsible from the spoilage of food products are needed to be analyzed in the plant. The product traceability is a key element to be developed for food safety systems. The ability of trace back may be one of the most important weapons in determining of origin of contaminated or adulterated food. The ICMSF recommends that spices should be treated as raw agricultural commodities and as such the ultimate use of products will dictate the specifications. For example black pepper that contains a high concentration of spore forming bacteria may be suitable as a table condiment for seasoning cooked foods that will be eaten immediately, but maybe unsuitable for canned food processor. More swab samples should be taken from the production plants and this control should be done more frequently. Swabs should be taken for *Listeria monocytogenes* from the food contact surfaces where the *L. monocytogenes* problem has been experienced. Swabs samples should also be taken for *Enterobacteriaceae* and *Salmonella* from abattoirs e.g. chicken slaughterhouse.

Government agencies, academia and industrial microbiologists should establish controls in-process in a Hazard Analysis and Critical Control Point (HACCP) system to assure the product integrity, rather than reliance on end-product testing for compliance to specifications. HACCP verification and validation activities must be improved. The strengthening of HACCP systems that encompass all stages of production, processing and distribution will serve to further enhance the microbial safety of these products. If any company extends its product portfolio, some revisions should be done in present monitoring system. As new technologies and tests are introduced into the complicated arena of laboratory testing, it becomes increasingly difficult for regulatory and advisory agencies to provide specific safety regulations and guidelines for each new situation. It is, therefore, the responsibility of the laboratory itself to develop its own guidelines

and work practices to ensure a safe work environment for all employees. In addition, educating of consumers as to the importance of correct food handling practices will also help to prevent the spoilage and illness incidents. Furthermore, the food poisoning network should be organized among the related authorities. Collaboration in this topic should also be improved between industry and universities.

METHODS USED FOR RISK ASSESSMENT

HACCP, ISO2200/2005, IEC 17025 and BRC standards methods are especially used in all countries. Some companies have own standards and/or cleaning and sanitation programs. As new technologies and tests are introduced into the complicated arena of laboratory testing, it becomes increasingly difficult for regulatory and advisory agencies to provide specific safety regulations and guidelines for each new situation. It is, therefore, the responsibility of the laboratory itself to develop its own guidelines and work practices to ensure a safe work environment for all employees. Risk assessment is carried out by the HACCP team that meets when necessary – such as a modification in the system and determines the risks that may appear and the procedures to keep those risks from becoming actual problems. For environmental safety risks we have a system based on the requirements of the ISO 14001 standard.

Risk Assessment in laboratory: In the laboratory, the risk assessment for each separate procedure and experiment are done. In the laboratory, we use routine tests such as counting of total aerobic, coliform, yeast and moulds. Sterilized equipment are used such as sterile pipettes, sterile petridishes. The plating of micro-organisms is conducted under laminar flow hood. To develop effective strategies that continually guarantee employees a safe work environment, the performance of risk assessments must be an integral and on-going part of laboratory operation. The risk assessments should be carried out at regular intervals, at least annually, but more frequently if problems are discovered. It should be performed whenever a change occurs in the laboratory such as a move or renovation, new worker, new infectious agent or new reagent, new piece of equipment. Tools useful in performing laboratory risk assessments are:

- → Reviewing laboratory records
- → Injury, illness, and surveillance reports

→ Equipment maintenance records

→ Employee training records

→ Environmental monitoring records

→ Formal inspections by certifying agencies

→ Reviewing published materials, equipment manuals, manufacturers' bulletins and newsletters, product inserts, scientific journals, published safety manuals and guidelines

→ Observing laboratory operation (requires knowledge of relevant literature and experience with similar activities).

PREVENTIVE MEASURES

The companies which have been producing dried vegetables and spices should have HACCP, GMP, SSOP and ISO 22000. Preventive measures in plant are: GMP, process design, cleaning and disinfection, hygiene monitoring, protective clothing, physical separation of raw and cooked products and pest control. Equipment contacting to product should be cleaned easily. There is a specialized hygiene team which has daily/weekly/monthly/yearly hygiene program in plants. Authorized personnel should employ cleaning in place (CIP) systems in cleaning for the critical equipment e.g. bioreactors and fermentation tanks as well as storage tanks for yeast production. The cleaning agents and/or disinfectants should be approved to use in food industry. In the laboratory, we follow the cleaning and disinfection procedures and we wear laboratory coats (protective clothing) all the time. Training of personnel is another significant issue. Workers should always wear the protective clothing when they are in the processing line. Education of employees about good hygiene practice and HACCP program should be done, periodically. It should include use of protective clothing and consideration of preventive measures to insure product safety. As for, work safety every department employs specific equipment. In the laboratory microbiology safety equipment is used e.g. sterile rooms, sterile air cabinets, sterile gloves and breathing masks.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Peter Quantick from University of Lincoln, UK for his scientific recommendations and Jana Ramus from Chamber of Commerce and Industry of Slovenia for her group contribution.

Further Reading:

1. Anon. 1980. Microbial ecology of foods. Vol. 6, Food commodities. New York: Blackie Academic and Professional.
2. Anon. 1986. Micro-organisms in foods 2, sampling for microbiological analysis: principles and specific applications. 2nd ed. Toronto: University of Toronto Press.
3. Anon. 1994. Spice microbiology hazard background review. American Spice Trade Association, Inc., Technical bulletin No. 941024.
4. Anon. 1998a. Bacteriological analytical manual. 8th ed. Revision A. Gaithersburg: AOAC International.
5. Anon. 1998b. Report prepared under the responsibility of ILSI Europe Risk analysis in Microbiology Task Force. In: Louve, J.L., Stringer, N.F. & Baird-Parker, A.C. (Eds.). Food Safety management tools.
6. Anon. 2006. Biorisk management: Laboratory biosecurity guidance. www.who.org.
7. D'Aoust, J.Y. 1994. Salmonella and the international food trade. Int. J. Food Microbiol., 24, 11–31.
8. Guarino, P.A. 1973. Microbiology of spices, herbs and related materials. Spec. Rep. No. 13. Proceedings of the 7th Annual Symposium, Fungi and Foods. Geneva (NY): New York State Agricultural Experiment Station.
9. Hefnaway, Y.A., Moustafa, S. & Marth, E.H. 1993. Sensitivity of *Listeria monocytogenes* to selected spices. J. Food Prot., 56, 876–878.
10. Ismaeil, A. & Pierson, M.D. 1990. Inhibition of growth and germination of *C. botulinum* 33A, 40B and 1623E by essential oil of spices. J. Food Sci., 55, 1676–1678.

11. Kiss, I. & Farkas, J. 1988. Irradiation as a method for decontamination of spices. Food Rev. Int., 4, 77–92.

12. Kovacs-Domjan, H. 1988. Microbiological investigations of paprika and pepper with special regard to spore formers including *B. cereus*, Acta Alimentaria, 17, 257–264.

13. Liu, Z.H. & Nakano, H. 1996. Antibacterial activity of spice extracts against food-related bacteria. J. Fac. Appl. Bio. Sci., 35, 181–190.

14. Pafumi, J. 1986. Assessment of the microbiological quality of spices and herbs. J. Food Prot., 49, 958–963.

15. Patkar, K.L., Usha, C.M., Shetty, H. S., Paster, N. & Lacey, J. 1993. Effect of spice essential oils on growth and aflatoxin B1 production by *Aspergillus flavus*. Lett. Appl. Microbiol., 17, 49–51.

16. Pruthi, J.S. 1980. Spices and condiments: chemistry, microbiology and technology. Adv. Food Res. Suppl. 4. New York: Academic Press.

17. Shelef, L.A., Naglik, O.A. & Bogen, D.W. 1980. Sensitivity of some common food borne bacteria to the spices sage, rosemary and allspice. J. Food Science, 45, 1042–1044.

18. Tainter, D.R. & Grenis, A.T. 1993. Spices and seasonings: a food technology handbook. New York: VCH Publishers, Inc.

19. Vajdi, M. & Pereira, R.R. 1973. Comparative effects of ethylene oxide, gamma irradiation and microwave treatments on selected spices. J. Food Sci., 38, 893–895.

20. Weber, F.E. 1980. Controlling microorganisms in spices. Cereal Foods World, 25, 319–321.

APPENDIX 1: PARTICIPANT LIST

1. Esra Agel, Tübitak, Gebze, Kocaeli, Turkey
2. Günay Akdoğan, Balparmak, Istanbul, Turkey
3. Emine Aksan, University of Mustafa Kemal, Alahan, Antakya-Hatay, Turkey
4. Ayşe Handan Dinçer Baysal, Izmir Institute of Technology, Urla, Izmir, Turkey
5. Duygu Baş, Istanbul University, Istanbul, Turkey
6. Ziya Gökalp Ceylan, Atatürk University, Erzurum, Turkey
7. Karmen Dugar, Pomurske mlekarne, Murska Sobota, Slovenia
8. Helen Ehavald, AS Fazer Bakeries Estonia, Tallinn, Estonia
9. Erdal Eroglu, Izmir Institute of Technology, Urla, Izmir, Turkey
10. Sebastjan Filip, Pekarna Pečjak d.o.o., Trzin, Slovenia
11. Irena Fonda, FONDA.SI d.o.o., Portorož, Slovenia
12. Savvas Gennaris, EPTA Laboratories and Veterinary Diagnostics, Nicosia, Cyprus (visiting scientist at VTT Technical Research Centre of Finland, Espoo, Finland)
13. Metin Guldas, Uludağ University, Karacabey, Bursa, Turkey
14. Kadrin Juhkam, Estonian University of Life Sciences, Tartu, Estonia
15. Mateja Juvančič, Ljubljanske mlekarne d.d., Ljubljana, Slovenia
16. Asli Kisikkaya, Tübitak, Gebze, Kocaeli, Turkey
17. Fatma Köksal, Istanbul University, Istanbul, Turkey
18. Figen Korel, Izmir Institute of Technology, Urla, Izmir, Turkey
19. Ana Kramar, University of Ljubljana, Ljubljana, Slovenia
20. Liina Kutsar, Tallinn University of Technology, Tallinn, Estonia
21. Vasiliki Lagouri, Technological Educational Institute of Thessaloniki, Thessaloniki, Greece
22. Solmaz Lee, Food Research and Control Institute, Hurriyet, Bursa, Turkey
23. Elżbieta Maćkiw, National Food and Nutrition Institute, Warszawa, Poland
24. Monika Marin, Droga Kolinska d.d., Ljubljana, Slovenia
25. Marite Mets, Tallinn University of Technology, Tallinn, Estonia
26. Primož Mokorel, ETA Food Industry, Kamnik, Slovenia
27. Ceyda Pembeci Kodolbaş, Tübitak, Gebze, Kocaeli, Turkey
28. Gregor Perne, Pivovarna Union d.d., Ljubljana, Slovenia
29. Mati Roasto, Estonian University of Life Sciences, Tartu, Estonia
30. Ferda Seyhan, Tübitak, Gebze, Kocaeli, Turkey
31. Jaroslav Šimek, Institute of Public Health in Ostrava, Ostrava, Czech Republic
32. Terje Tamme, Estonian University of Life Sciences, Tartu, Estonia

33. Hasan Ture, Izmir Institute of Technology, Urla, Izmir, Turkey
34. Irfan Türetgen, Istanbul University, Istanbul, Turkey
35. Roxana Ursu, Rompak Baker's Yeast Company, Pascani, Iasi, Romania
36. M. Fatih Uyar, Hacettepe University, School of Health Technology, Ankara, Turkey
37. Işil Var, School of Agriculture, University of Çukurova, Adana, Turkey
38. Cilka Vučak, Perutnina Ptuj d.d., Ptuj, Slovenia
39. Martina Weiss, Žito Group, Ljubljana, Slovenia
40. Hülya Ölmez, Tübitak, Gebze, Kocaeli, Turkey
41. Nil Pembe Özer, Kocaeli University, Izmit, Kocaeli, Turkey
42. Tarik Öztürk, Tübitak, Gebze, Kocaeli, Turkey
43. Şebnem Öztürkoğlu, Tübitak, Gebze, Kocaeli, Turkey

44. Mehlika Borcakli, Tübitak, Gebze, Kocaeli, Turkey
45. Kamil Tayfun Carli, Uludağ University, Bursa, Turkey
46. Ayşegül Eyigör, Uludağ University, Bursa, Turkey
47. Bo B. B. Jensen, BioCentrum-DTU, Kgs. Lyngby, Denmark
48. Renata Karpíšková, National Institute of Public Health, Brno, Czech Republic
49. Peter Quantick, University of Lincoln, Brayford Pool, Lincoln, UK
50. Laura Raaska, VTT Technical Research Centre of Finland, Espoo, Finland
51. Jana Ramuš, Chamber of Commerce and Industry of Slovenia, Ljubljana, Slovenia
52. Ivan Rychlik, Veterinary Research Institute, Brno, Czech Republic
53. Satu Salo, VTT Technical Research Centre of Finland, Espoo, Finland
54. Alpay Seyhan, JohnsonDiversey, Içerenköy, Istanbul, Turkey
55. Sonja Smole Možina, University of Ljubljana, Ljubljana, Slovenia
56. Tiina Veskus, Tallinn University of Technology, Tallinn, Estonia
57. Raivo Vokk, Tallinn University of Technology, Tallinn, Estonia
58. Gun Wirtanen, VTT Technical Research Centre of Finland, Espoo, Finland
59. Güner Özay, Tübitak, Gebze, Kocaeli, Turkey

APPENDIX 2: SEMINAR PROGRAMME

Monday, October 22, 2007

8.30–9.10	Registrartion & coffee/tea in the meeting lobby
9.10–9.45	Welcome and general information on the seminar; Dr. Gun Wirtanen, VTT Technical Research Centre of Finland (VTT), Espoo, Finland
9.45–10.15	Biofilm problems of pathogens in food processing lines; Dr. Gun Wirtanen, VTT Technical Research Centre of Finland, Espoo, Fınland
10.15–11.00	Modelling as a tool in evaluating hygienic design of food processing equipment; Assoc. Prof. Bo B. B. Jensen, BioCentrum-DTU, Kgs. Lyngby, Denmark
11.00–11.30	Good hygiene practices and hygienic design; Alpay Seyhan, JohnsonDiversey, Istanbul, Turkey
11.30–11.40	Brief introduction to group works; Dr. Gun Wirtanen, VTT Technical Research Centre of Finland, Espoo, Finland
11.40–13.00	Lunch
13.00–13.30	Experimental data and modelling in optimisation of tank cleaning; Dr. Satu Salo, VTT Technical Research Centre of Finland, Espoo, Finland
13.30–14.00	Efficacy of cleaning agents and disinfectants used in decontamination procedures in food industry; Dr. Gun Wirtanen, VTT Technical Research Centre of Finland, Espoo, Finland
14.00–14.30	Coffee/tea break
14.30–15.00	Detection of *Salmonella* in poultry – measures performed within risk assessment in Turkey; Assoc. Prof. Ayşegül Eyigör, Uludağ University, Bursa, Turkey
15.00–15.30	Prevelance and mechanisms of resistance of *Campylobacter* sp. in poultry and water; Assoc. Prof. Sonja Smole Možina, University of Ljubljana, Ljubljana, Slovenia
15.30–16.00	*Listeria* in ready-to-eat products; Dr. Renata Karpíšková, National Institute of Public Health Center for Food Chain Hygiene, Brno, Czech Republic
16.00–16.30	Coffee/tea break
16.30–17.00	Prevention of toxigenic moulds in grains, nuts and dried foods; Assoc. Prof. Güner Özay & Dr. Ferda Seyhan, Tübitak, Gebze, Kocaeli, Turkey

17.00–17.30	Protective cultures and antimicrobial activities in foods; Dr. Mehlika Borcakli, Tübitak, Gebze, Kocaeli, Turkey
18.00–20.30	Dinner

Tuesday, October 23, 2007

9.00–9.30	Registration for the second day & Coffee/tea served in the meeting lobby
9.30–10.15	Basic principles of microbial risk assessment; Dr. Peter Quantick, University of Lincoln, Lincoln, UK
10.15–10.45	The HYGRAM® -system – a practıcal tool for risk assessment in food industry; Dr. Laura Raaska, VTT Technical Research Centre of Finland, Espoo, Finland
10.45–11.15	Coffee/tea served in the in the meeting lobby
11.15–12.00	Past, present and future of HACCP systems; Dr. Peter Quantick, University of Lincoln, Lincoln, UK
12.00–12.30	Viruses as a cause of foodborne diseases; Dr. Ivan Psikal & Dr. Ivan Rychlik, VRI, Brno, Czech Republic
12.30–13.30	Lunch
13.30–13.45	Introduction to group works; Dr. Gun Wirtanen, VTT Technical Research Centre of Finland, Espoo, Finland
13.45–16.15	Group works including preparation of presentations
15.00–15.45	Coffee/tea served in the in the meeting lobby
16.15–17.30	Presentations given by the groups
17.30–18.00	Concluding remarks given by Prof. Raivo Vokk, Tallinn University of Technology, Tallinn, Estonia

Series title, number and report code of publication

VTT Symposium 251
VTT–SYMP–251

Author(s)
Wirtanen, Gun & Salo, Satu (Eds.)

Title

RISK ASSESSMENT OF MICROBIAL PROBLEMS AND PREVENTIVE ACTIONS IN FOOD INDUSTRY

Abstract

Harmful microbes may enter the manufacturing process and reach the end product in several ways e.g. through raw materials, air in the manufacturing area, chemicals employed, process surfaces or factory personnel. The elimination of microbes growing on surfaces, this growth is also known as biofilm formation, in both direct and indirect contact with the food products produced is a very difficult and demanding task because many factors e.g. temperature, time, mechanical and chemical forces affect the microbial detachment. The target of microbial control in a process line is twofold: to prevent the entrance and establishment of harmful microbes on process surfaces and to limit the number or activity of microbes by controlling deposit formation. The second open seminar *Risk assessment of microbiological problems and preventive actions in food industry*, which was held in Istanbul 22nd–23rd of October 2007, focused on risk assessment, Good Manufacturing Practises (GMP), Hazard Analysis Critical Control Point (HACCP) as well as hygienic design and other primary tools used in controlling microbiological hazards in food industry. Identification of definitive critical control points, however, is difficult and the implementation of effective HACCP programs can be complex and slow. In EU Member States HACCP programs have been implemented for years in food, packaging and other food related processing industries e.g. lubricant industry. As can be seen from these proceedings significant experience was distributed and discussed in this seminar. The abstracts and group works of the participants are also published in this publication.

ISBN
978-951-38-6325-8 (soft back ed.)
978-951-38-6326-5 (URL: http://www.vtt.fi/publications/index.jsp)

Series title and ISSN	Project number
VTT Symposium 0357-9387 (soft back ed.) 1455-0873 (URL: http://www.vtt.fi/publications/index.jsp)	8673

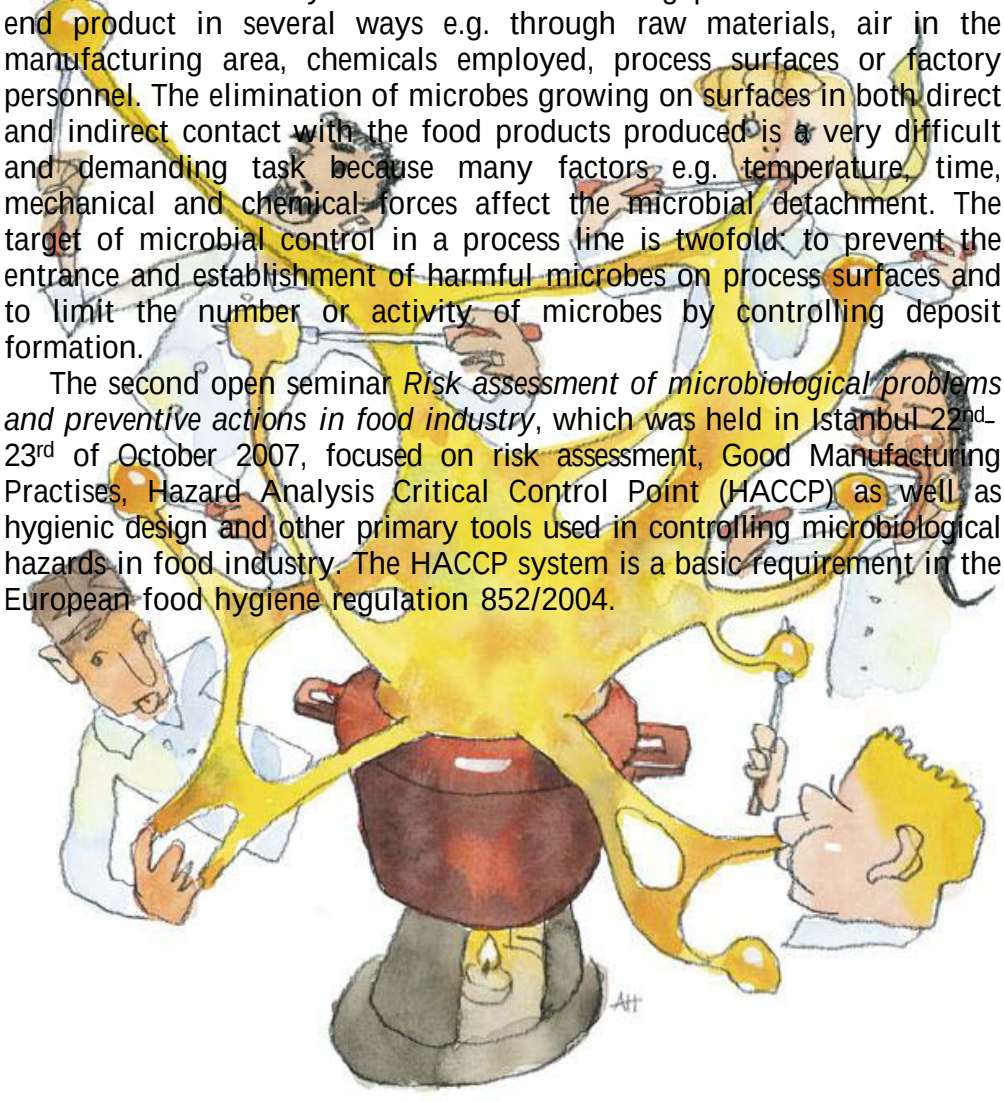
Date	Language	Pages
January 2008	English	176 p. + app. 4 p.

Name of project	Commissioned by
SAFOODNET	SAFOODNET; FP6-022808-2006

Keywords	Publisher
risk assessment, food processing, good hygiene practices, hygienic design, contamination routes, pathogens, microbial contaminants, biofilm, cleaning, disinfection, antimicrobial effects	VTT P.O. Box 1000, FI-02044 VTT, Finland Phone internat. +358 20 722 4520 Fax +358 20 722 4374

Harmful microbes may enter the manufacturing process and reach the end product in several ways e.g. through raw materials, air in the manufacturing area, chemicals employed, process surfaces or factory personnel. The elimination of microbes growing on surfaces in both direct and indirect contact with the food products produced is a very difficult and demanding task because many factors e.g. temperature, time, mechanical and chemical forces affect the microbial detachment. The target of microbial control in a process line is twofold: to prevent the entrance and establishment of harmful microbes on process surfaces and to limit the number or activity of microbes by controlling deposit formation.

The second open seminar *Risk assessment of microbiological problems and preventive actions in food industry*, which was held in Istanbul 22nd–23rd of October 2007, focused on risk assessment, Good Manufacturing Practises, Hazard Analysis Critical Control Point (HACCP) as well as hygienic design and other primary tools used in controlling microbiological hazards in food industry. The HACCP system is a basic requirement in the European food hygiene regulation 852/2004.



SAFOODNET

Julkaisu on saatavana	Publikationen distribueras av	This publication is available from
VTT PL 1000 02044 VTT Puh. 020 722 4520 http://www.vtt.fi	VTT PB 1000 02044 VTT Tel. 020 722 4520 http://www.vtt.fi	VTT P.O. Box 1000 FI-02044 VTT, Finland Phone internat. + 358 20 722 4520 http://www.vtt.fi

ISBN 978-951-38-6325-8 (soft back ed.)
ISSN 0357-9387 (soft back ed.)

ISBN 978-951-38-6326-5 (URL: http://www.vtt.fi/publications/index.jsp)
ISSN 1455-0873 (URL: http://www.vtt.fi/publications/index.jsp)