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VTT SYMPOSIUM 248

MICROBIAL CONTAMINANTS & CONTAMINATION ROUTES IN FOOD INDUSTRY

1st Open Seminar arranged by SAFOODNET – Food Safety and Hygiene Networking within New Member States and Associated Candidate Countries

VTT

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Keywords:
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1ST OPEN SEMINAR ARRANGED BY SAFOODNET – FOOD SAFETY AND HYGIENE NETWORKING WITHIN NEW MEMBER STATES AND ASSOCIATED CANDIDATE COUNTRIES; FP6-022808-2006

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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 first seminar arranged by SAFOODNET to a targeted audience of industrial, research and authority people is titled "Microbial contaminants and contamination routes in food industry" and it is dealing with characteristics of important contaminants, contamination routes and factors enhancing microbial contamination of process line. The forthcoming activities in SAFOODNET-project are 2 open seminars in risk assessment and management to disseminate safety issues; 2 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 EG to suggest and evaluate pilot actions for future needs in RTD activities; performance of pilot actions in food safety suggested by the EG in food industry especially in SMEs as a base for future RTD activities and building-up a new channel for scientific co-operation within the new EU.

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APPENDIX 2: SEMINAR PROGRAMME

LECTURES

BIOFILM FORMATION IN FOOD PROCESSES

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Microbes inhabiting contact and environmental sites in food processing are mostly harmful, because microbial communities that form in critical places contaminate the surfaces and consequently the products made in that particular process. Some microbes naturally have a higher tendency to produce biofilm than others, but biofilm can generally be produced by any microbe under suitable conditions i.e. on moist surfaces with some nutrients. This is the case especially if the process equipment is not hygienically integrated in the process line or the cleaning and disinfection procedures are not properly designed to remove the organic soil from the process surfaces. Equipment that causes problems in food processing and packaging include slicing and cutting equipment, filling and packing machines, conveyors, plate heat exchangers and tanks with piping. *Listeria monocytogenes* has been found on equipment and process surfaces, which are difficult to clean. Such equipment can thus cause microbial contamination in food processing. Therefore the design of the equipment and process line in the food processing and packaging industry is important for preventing formation of the biofilm and so improving process and production hygiene. Furthermore, pathogens in biofilms have been found to be more resistant to many types of disinfectants.

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. It is also important to remember that about 85–96% of a biofilm consists of water, which means that only 2–5% of the total biofilm volume is detectable on dry surfaces. A biofilm consists of microbial cell clusters with a network of internal channels or voids in the extracellular polysaccharide and glycoprotein matrix, which allows nutrients and oxygen to be transported from the bulk liquid to the cells. Once a biofilm has been formed, it can be a source of contamination for foods passing through the same processing line. At present the most efficient means for

limiting the growth of microbes are good production hygiene, the sensible running of the process line, and the well-designed use of cleaning and decontamination processes. Common foodborne pathogens such as *Bacillus cereus, Staphylococcus aureus, L. monocytogenes, Mycobacterium paratuberculosis, Clostridium perfringens, Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Campylobacter jejuni,* and *Yersinia enterocolitica* have readily been found to produce biofilms on surfaces. This presentation deals with biofilm formation of common foodborne pathogens.

Salmonella is a non-spore-forming rod-shaped, mostly motile Gram-negative bacterium belonging to the family *Enterobacteriaceae,* in which approximately 2,200 serotypes are recognised. Foods commonly associated with the disease include raw meats, poultry, eggs, milk and dairy products. Milk-borne salmonellosis is common in parts of the world where milk is neither boiled nor pasteurised. It occurs, but much less frequently, in developed countries where the main products implicated are pasteurised milk, powdered milk and certain cheeses. Several groups have reported that *Salmonella* has formed biofilm on various types of surfaces used in the food processing industry. These studies have shown that *Salmonella* spp. can form biofilms on food contact surfaces and that the cells in biofilms are much more resistant to sanitizers compared with planktonic cells.

E. coli is a Gram negative, rod-shaped bacterium. Because many microbes from faeces are pathogenic in animals and humans, the presence of the intestinal bacterium *E. coli* in water and foods indicates a potential hygiene hazard. Most strains of *E. coli* are harmless. However, a few strains with well-characterised traits are known to be associated with pathogenicity. Those causing the greatest concern in water and foods are the five major groups of intestinal pathogens i.e. the enterohaemorrhagic, the enterotoxigenic, the enteroinvasive, the enteropathogenic and the enteroaggregative *E. coli*. *E. coli* has been isolated from a large number of foods and drinks e.g. dairy products, fresh soft cheeses, Camembert and Brie cheeses, vegetables, meat, fermented meat sausage, poultry and fish products, water and apple cider. These agents can cause outbreaks of diarrhoea. However, food-poisoning outbreaks caused by dairy products contaminated with *E. coli* have been rare. *E. coli* O157:H7 has been isolated from raw milk and bulk tank milk samples in the range of 0 to 10%. *E. coli* O157:H7 is often transmitted to humans via unpasteurised milk. *E. coli* can also survive for

extended periods of time in several types of acidic foods e.g. cheese and yogurt. Acid-adapted *E. coli* O157:H7 has shown enhanced survival and prevalence in biofilms on stainless steel surfaces.

Campylobacter spp. are microaerophilic, very small, curved and thin Gram negative rods. Ingesting milk with as few as 500–800 cells can cause illness. Since the infective dose is quite low and the food may in many cases contain only a few cells, liquid enrichment methods are normally required before plating on a selective medium in order to detect contamination with *C. jejuni* or *C. coli.* Successful detection of these organisms requires incubation at 42 °C under microaerophilic conditions. Laboratory tests have shown that in optimum circumstances campylobacter is able to form a biofilm on stainless steel and glass in 2 days. The number of viable *C. jejuni* determined by using a direct viable count was greater than by using culturing techniques, which indicates that *C. jejuni* cells can form a viable but nonculturable state within the biofilm. Both determination methods showed that biofilms enhance the survival of *C. jejuni* during a 7-day period at 12 ºC and 23 ºC. Food residues or moisture improve the chances of survival by campylobacter on surfaces. It also survived very well on wooden surfaces because the pores in the wood protect the cells from oxygen.

L. monocytogenes is a facultatively anaerobic Gram-positive, non-spore-forming short rod that is widely distributed in nature. It is a non-host specific pathogen. Listeriosis may occur sporadically or epidemically. The organism has been isolated from raw milk, mastitic milk and pasteurised milk. Foodstuffs associated with listeriosis outbreaks also include cold-smoked and gravad rainbow trout products, sliced cold cuts, soft cheese, butter, ice-cream and coleslaw. Examples of epidemic sources are: coleslaw in Canada 1981, unpasteurised milk in the USA 1983, Mexican-style soft cheese in USA 1985, a pork product in France 1992, chocolate milk in the USA 1994, soft cheese in Switzerland 1995, rainbow trout in Sweden 1997, corn in Italy 1997, hot dogs in the USA 1998–99 and butter in Finland 1999. Of the 13 different *L. monocytogenes* serotypes, only three (1/2a, 1/2b and 4b) have been predominantly implicated in human diseases. It has been reported that healthy people can be carriers of *L. monocytogenes*. *L. monocytogenes* is able to grow in many environments: it can grow at low oxygen tensions, in high salt concentrations and over a wide range of pH and temperatures. The bacterium can survive for a limited time in up to 25% salt at 4 °C. Hygiene monitoring in

the food processing industry is important, because *L. monocytogenes* can colonise and form biofilms in food processing environments and on surfaces. *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.

S. aureus is an opportunistic pathogen, which is a Gram-positive, aerobic, non-spore-forming catalase positive rod. Nasal and skin carriages are frequent vehicles in the transportation of *S. aureus.* Its occurrence in sour milk products such as yoghurt is worthy of investigation, as it is present in relatively high numbers in raw milk. Staphylococci have been shown to grow rapidly during the initial fermentation. Similar behaviour by *S. aureus* has previously been reported in both yoghurt and cheese. *S. aureus* was isolated in a total of 7% of food contact sites and 8% of environmental sites from 10 SMEs that produce high risk foods in the United Kingdom. The source of *S. aureus* almost always originated from food handlers or from utensils previously contaminated by humans. Contamination of food can occur as a result of poor hygienic practices in any part of the food chain. Resistance to oxidative disinfectants has mainly been associated with biofilm formation. For a 4-log kill of *S. aureus* in biofilms on stainless steel, polystyrene and glass in a nutrient flow 50 and 600 times higher concentrations of benzalkonium chloride and hypochlorite were needed than for suspended cells, respectively.

B. cereus is a Gram-positive, aerobic, spore-forming rod, normally present in soil, dust, and water. *B. cereus* cells are large and motile. The organism produces a number of toxins with distinct diarrhoeal and emetic syndromes. *B. cereus* occurs extensively in the environment but despite the fact that it is a common contaminant in raw milk, food-poisoning outbreaks caused by dairy products contaminated with *B. cereus* have been rare. In a dairy product survey *B. cereus* was found in 52% of ice-creams, 35% of soft ice creams, 29% of milk powders, 17% of fermented milks and 2% of pasteurised milks and fruit-flavoured milks. Indicators of a prolonged contamination problem caused by mesophilic *B. cereus* strains early in the production chain have also been shown in dairy plants. Additional contamination of milk by the *B. cereus* biofilm has been shown to occur in the filling machine. Different *Bacillus* spp., and among them *B. cereus*, have been found on liquid packaging boards and blanks and could thus be an

additional source of biofilms containing *Bacillus* spp. Furthermore, spores of *B. cereus* are reported to possess a pronounced ability to adhere to the surface of stainless steel, which is commonly used in food processing. Spores of *B. cereus* have been shown to adhere, germinate and multiply on the stainless steel surfaces of a tube heat exchanger. The attachment of *B. cereus* in process lines may act as a continual source of post-pasteurisation contamination.

C. perfringens is a spore-forming, Gram-positive, anaerobic, non-motile rod which forms large, regular, round and slightly opaque and shiny colonies on the surface of agar. There are 5 types of *C. perfringens*: A, B, C, D and E, which produce different types of toxins. Spores of some strains are resistant to heat treatment at 100 °C for more than l hour. Cases of *C. perfringens* food poisoning from new food sources, because it is so adaptable and prolific, have helped to show how our perceptions and understanding of safe food change with new knowledge. *C. perfringens* can be found in the normal flora of the intestinal tracts of both animals and humans, as well as in soil, clothing, and skin. It has been found in virtually all environments tested, including water, milk and dust. In view of its widespread presence in moist soil, its presence in air and dust in kitchens, catering and food processing environments is not surprising. Food products besides dairy products commonly contaminated with *C. perfringens* are poultry, fish and vegetables.

The genus *Mycobacterium* contains approximately 50 species, which are divided into rapid growers, slow growers and the human leprosy bacillus. Mycobacteria are weakly Gram-positive, non-motile, slender, non-spore-forming, rod-shaped, aerobic and free-living in soil and water. They are widely distributed in nature and have been isolated e.g. from natural and piped waters, unpasteurised milk, butter and vegetables. They do not produce appreciable amounts of toxin substances and do not cause food poisoning. Infections in humans and animals may be caused by most of the slowly growing mycobacteria e.g. *M. avium* and *M. xenopi*. The only rapidly growing pathogenic species are *M. chelonae* and *M. fortuitum*. A pilot plant pasteuriser was used to examine the heat resistance of *M. avium* subsp. *paratuberculosis* (*M. paratuberculosis*) during HTST pasteurisation using raw milk samples under various time and temperature conditions. Results indicated that low numbers of *M. paratuberculosis* may also survive extreme HTST treatments. The ability of mycobacteria to colonise experimental drinking water distribution systems has been investigated, and it

was found to be present in biofilm within an hour of introduction for over 9 weeks. It was constantly present in outlet water samples and in biofilm samples.

References to this extended abstract can be found in the book chapter: Wirtanen, G. & Salo, S. 2005. Biofilm risks. In: Handbook of hygiene control in the food industry. Lelieveld, H., Mostert, T. & Holah, J. (eds.). Cambridge: Woodhead Publishing Ltd. Pp. 46–68. ISBN 1-85573-957-7.

MICROBIAL ECOLOGY IN MANUFACTURING PAPER-BASED PACKAGING MATERIALS FOR USE IN FOOD INDUSTRY

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Packaging serves as a major defence against external hazards. However undesirable interactions between packaging material and food can give rise to potential problems e.g. migration of packaging components or penetration of micro-organisms through packages. The importance of hygiene and microbial management in the industry manufacturing packaging material has increased considerably as a result of the changes in legislation, tighter international competition and increasing customer requirements. The contact time between package with food has extended due to longer selling periods and extended transport time from production plant to market. Furthermore the package is not any more only used as a cover of food product during transportation and storing but often also as a tray in which food can be warmed up and served as well. Simultaneously today consumer prefers minimally processed food with no preservatives, low fat, sugar and salt content and a long time of use making in a way food more susceptible for microbial contamination. Important microbial growth enhancing changes such as increased use of recycled fibre as a raw material and decreased consumption of water (closing of water circulation system) has taken place as well in manufacturing of paper and board that also stress the importance of hygiene and efficient microbial management. Due to these important changes manufacturers of food packaging materials as important raw material suppliers for food industry have become increasingly conscious of customer demands relating to concerns of food safety. Today, most food and packaging material plants have a hygiene and safety management system built on the HACCP concept and good manufacturing practice systems.

The date a legal requirement exists for chemical quality of raw materials and food packages, but microbiological requirements are usually missing. The legislation on food contact materials does not give any specifications for the

microbiological quality of the materials. The Policy Statement of Council of Europe (CoE) concerning paper and board materials and articles intended to come into contact with food states that materials intended to come in contact with food should be of suitable microbiological quality taking into account the intended end use and that for materials intended to come in contact with aqueous and/or fatty foodstuffs particular attention should be paid to pathogens.

The risk connected to packaging material is the potential transfer of food spoilage or pathogenic organisms to the packed food. Most packaging materials have proven to be completely impervious to micro-organisms. About 1% of the micro-organisms found in the board has been estimated to transfer. This indicates that transfer is possible but at low rate. Moreover, the microbial load of fresh food products is incomparably large relative to the amounts of micro-organisms that could permeate through package that this phenomenon can usually be neglected However even a low number of bacteria in the packaging material could be of concern for e.g. aseptic foods, if bacteria migrate across the package. The routes of contamination from the packaging material to food include the surface, cutting dust or direct contact with the raw edge of the paperboard.

Production of fibre-based food contact materials include several phases in which microbiological problems can occur. However it must be stressed that the threat of microbial contamination from packaging materials is more theoretical while food itself is always the most prominent source of microbial contamination. There are no published cases were micro-organisms originating from packaging material have migrated into the food, multiplied there and caused illness of a human. However there are cases when micro-organisms originating from packaging materials have spoiled the food product. Paper and board machines offer micro-organisms a favourable environment to grow and multiply, and hence their numbers may be quite high without causing significant problems for runnability or end product safety. Therefore, it is more important to determine the nature of the micro-organisms (e.g. pathogen, slime producer, toxin former, producer of off-odours) than their exact numbers.

Microbiological problems in the paper industry can be grouped as follows:

1. Micro-organisms causing spoilage of raw materials, since many micro-organisms break down cellulose fibres, starch, casein and rosin sizing e.g. *Pantoea agglomerans* and *Bacillus subtilis* spoil starch.
2. Micro-organisms causing problems in the process: producers of slime and deposits e.g. *Burkholderia cepacia*, *Deinococcus* spp. and *Bacillus* spp.
3. Micro-organisms threatening process safety: micro-organisms harmful to human health in raw materials, process environment and end products e.g. *Bacillus cereus, Klebsiella pneumoniae* and *Staphylococcus aureus.*
4. Micro-organisms reducing the quality of end product: micro-organisms harmful to human health and their metabolic products (toxins), micro-organisms that can cause colour defects in products and micro-organisms harmful to the hygiene of the end product e.g. *Bacillus cereus*, *Staphylococcus aureus*, coliforms and moulds.
5. Micro-organisms that can cause smell or taste defects, such as the volatile, foul-smelling compounds produced during microbial metabolism e.g. *Clostridium* spp., *Desulfovibrio* spp. and actinobacteria.

In paper machine environment the micro-organisms are to be found colonising surfaces in organised biofilm. The microbial ecology and parameters that affect the well-being of the microbial community in industrial environments is poorly known. Microbial ecology of industrial environments is challenging to be studied because great variation in the cell density and composition of microbial population exist over space and time as natural and environmental factors affect microbial growth and as a consequence the qualitative and quantitative composition of the microbial community. However to be able to control the microbial growth efficiently with very targeted actions we need knowledge on the species, their characteristics and changes in the microbial community over time and space. One approach is to perform microbiological surveys were samples are taken from the raw materials, process environment and the end product, micro-organisms detected and identified by using both conventional and modern methods based on molecular biology.

Based on research results obtained hitherto the numbers of potential pathogens are very low, most of the potential pathogens have been shown to be not virulent

and the migration of micro-organisms from packaging materials into food is very unlikely. However competence in microbiology is required to perform reliable risk assessment and to focus the risk assessment measures efficiently and cost-effectively. Furthermore microbiological risk assessment and risk management activities at industry level require significant amount of microbiological analysis performed by using sufficiently sensitive and rapid detection methods. While traditional cultivation methods are far too slow compared to the turnover of fibre-based products and in many cases not sensitive enough to detect pathogens that may cause illness at very low concentration there has been a clear need for novel rapid and sensitive detection as well identification methods.

THE RELEVANCE OF PSYCHROTROPHIC SPOILAGE MICROBES IN FOOD HYGIENE

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Microbial metabolism of organic mater is a naturally occurring biological process. When this activity leads to unappealing and unacceptable foodstuff, this process is considered microbial spoilage. Since many foods provide ideal conditions for the growth of micro-organisms, spoilage is a constant concern in the modern food processing industry causing severe economic losses to the manufacturers and retailers. Microbial spoilage is also a concern to the world's food supply contributing to a vast amount food that is wasted. This presentation will focus on bacteria that are to grow and cause spoilage in refrigerated foods, with particular reference to lactic acid bacteria. The relevance of psychrotrophic spoilage LAB in food hygiene will be addressed in examples of contamination studies carried out in collaboration with the Finnish food operators.

Microbial food spoilage is a complex process. Bacterial growth to high numbers is considered a prerequisite for the development of sensory defects in many different types of products. The sensory signs of bacterial spoilage are many and varied; they may be visual (slime, swelling, discoloration), or be apparent by smell or taste (off-odours and off-flavours). These signs are typically a result of the accumulating by- and end-products formed during metabolic activity of bacteria. Many studies have shown that from the great variety of bacteria contaminants present in the initial population of a cold-stored product, only a small fraction are able to grow and predominate the so called "spoilage population". The bacteria responsible for quality deteriorations are often called "specific spoilage organisms" (SSO). Many factors together, such as product type (physical and chemical characteristics, preservatives) and the way it is processed, packaged and stored select the bacteria that are able to survive, compete and grow to high levels, and hence cause spoilage.

In large-scale manufacturing of meat and meat products, the objective is often to achieve the longest possible shelf-life to meet the demands of trade and logistics. The traditional way to manage microbial spoilage of meat products relies on good hygiene, appropriate cold-storage and advanced packaging techniques such as packaging under vacuum or modified atmospheres. Due to these current packaging techniques, psychrotrophic lactic acid bacteria (LAB) usually predominate the microbial populations in cold-stored, packaged meats whereas the growth of aerobic, gram-negative organisms (*e.g. Pseudomonas* spp. and *Shewanella putrefaciens*) is restricted. In case of temperature abuse, *Enterobacteriaceae* may also reach high levels and contribute to rapid spoilage.

Psychrotrophic LAB are a versatile group of organisms. Depending on the nutrients available and the various processing steps (heat treatment, preservatives) conducted, the growth of specific spoilage LAB can be very rapid and they may cause severe quality deteriorations. The genera associated with quality deteriorations in meat and meat products include *Carnobacterium, Lactobacillus* and *Leuconostoc*. LAB spoilage of meats is typically manifested as sour off-odours and off-flavours attributed to lactic acid, acetic acid or volatile fatty acids. This “fermenting type of spoilage” is often accompanied by gas accumulation and bulging of the package, or formation of visible slime or green discoloration on the meat surface. The growth of spoilage LAB in meat products is difficult to control due to their resistance to environmental conditions that would inhibit most other bacteria such as low pH, refrigeration temperatures and packaging in the absence of oxygen. In general, LAB are also considered relatively resistant to nitrite which is used by meat industry to control bacterial growth in various processed meat products.

Food may become contaminated with spoilage LAB at all stages of processing (harvesting, processing, storage). Since many of the psychrotrophic spoilage bacteria are wide-spread in nature, contaminated raw material or ingredients are often thought to be major sources of spoilage bacteria. Besides being brought into production area along with the raw materials, spoilage bacteria can be recovered in the processing plant air or they may contaminate the product via workers. Spoilage bacteria may also build up in high numbers in processing equipments, and processing environment may even select certain types of SSO to form an in-house population. In many cases, control of SSO is dependent on maintaining good plant sanitation and high-quality ingredients. To avoid

contamination, it is also advisable to handle fermented meat products separately from cooked meats.

Solving food hygienic problems caused by spoilage bacteria has proven challenging. Psychrotrophic bacteria are not equal in their spoilage potential; therefore contamination analysis requires conventional sampling and culturing techniques together with molecular typing methods. A careful sampling plan is also essential to detect the spoilage bacteria among the background microbial populations and to trace their routes through the production chain. A set of molecular typing or characterization methods and an identification database is often needed, since for example LAB or *Pseudomonas* spp. cannot be reliably identified by conventional phenotypic techniques. To study spoilage LAB diversities, we have established an identification database at Department of Food and Environmental hygiene, University of Helsinki, Finland. In this library, ribotyping patterns (= 16S and 23S rRNA gene HindIII restriction fragment length polymorphism patterns) are used as the operational taxonomic units in the numerical analysis and currently it comprises ribopatterns of all relevant food-associated LAB including over 200 type and reference strains. To identify and characterize LAB isolates, the similarities between new strains (ribopatterns) and the type strains can be visualized in the form of dendrogram. Once the contaminants or the SSO have been identified, control of food spoilage requires understanding of their ecology, physiological properties and metabolism. This knowledge is fundamental to determine and implement improvements to prevent the contamination and hinder the survival or growth of the SSO in the product.

Bacterial spoilage continues to be an important factor limiting the shelf-life of fresh meat and meat products. Many meat operators consider a spoilage incident potentially as damaging to the product brand as a foodborne outbreak. Since foodborne pathogens are rarely responsible for spoilage, and most of the psychrotrophic spoilage bacteria are rarely associated with foodborne outbreaks, microbiological safety and microbial spoilage are, however, often considered two separate issues. In general, there are no management tools systematically planned out to control spoilage bacterial contamination during meat processing. In many cases, management of spoilage bacterial contamination is based on good manufacturing process and hygienic practices. However, experience and research have shown that good hygienic practices alone cannot guarantee complete avoidance contamination with certain spoilage bacteria, such as LAB.

More information about psychrotrophic spoilage bacteria thriving in food ecosystems and their contamination routes are necessary to determine adequate management decisions with regard to controlling this type of spoilage. However, pre-existing microbial control strategies, such as hygiene prerequisites and risk assessment approaches, which have been designed to control food safety could also be applied in managing microbial food spoilage.

IMPACT OF COMPOSITION AND METABOLITES OF INTESTINAL MICROBIOTA ON FOODBORNE INFECTIONS

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Several scientists of the early 20th century (L. Pasteur, I. Metchnikoff, T. Escherich) concluded that variety of diseases could be attributed to the disruption of the indigenous microflora, allowing pathogenic microorganisms to multiply in host and cause disease. Further, with development of antimicrobial therapy it was clearly shown that the disruption of normal microbial ecology of gut increased the prevalence of enteric infections and there were emerging protracted cases of gastroenteritis. Very complicated and variable pathogenesis of enteric, mainly food borne infections has still dimmed the relationship between host normal microbiota and the hosts' susceptibility to infection. The composition of intestinal microflora e.g. microbiota (IMF) of healthy individual comprises around 2000 species, their communities express both common and individual features in a host. The lecture deals with the definitions of normal indigenous intestinal microbiota, its differences in particular niches of the intestine and the genetically determined stability of host microflora. The functions of IMF, particularly the role of microbial metabolism in health and disease has been largely studied *in vitro*, in experimental models, volunteer and clinical trials. The main impact comprises the influences on GI mucosal morphology, digestion, peristaltics, host metabolism, immunity and improvement of colonisation resistance. New methods and tools have provided investigators with rich databases on *composition* of IMF collected by the culture based methods, molecular tools (FISH, PCR-DGGE, PFGE, RT-PCR, etc. mRNA expression), by morphological evaluation (Gram staining, electronmicroscopy, immuno-morphological detection by specific antibodies). For studies of IMF *metabolism* several methods have been applied: the culture based methods for detection of organic acids, lipo-and proteolytic activity but also the estimation of the metabolic endproducts by gas-chromatography, high pressure liquid chromatography, Maldi-TOFF spectrometry, SDS-PAGE. *Host–IMF interactions* could be evaluated by morphological

assessment of physiological/pathological lesions in organs, assessment of cellular defence (Macrophages, PMN leukocytes, T and B lymphocytes in tissues, searching for serum antibodies, interleukines, MF, PMN, Toll like receptor, TLR activation.

Mechanisms of action of IMF against pathogens can be divided as follows:

- Indigenous IMF specific *competitive exclusion* of infectious agents (anti-adhesion factors, competition for nutrients, antimicrobial metabolites, bacteriocins, suppression of toxin production, altogether sustaining the balance of composition of micro-flora). Still, a few is known about the stability of metabolism – exceptional in some diseases.
- *IMF and host-specific immune response* towards foreign antigens of microbial origin by enhancing the activity of macrophages, increasing the amount of natural killer and NKT -cells and the level of interferon-γ, stimulating the production of IgA, IgG and IgM antibodies.
- In host epithelial cells the induction of some *non-specific protective factors* as cytokines, mucus, short chain fatty acids (SCFA), epithelial tight junctions and cells protecting factors like zonulins and defensins against pathogens.

The normal state of host microbial ecology (relations between IMF and host characteristics) in different body locations is described as eubiosis. In eubiosis (good life) the particular MF of different body regions is expressing several well balanced functional characteristics that are controlling the morphology, several mucosal functions, the natural and adapted immune mechanisms, altogether resulting in general welfare of healthy persons. In opposite, the imbalance of microflora, resulting in impaired microbial ecology and disturbance or loss of general welfare, was in Europe called dysbiosis (formerly dysbacteriosis by Nissle in Germany). In Anglo-American literature the imbalance in the normal relationships between various groups of resident and transient microorganisms are characterized by clinical syndromes or disease entities (malnutrition, antibiotic associated diarrhea, inflammatory bowel disease etc.). The IMF associated infectious pathologies can be induced either by:

- multiplication of facultatively pathogenic host microbes or
- infections by enteric pathogens where host derived altered function of normal microflora or
- deprivation of some important components of normal microflora.

The deprivation of colonisation resistance (CR) by IMF is the real basis for increased susceptibility to different infectous agents, incl food-borne pathogens.

Metschnikov (1907) has proposed that re-establishing the disturbed composition or metabolism of micro-biota might prevent disease and restore health. A century later the concept of microbial interference therapy (MIT) has well survived: maintenance of health or restoration of diseased functions of host by introducing living microbes. During centuries people have consumed different fermented milkproducts. It is now known that several bioactive peptides against high blood pressure, blood coagulation, depression are available in fermented by lactobacilli milk. Probiotics are one category of functional food. Probiotics (meaning "for life") can be defined as live bacteria of the normal human microbiota that beneficially affect the host health by improving the microbial balance**.** The main aims are: a) to enrich the composition of human microbiota with non-harmful biologically active bacteria; b) maintain sound intestinal health by fighting against infectious microbes; c) restore the imbalance of microflora after infections and antibiotic therapy. Antagonistic activity of probiotic bacteria against intestinal pathogens helps to prevent intestinal infections. Correction of imbalance of microflora by probiotics reduces the number of infections with facultative pathogens accidentally colonising host after antibiotic therapy or due to viral infection. Probiotic bacteria are applied in food and pharmaceutical industry. In food industry they provide additional healthy value to different milk-based products. Pharmaceutical biotherapeutic preparations (food additives) of probiotic bacteria can be consumed alone or in combination with prebiotics (fructo-oligosaccharides). The latter non-absorbable carbohydrates reach the colon and provide a good substrate for probiotics. These combinations are called synbiotics. Probiotics can be used also with different medicines or other substances if they did not distrub their influence yet provide some additive value for usual treatment schemes. Probiotics as adjunct to antimicrobial therapy have been applied in many infectious diseases, including travellers´diarrhea, antibiotic-associated colitis, *Clostridium difficile* related infections, rotavirus enteritis, urinary tract infections, vaginosis and many other diseases of infectious origin.

In different clinical trials the efficacy of probiotic therapy varies largely. Reviewing 46 double blind, randomized, prospective clinical studies with probiotics the successful clinical outcome was found only in 56% of trials. In the

lecture the putative reasons of some failures are discussed which are seemingly mainly associated with different pathogenesis of enteric, incl. food-borne infections. Different enteric pathogens can cause gastroenteritis, diarrhea or intoxication either by adhering or secreting the enterotoxines and disturbing the absorptive function (viruses to intestinal villi of small intestine, ETEC, EPEC, Salmonella), or causing the denudation of mucosal villi by cytotoxins (EIEC, campylobacters, shigellas, salmonellas), intruding into cells, causing inflammation and generalisation of infection (yersinias, *Salmonella typhi*, EHEC) or causing haemolytic toxemia or neurological symptoms (*Shigella dysenteriae*, EHEC). Considering the variability of the main attack to host it is understandable that interference therapy by probiotics has its chance for effect only in some types of GI infections. Some possibilities for reduction of the intestinal infections by probiotic lactic acid bacteria are described. Problems to be solved include the selection of probiotics with specific antimicrobial properties, for particular target bacteria/infections and for action in defined ecological niches. This has to stimulate the investigators for more profound understanding of microbial ecology, pathogenesis of infections and immune answer.

The elaboration of the antimicrobial and antioxidative probiotic *Lactobacillus fermentum* ME-3 (DSM 14241) ME-3 has been performed under the regulations of WHO/FAO (2002), following the strain identification and molecular typing, assessment of the functional characteristics and safety by laboratory tests, experimental animals and volunteers, conducting several clinical trials in volunteers and diseased persons (urinary tract infection, allergy, brain stroke), development of the necessary technology for industrial incorporation into products and their control and at last, participating in market campaigns and surveillance of consumers interests. The health claims of ME-3 contain reduction of risk for salmonellosis, shigellosis and *E. coli* derived infections from one hand and the enhanced anti-oxidative function of intestinal mucosa and blood sera of host, comprising antiatherogenicity, on the other hand.

CLASSIFICATION AND DIFFERENTIATION OF *SALMONELLA* SP. USING DNA BASED TECHNIQUES

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An increased demand for differentiation of pathogenic microorganisms circulating in the environment naturally results into an increased demand on new typing methods. Although the traditional methods like biochemical characterisation, serotyping or phage typing still remain valuable tools in differentiation of bacterial pathogens, these are frequently supplemented by the methods based on the analysis of genetic material. This is true for *Salmonella* sp., as well as many other pathogenic bacteria. *Salmonella* can be subtyped either by isolation and characterisation of plasmid DNA or chromosomal DNA.

Plasmids of *Salmonella* vary in size from 2 to more than 200 kb. The best described group of plasmids are the virulence plasmids (50–100 kb in size) present in serovars Enteritidis, Typhimurium, Dublin, Cholerae-suis, Gallinarum, Pullorum and Abortus-ovis. They all encode *spvRABCD* genes involved in intra-macrophage survival of *Salmonella*. Another group of high molecular weight plasmids are plasmids responsible for antibiotic resistance. Since most of these plasmids are conjugative, besides storage of genetic information, they contribute to the spread of genes in bacterial populations. The low molecular weight plasmids are the last group of plasmids found in *Salmonella*. Some of them have been shown to increase resistance to phage infection due to the presence of restriction modification systems. Despite limited knowledge on their function, their presence or absence is frequently used for strain differentiation in epidemiological studies. Purified plasmids can be resolved directly by agarose gel electrophoresis. Alternatively, prior the electrophoresis, to increase the discriminatory power, plasmid DNA can be digested by restriction endonucleases. Limiting factor for plasmid profile analysis in *Salmonella* is the fact that the low molecular weight plasmids are found only in about 10% of *Salmonella* field strains. When chromosomal DNA is used as a target for bacterial strain differentiation, simple restriction

endonuclease digestion combined with conventional electrophoresis cannot be applied because of the size of chromosomal DNA. However there are several way how to overcome this complication.

The first possibility is to separate digested DNA by conventional electrophoresis, blot the DNA fragments onto a nylon membrane and hybridise the membrane with a suitable probe. To enable subsequent visualisation, the probe must be labeled in some way. Although currently the non radioactive ways of labeling are preferred, for explanation and understanding rather obsolete way of radioactive labeling is more appropriate. The probe must contain a sequence which appears in a reasonable number of copies in a bacterial chromosome. Suitable probe can be therefore represented by genes coding for rRNA which appears in 7 copies in *Salmonella* Typhimurium chromosome, or IS200 sequence. If the probe contains the rRNA sequence, the protocol is usually described as ribotyping.

Since the hybridisation is rather time consuming and laborious procedure, alternatives were sought. Current gold standard of DNA typing in *Salmonella* is the macrorestriction analysis. During this procedure, the chromosomal DNA is carefully purified from bacteria embedded in agarose plugs and digested by restriction endonucleases which cut the DNA at limited number of positions. This is usually achieved with restriction endonucleases recognizing 8 bp long sequence like NotI or SpeI. Sometimes even some 6 bp long sequences appear very rarely in bacterial genomes and restriction endonucleases recognizing such sequences can be used for macrorestriction analysis as well. Typical example for this is *Salmonella* chromosomal DNA and XbaI restriction endonuclease. Long DNA fragments generated by the "rare cutters" are then separated by pulsed field gel electrophoresis, which enables separation of DNA fragments hundreds of thousand bp in size.

Spectrum of DNA fragments obtained either by ribotyping or after the macrorestriction analysis is sometimes difficult to compare between different laboratories. This is why, despite a widespread and dominant use of these two protocols in bacterial DNA based typing, additional alternatives are being sought. Three alternatives are worth at least brief mentioning.

The first one is based on sequencing of multiple house keeping genes and comparison of obtained DNA sequences. Advantage of this approach is ultimate information which can be shared by any laboratory. This approach has been used for example for the description of recent evolution of *Salmonella* sp.

Another alternative is based on PCR detection of mobile DNA sequences. These can be different plasmids, insertion sequences or integrated prophages. Indeed, when we compared complete chromosomal sequences of 2 strains of *Salmonella* Typhimurium, we observed that with a single exception, the remaining differences observed by the macrorestriction analysis can be explained by the acquisition or loss of prophages. When we performed multiple PCRs specific for prophage sequences, we observed nearly the same discriminatory power as for the macrorestriction analysis while the results were expressed by a digital code easily comparable by any laboratory.

The last technique which can be used for strain characterization is the microarray genomotyping. Great advantage of this method is that the whole genome of a bacterial strain under investigation is analysed in one time. During this procedure, labeled DNA from a strain under investigation is let to hybridise with a DNA chip containing a whole genome of *Salmonella*. Disadvantage at the moment is a rather high cost of the analysis and also the fact, that only the genes present on the DNA chip but absent in the genome of investigated strain can be detected. If the strain under investigation posses additional genes which are not present on the chip, these will remain unrecognised.

In a summary, the most widespread method used in DNA typing of *Salmonella* as well as many other pathogenic bacteria is the macrorestriction analysis. Occasionally, plasmid profile analysis and/or ribotyping are being used in some laboratories. Besides these, several other protocols of interest are tested but have not reached that wide application so far.

ENTERIC PATHOGENS – PREVALENCE IN FOOD PRODUCTS AND MECHANISMS OF SUPPRESSION BY PROBIOTIC LACTIC ACID BACTERIA

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Foodborne disease, e.g. infection (FBD) is a major cause of illness and death worldwide. FBD is caused by pathogenic microorganisms if consuming contaminated food or drink. FBD outbreak means infection where two or more different persons get infected after consuming contaminated food. Outbreaks reported as food-borne, involve a single vehicle of infection and should be identified by the epidemiological or microbiologic investigations. However, most frequently they appear in form of sporadic cases that are not registered at all (under-diagnostics). The FBD can emerge as noninfectious food poisoning (hard metals), infections with live bacteria or intoxication with preformed toxins (botulotoxin) or some toxic metabolites (putrescine, tyramine) produced by contaminating bacteria.

Surveillance: The greatest challenge to protect population from food-borne infection is to spread information and knowledge about the sources and routes of transmission of pathogens into food products. The exclusion and control of these well-estimated risk factors helps to develop safe food all over the world and to reduce the socio-economic burden of diseases. WHO suggests applying the effective control of food-borne disease by "using the evaluated information about food-borne hazards and the incidence of food-borne disease". Really, there are available the estimates of the contribution of specific pathogens to the overall extent of food-borne infection also at the national level in Estonia, Sweden and Finland.

Socioeconomic impact: FBD figure mainly gastroenteritis, rarely food intoxication. The typical cost of gastroenteritis is measured by disability adjusted life years (DALY). In the Dutch population for instance the costs are 343 million EUR, 77 EUR per case in 1999. The recent developments are associated with

EU programs highlighting all stages of the food chain to assure food safety. To understand this relationship "from farm to fork" the epidemiological and clinical studies are welcome, taking into account the national differences.

Incidence of FBD: In European Northern countries and Baltic States the main food-borne pathogenic bacteria belong to the microbial family *Enterobacteriaceae*, including *Salmonella sp., Campylobacterium sp., Shigella* sp. and *Yersinia enterocolitica.* Altogether these bacteria are classified as gram-negative enteric bacteria. These bacteria are the emerging or remerging pathogens worldwide whose significance is on the rise. The application of molecular tools for their detection has received great attention. Of importance is also the life threatening Gram-positive food-borne pathogen of *Listeria sp.* However, the incidence of the listeria-derived infections is fortunately quite rare in Northern countries and Estonia.

The pathogenesis of food-borne infection starts from consuming contaminated food where the content of pathogenic bacteria is crucial for development of disease. The *Salmonella* sp. needs very high population level of bacteria in food (more than 10^5 microbial cells/ml) to achieve the proper infectious dose $>10^7$ bacteria by consumption of 100–200 ml of the food. In contrast, the infectious dose of *Shigella* species is quite low, figuring only 1000 microbial cells. The infectious dose of *Campylobacterium, Yersinia* and *Listeria* largely depend on the virulence factors of particular food contaminant pathogens and the food environment, also the contact time and conditions for germinating bacteria. In food industry the conditions are usually well described for safe food production.

Foods and risk of FBD: There are a few studies where the risk of different foodborne diseases from different kinds of foods is evaluated (Adak *et al.*, 2005). In England and Wales, at 1996–2000 the most frequent etiologic agents of FBD were campylobacteria, followed by twice lower incidence of *Clostridium perfringens* and *Yersinia* spp. infections, the *Salmonella* sp. together with different *E. coli* infections (ETEC, EPEC, EIEC, EagEC) figuring the 4th and 5th in the list. However, it should be mentioned that in nearly 50% of cases the infectious agent was left unknown though the microbiological diagnostics is very highly evaluated in UK. The similar data are provided also in textbooks. The *Listeria monocytogenes* driven infections were quite rare (221 cases), yet the mortality rate was 30%! In this paper the foods were classified into broad

food groups, such as poultry, and more specific food types, e.g. chicken, and the percentage of outbreaks due to each type of food for each pathogen was calculated. This offers a good overview about disease impact according to food: out of 1,724,315 studied cases for the 5y period there were only 4% of cases where the food handlers were the source of infections; in the cases left, most illness was attributed to eating poultry (30%), complex foods (27%) and red meat (17%). These studies show mainly the national/ethnic habits in food consumption. Therefore it is not astonishing that chicken consumption accounted for more disease, deaths and healthcare usage than any other food type in UK. Only 5% of patients were infected by eating plant-based foods e.g. vegetables, fruit and rice and it seems that the consumption of milk products is not very high as only 6% of FBD were associated with dairy products. In Estonia, quite specific food was incriminated to cause outbreaks: the combined food as puddings, creams, sweets, potato salads.

Reviewing the literature of the recent food outbreaks worldwide, there were found very similar data to the common textbooks, concerning the association of specific pathogens with particular food. *Campylobacter sp*. caused FBD is found after consuming poultry, in US annually >1 million people are infected, developing a long duration diarrhea; poultry has been the main source also in Denmark (2004); in Sweden the campylobacter gastroenteritis is clearly linked with water supply and livestock (2004). For *Salmonella enteritidis* – the main contaminated foods listed are hens eggs (Denmark, 2004), complex food, e.g. school-lunch dessert (Japan, 2001), eggs-salad (US, 2003) but also as a quite unusual foodstuff – the imported peanuts from Australia, causing the spread of FBD in Canada, England and Scotland (2001). The problem of antibiotic resistance of salmonellas and failure of treatment, development of carriage is the serious problem bound to FBD. Quite unexpectedly the FBD was caused by Halva from Sesame seeds produced in Turkey and afterwards the contaminated Halva food product had been imported to Germany, Sweden, Norway and Australia. Though *Listeria* sp. infections do emerge rarely but the threat for pregnant women, neonates and elderly persons with immunodeficiency can become a growing problem. In Japan, a nationwide survey of human *Listeria monocytogenes* infection revealed 83 cases of listeriosis per year with an incidence of 0.65 cases per million of the population (2002). *Shigella* sp. infections are usually associated with food handlers: in US it was described a multi-state outbreak in 2000 by commercially prepared food, a dip with cheese

which was hand-broken to be added to dip. The education of large populations could help to avoid FBD caused by handlers in manufactures with decreased hygiene standards.

The dangerous food-borne agent *E. coli* O157:H7 caused infections are not very frequent in Nordic and Baltic countries, showing only some single cases that are mainly associated with consumption of red meat and milk. *E. coli* O157:H7 (EHEC) has been linked to salami in Canada (1999), to milk in Denmark (2004), found among staff of a large hospital in Sweden (2004). Very similar results on water contamination by EHEC in farms wells were assessed both in US (2003) and Scotland (2006). The waterborne outbreaks clearly need more attention, as in local food production the water is very closely associated with food chain. In the sparse population of Northern Scotland, Sweden, Norway and Finland, the groundwater is used without disinfection and the bacteria could survive for long time. *Campylobacter jejuni* and *C. coli* caused three waterborne outbreaks in Finland (2003). The viruses (Norovirus, Norwalk-like virus) are causing massive water related outbreaks of gastroenteritis in different Nordic countries, the viruses can also be transmitted to foods.

<u>Improvement of food safety by LAB probiotics:</u> Probiotics are live bacteria of human origin having beneficial influence on host's health. Viable lactic acid bacteria (LAB) are largely used for designing functional food, e.g. probiotics. In community there is a growing need for functional food (FF) to get support against infections, atherosclerosis associated diseases like cardiovascular heart disease, brain stroke but also against metabolic syndrome, obesity and cancer (Ljungh and Wadström, 2006) Thus, consumers interest, granting the success in market, is a good starting point for development of probiotics. However, only some 10 to 15 y lasting evidence based research can result in internationally accepted product. Next, the innovative thinking of stakeholders and enterprises is of utmost importance to manage with financial pressure and nationwide strict health claim regulations. Recently the consensus for health claims on FF products was elaborated by FUFOSE and PASSCLAIM (DOI10.1007/s00394-005-1104-3) allowing the use of the health claims of either enhanced function or disease risk reduction.

The National culture collection of lactobacilli at Dept. Microbiology of University of Tartu provides excellent basis for search of LAB strains of human

origin with high antimicrobial activity. However, the development of a probiotic has to pass the long route from scientific ideas, nation-wide evidence based research, patenting process, transfer of technology to dairy and cheese enterprises, before to be delivered into the hands of consumer.

A meanwhile step in probiotic development affording safety of food products is described. The food product serving as carrier of probiotic strain (to be delivered to host organism) can get some additive value from the functional properties of the probiotic candidate strain, in order to strengthen the food safety.

Mechanisms of action of probiotics against pathogens in raw material and food products comprise specific *competitive exclusion* of infectious agents: a) competition for nutrients, b) production of antimicrobial metabolites, bacteriocins, c) suppression of toxin production by contaminating pathogens, altogether sustaining the balance of composition of specific micro-flora developed in food-product.

Together with Estonian Technology Agency we have elaborated several products which safety is granted due to the high antagonistic activity of incorporated LAB probiotic candidate strains. The metabolites, the organoleptic indices and laboratory, experimental animal studies for safety should be performed to get acceptance from local Veterinary and Food Board and Consumer protection Board.

Effect of Maintenance Routines in Food Processing on Production Hygiene

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Contamination of foods by food handlers has been identified as one of the most important cause of foodborne outbreaks. The regulation on food hygiene (EU) No 852/2004, Annex II states, that food-handlers must be supervised and instructed in food hygiene matters commensurate with their work activity. Contamination from personnel can be direct or indirect. Direct contamination can happen by contact between the body and the food product. In indirect contamination, people act as vectors and transfer contamination from one area or surface to another .Maintenance personnel do not actually touch the raw materials or food products, but they presumably touch a multitude of surfaces in contact with unpacked products. Maintenance personnel dismantle machinery for cleaning procedures and reassemble it after cleaning in addition to maintaining the operation of machinery during production.

L. monocytogenes is a pathogenic bacterium that survives in low temperatures and is problematic for food industry. It attaches to and grows on different surfaces even at low temperatures, tolerates anaerobic conditions and a wide pH range and may persist in food processing equipment. *L. monocytogenes* can be transferred from processing surfaces to foods. In the presentation, results from two projects “Hygienic equipment in food industry” and “Food industry, safety and hygiene management”, co-ordinated by VTT, are presented.

A study was made on the hygienic working practices of maintenance personnel in Finnish food industry. The aim of this work was to study the level of hygiene knowledge and the hygienic practices of maintenance personnel in the food industry in order to identify aspects that could have a negative effect on equipment hygiene and that should be improved. A mail survey was sent in spring 2002 to maintenance personnel, food-handlers, quality managers and cleaning personnel in 106 Finnish food companies. Answers were obtained from

23.6% of the plants. Most of the maintenance personnel (n=59) work in the production area continuously (61%) or at least 5 times (23.7%) during a work shift. Of the maintenance personnel, 42.4% reported having touched surfaces with contact in food often or always and while working in the production area 55.9% of them usually wore gloves. However, only 13.6% washed their hands after smoking and 23.7% before starting to work without gloves. 33.9% of maintenance personnel found they had not received enough information about hygiene commensurate with their work. Studies about food hygiene were not included in their basic education (89.9%). Most (63.6%, n=55) of the maintenance personnel knew where they could find the hygiene rules. One third (32.2%) of the respondents answered that they washed their tools once a day or always after work. The others answered that they washed their tools more seldom and 32.2% only once a year or never. Almost all (89.8%) of the maintenance personnel had personal tools which they themselves cleaned. For tools that are in common use, no persons were in charge of the cleaning in 71.9% of all the cases reported. The quality managers were asked if they had ever received consumer complaints, which could be linked directly to the work of maintenance personnel. Such complaints were rarely or seldom reported by 62.5% of them (n=16).

Based on the results, hygienic working practices of maintenance personnel should be improved and more hygiene education is needed. Clear hygiene rules targeted to maintenance personnel should be available for all maintenance personnel. Maintenance personnel are a potential source of contamination of food products due to the nature of their work. They move a lot between the production area and their workshop and they have to touch a lot production surfaces. The proper use of protective clothing, washing of hands and tools as well as avoiding foreign bodies left on the production lines should be targeted when the hygienic working practices are developed for maintenance personnel. In addition to the questionnaires, the working practices of maintenance personnel were studied at four food companies and microbial samples of the tools, protective clothing, hands and working environment were analysed for total aerobic bacteria, *Enterobacteriaceae* and *L. monocytogenes*. The amount of aerobic bacteria on maintenance personnel's clothes, work environment and tools, was, on average high (5–14 cfu/cm^2). *Enterobacteriaceae* were not found in samples except high numbers on one of the gloves (100 cfu/cm^2). *L. monocytogenes* was found from a tool, a screwdriver, which was in common

use by maintenance personnel which emphasizes need for regular cleaning and disinfecting of the tools. *Listeria* spp. was found from another tool and protective clothing of personnel as well indicting, that maintenance personnel have possibility to transfer *Listeria* in the food plant.

Another study was made on survival and growth of *L. monocytogenes* in lubricants used in the food industry. Lubricants in food-processing are needed in maintenance of the equipment to e.g. decrease friction and wear and to increase the efficiency of systems. The survival and growth of three *L. monocytogenes* strains in 10 lubricants (synthetic and mineral-oil based) used in the food industry, and rapeseed oil, was investigated at room temperature (20 °C) and refrigerated (5 °C). Additionally, the transfer of *L. monocytogenes* from lubricants to stainless steel surfaces and vice versa was investigated. Though the amount of *L. monocytogenes* in most lubricants, both pure and soiled, decreased significantly ($p < 0.05$) during the 14-d test period, lubricants may act as sources of contamination on the basis of the results obtained on the survival of *L. monocytogenes*. In general, temperature had significant effect ($p < 0.05$) on listericidal effect of lubricants contrary to soiling ($p > 0.05$), however the effect of both factors was dependent on lubricant ($p < 0.05$). The results clearly showed that *L. monocytogenes* survived in synthetic conveyer belt lubricant diluted in water. In addition, *L. monocytogenes* was transferred significantly ($p < 0.05$) from stainless steel surfaces into conveyer-belt lubricants and into mineral-oil based hydraulic oil and was transferred from lubricants to stainless steel surfaces as well. The results emphasize need for careful use of lubricants during maintenance work and need for regular change of lubricants and cleaning and disinfecting of surfaces between the change. Further as conclusion of the studies made, improvements in hygienic working practices of maintenance personnel are required with respect to minimizing the occurrence of *L. monocytogenes*, as well.

References to this extended abstract can be found in the articles: Aarnisalo, K., Tallavaara, K., Wirtanen, G., Maijala, R. & Raaska, L. 2006. The hygienic working practices of maintenance personnel and equipment hygiene in the Finnish food industry. Food Control, 17, 1001–1011 & Aarnisalo, K., Raaska, L. & Wirtanen, G. 2007. Survival and growth of Listeria monocytogenes *in lubricants used in the food industry. Food Control, 18, 1019–1025.*

HYGIENICALLY INTEGRATED SYSTEMS

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The demands that are placed on product and process within the food production industry are interconnected. Therefore, it is not appropriate to use a mainly sequential approach to designing a production facility, where you first address the primary function of the product, i.e. product quality, and then consider other issues like safety, hygienic design, cleaning, flexibility, and traceability. There is a need for integration of these issues, in order to make the best decisions and to balance the different needs.

Frequently during the sequence of designing, fabricating, installing, contracting, making design changes, or maintaining a production assembly, a line, or a facility, poor decisions are made because the sequential approach to problem-solving is used. In that case, we may unintentionally create hazards in the process line, like leaving a valve on a branch closed, thus creating a dead end, or just placing equipment incorrectly, making cleaning very difficult.

Another important issue in obtaining an optimally running line is to make sure that it is operated in a systematic way. One way to ensure high performance is to carry out implementation of HACCP and GMP, which deals primarily with hygiene, cleaning and critical control point monitoring. Furthermore, high performance is ensured by employing change management, by establishing and maintaining documentation with regard to installation, automation, operation, maintenance and cleaning and by proving the operation and performance of the equipment before routine use.

In order to deal with these issues a guideline was developed by a subgroup of the European Hygienic Engineering & Design Group (EHEDG). Part of the scope of the guideline is to a) describe integration of entities, up to and including the manufacture and supply of goods, in order to produce safe food or related products cost effectively; and b) describe integration topics that can affect

hygienic design, for example installation, operation, automation, cleaning and maintenance, especially those that are common or a frequent cause of failure.

The guideline has the task of linking and supporting currently available EHEDG guidelines, laws and standards (such as EN1672-2). Entitled "Integration of Hygienic and Aseptic Systems", the document is in review stage and will be published early this year.

The guideline established that the hygienic system being developed must conform to all specified requirements (the specified requirements may originate from, for example, legislation, users, product quality or safety). The integrated approach also includes determining specifications for issues such as product flow, control strategy, automation, maintenance, change management, and training of personnel. Furthermore, hygiene risk assessment is a necessity (for installed manufacturing systems, HACCP). A failure-mode-and-effect analysis (FMEA), which is a structured equipment-based safety tool based on risk assessment of the consequences of failure of any entities within a process, may also be carried out.

The integration process comprises of a set of actions that are done in order to successfully complete a hygienic integration of entities. These actions are for example to define stakeholder requirements, analyze and specify design, install entity, qualify installation, validate and qualify operation etc.

For each integration action entities must have at least a prospective validation identifying probable failure modes. Hygienic integration should be carried out on a modular basis, with entities that have already satisfied the hygiene requirement for integration. Instructions must cover: installation, operation, cleaning, sterilization (if applicable) and maintenance. Concurrency with design and validation activities other than those concerned with hygiene is a prerequisite. For an unassigned module or assembly, the provisionally intended process or processes and product(s) must be defined in a prospective list.

Going through for example the action to define stakeholder requirements, the list of requirements should produce a conceptual design of the entity or entities under examination. Every time such a step is completed, the flow diagram takes the user through a confirmation step, making sure there is compliance between

intake information and the outcome from this analysis. For example, if the user in the conceptual design forgot to take some legislation issues into consideration, the user should be able to notice this before going on to specify the design. The flow chart also prompts a record of data produced during the decision process and also a record of the decision itself.

When the verification of the hygienic performance is done (this is the last action point in the integration step), the entity has successfully been integrated. It can then be implemented in the specific process it was assigned for, or handed over to the user. If the entity has not been assigned to a particular product or process, then it can just be added to the library of hygienically verified entities.

The intention of the guideline recommendations is to avoid hazards that might otherwise be produced, which can be of microbiological, chemical or particulate nature in the consumable product. Hereby, the risks of recalls, lawsuits, and damage to reputation are also minimized. By supporting the integrated approach the guideline is also intended to reduce other non-safety problems, for example, environmental impact, costs, and excessive use of resources like water, chemicals or energy. The overall outcome of a successful implementation of this approach is optimal cost efficiency of both the construction phase and the finished design.

METHODS FOR EVALUATION OF PROCESS HYGIENE

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The control of quality is essential in order to provide wholesome and safe food products to consumers. The intention of food companies is to minimize the risk of epidemics and spoiled food stuffs caused by pathogens and other harmful microbes. The requirements for better hygiene has raised via development of food industry which includes prolonged self life, centred production and long transportation, automated cleaning systems, reduced cleaning time, demands for environment safe cleaning agents.

Hygiene can be defined with different aspects such as the amount of dirt, protein, polysaccharide and organic residuals, biofilm, dead and/or live microbes generally or specific pathogens and other harmful microbes. Also, hygiene can be detected from different sources; from final product, raw materials, process equipment, environment surfaces, air and personnel. This variation means that sample to be diagnosed can be in gas, liquid or solid form or the sample can be a surface of equipment. Sampling from surfaces is very challenging; either the measuring has to be done direct from surface quickly and without harming the surface or the dirt has to be detached from the surface as properly as possible without harming surface or microbes to be detected.

Hygiene detection of the food processing equipment and surfaces is one important part of the quality control. By sampling the surfaces it can be possible to prevent bad quality, it is a big help in tracing the contamination sources and in optimizing cleaning systems. For choosing a suitable method for detection it is important to know what kind of information is needed. Also it is important to figure out the wideness of sampling (the amount of samples and the frequency of sampling) when choosing the method. Quantification of micro-organisms from surfaces is difficult because of strong microbial adherence and because the cells grow in layers, forming biofilms. By collecting data it is possible to notice the changes in trendlines. Also the setting of limit values is depending on the sample

and the method used. The threshold of detection of adhering micro-organisms can vary according to the enumeration technique employed, and some techniques underestimate the number of micro-organisms on a surface.

The problems associated with the repeatability and reproducibility of studying biofilm microbes are well known. The methods currently most often used in surface hygiene control are the cultivation method based on detached samples (swab, sponge, biofilm reagents, ultrasonics) and contact plates or slides, the ATP bioluminescence method (measurements directly from the surface or from swabbed sample), protein residues, impedance based method and microscopying methods such as epifluorescence microscopying.

Various researches have been made to test and develop alternative methods for sampling from surfaces at VTT within several projects. Often used test matrix has been microbiological soil dried or grown onto surface of stainless steel plate. The reference method has been the conventional cultivation using swabbed sample.

SWABS AND SPONGES

The sample for hygiene controlling is generally detached from surface with sterile swab or sponge. In the swabbing method the process area of interest is swept with cotton, dacron, ryon or alginate tipped stick to collect possible micro-organisms. The collected micro-organisms are then released into an appropriate solution for subsequent cultivation. The advantage of the method is that it provides good access in confined process areas. The method is also easily adaptable to many detection techniques.

Comparison of three types of swabs was done by testing cotton-, dacron-, and alginate-tipped swabs. The results of cultivation and epifluorescence microscopying after swabbing showed that there were not big differences between different types of swabs. Thus microscopying shows that cotton tipped swab removes slightly more biofilm than the others. The special property of calcium alginate swab is that it is degrading easily. That is advantage when the cells have to be released into an appropriate solution for cultivation but a disadvantage during swabbing.

DETERGENT BASED SOLUTIONS FOR DETACHING BIOFILM

Various substances are known to improve the detachment of biofilms. The mild alcohol-, surfactant- and enzyme-based detergent blends has been tested and these detergent based solutions can be used to improve the detachment of biofilms either with swabs moistened in detergent solution or by spraying detergent solution onto sampling surface before swabbing with dry swab.

Contact plates can be used for flat surfaces; in this case the assumption is made that the micro-organisms stick to the agar surface and start to multiply. The method is easy to use and labour saving because it is not necessary to transfer the microbes from the swab to the cultivation medium. However, this method is also based on detachment of surface-bound micro-organisms, which is the limiting factor in the swab method.

Ultrasonication has proved to be an effective technique for the removal of biofilms from surfaces. In ultrasonication high-frequency vibration leads to strong formation of very small bubbles that hit the surface at high speed, which in turn causes detachment of surface-bound micro-organisms and biofilms. The technique has mainly been applied for detaching biofilm bacteria from test slides in bath sonicators. The method cannot be applied as such for routine control of process hygiene.

It has been reported that cells counted by direct microscopy consistently give results one log unit higher than the cultivation method. In our experiments epifluorescence microscopy clearly revealed that even vigorous swabbing only detached a small part of the actual biofilm and cells within it. This means that techniques based on swabbing only provide limited information about the true surface hygiene.

The advantage of measuring ATP directly from the surface is that the assessment can be undertaken while the micro-organisms remain surface-bound. The ATP method is especially suitable for hygiene monitoring, as it detects both living micro-organisms and other organic residues in the sample. However, a severe deficiency of the method is its rather high detection limit for bacteria that may be further increased in the case of cells stressed by cleaning or disinfection. Thus

the method is rather unreliable in situations in which even very low numbers of residual micro-organisms after cleaning are to be monitored.

An obstacle to the use of both the contact plate technique and ATP measurements directly from surfaces is that both methods require a flat, even surface of a certain dimension. Unfortunately, critical points in the hygienic aspect are often poorly accessible, typically located in confined spaces in the process.

MOLECULAR TECHNIQUES AND MICROSCOPY IN BACTERIAL DETECTION AND TYPING

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Microorganisms in the food industry are mostly harmless but some may be harmful to the process and/or safety of the product. Therefore, the control of harmful microorganisms is essential. Industrial processes that deal with any biological material provide nutrients and conditions for microorganisms to grow either as plankton cells in the circulating process waters or as sessile in the shelter of biofilm on surfaces. Due to the spatial and temporal heterogeneity of industrial samples and technical problems related to sampling in the industrial environment, obtaining a representative sample from certain foods and food-related industry is a demanding task. In addition, microbes are often tightly attached to the surfaces and the process equipment may contain parts that are difficult to access.

Fluorescence microscopy is widely used in microbial ecology studies. There are several advantages in the use of fluorescence microscopy. It is fast and rather easy to use, it allows the visualization of spatial distribution of cells in the sample, and with suitable combination of fluorescent stains, the differentiation between viable and dead cells is possible. However, direct identification of microbes is not possible with conventional fluorescent stains. Distinguishing cells on the basis of morphology is therefore important, because fluorochromes are not specific for bacterial species or genera. Numerous fluorescent stains are used for detection of both biofilm and plankton cell samples to study the viability and/or the total number of microorganisms. The most commonly used stains for the detection of total number of bacteria are acridine orange (AO) and 4',6-diamidino-2-phenylindole (DAPI). There are several stains that target either viable bacteria or non-viable bacteria. The most commonly used viability-staining system for industrial samples is LIVE/DEAD *Bac*Light Viability Kit (Molecular Probes, USA).

A molecular technique used for detection of pathogens must be capable of detecting low numbers of target bacteria in samples which may contain considerable background of interfering microorganisms and several matrix-derived compounds that may hamper the detection. In microbial community analysis the method should allow detection of different groups or species present in the ecosystem with similar efficacy to avoid biases in evaluation of species distribution and complexity of the microbiota. Biases may already be introduced by sample handling, as well as during the extraction of nucleic acids from microbes in the sample. Sensitivity of the molecular method can be improved by enrichment culture, but this also precludes attempts to quantify the number of target organisms in the sample. Molecular techniques can be utilized in the detection and identification of microbes in two ways: a) identification is performed directly from sample material, or b) identification is based on combined culture and molecular detection. A sample matrix studied plays an important role when the decision between the two choices is made. If the matrix is known to contain factors that can inhibit e.g. PCR reaction and which are difficult to remove, it is often best to use the combination of a culture technique and a suitable molecular technique. There are two major techniques applied in the molecular detection and identification of bacteria, PCR and hybridization. When molecular tools were first introduced for detection and identification of microbes, hybridization methods were widely applied. The rapid evolvement of PCR techniques has led to the present situation where hybridization is mainly used in combination with PCR. However, a technique called *in situ* hybridization, in which bacteria are detected in their natural microhabitat, has proved useful in applications where enumeration of the target organisms is warranted. Recent development of DNA microarrays allows, due to the automatisation of the procedure, simultaneous identification of huge number of specific sequences by hybridization.

Microbial ecology studies are often based on ribosomal RNA (rRNA) or rDNA sequences. 16S rDNA sequences can be used to infer phylogenetic relationships and to identify unknown microbes by database comparisons. It has also been proposed that rRNA content is appropriate for assessing changes in metabolically active bacterial populations, since rRNA content depends on bacterial activity. Besides rDNA gene, other target genes can be used for molecular detection of selected microbial groups/species from food and industrial samples. Genes associated with virulence factors, such as toxin producing

listeriolysin O (*hly*A) gene in *L. monocytogenes*, are commonly used for detection of food-borne pathogens. In addition, genes coding for physiological properties e.g. cold shock protein genes that are present in psychrotrophic *B. cereus* -group strains have been used as target molecules for detection.

Genetic fingerprinting techniques can be used to characterize bacterial communities or single bacterial isolates. Genetic fingerprinting techniques of microbial communities provide a pattern or a profile of the community diversity based upon the physical separation of unique nucleic acid sequences. Community analysis techniques are relatively easy and rapid to perform and they allow simultaneous analysis of multiple samples enabling the comparison of genetic diversities of microbial communities from different habitats, or studying the behaviour of individual communities over time. Community analysis can be performed with techniques such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), and single-stranded conformation polymorphism (SSCP). DGGE/TGGE analysis combines a direct visualization of bacterial diversity and the opportunity to subsequently identify community members by DNA fragment sequence analysis or hybridization with specific probes.

Fingerprinting of bacterial isolates can be performed by a variety of techniques including e.g. ribotyping, amplified ribosomal DNA restriction analysis (ARDRA), pulsed-field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD), repetitive element sequence-based-PCR (rep-PCR), and amplified fragment length polymorphism (AFLP). All these techniques aim at differentiating bacterial isolates at the subspecies level, preferably even at the strain-level. Prior to molecular techniques phenotypic methods such as biotyping and serotyping were used for the bacterial strain differentiation. These techniques are still used today, but with molecular techniques more reliable and often less laborious fingerprinting can be achieved. Regardless of whether phenotypic or genotypic techniques are applied, the fingerprinting is preceded by culture and single-colony subculture steps. Thus, even though PCR and hybridization can be used both in bacterial detection and fingerprinting, the techniques applied differ in a profound way: while detection methods are able to find the target organisms in a sample containing hundreds of other bacteria, fingerprinting methods are not genus or species specific and can therefore be applied to bacterial pure cultures only.

The emergence of new detection and real-time methods is linked to the need for a better assessment of the microbiological quality of products. This objective can be reached through an increase in detection specificity and a reduction in analysis time. In particular, the *in situ* techniques should enable progress in the understanding of the ecology of complex microbial communities in minimally disturbed samples. The most important weakness of culture-independent methods is that the taxonomic interpretation of data appears problematic. Although various new detection methods are applied to detect microorganisms from the industrial environment, the use of culture techniques will persist since the international standard methods for detection and enumeration of pathogens are based on cultivation. In addition, in many industrial quality control laboratories resources for the use of new molecular methods are inadequate.

In routine food control PCR-assays may shorten the time needed to identify e.g. *L. monocytogenes,* although enrichment may be necessary prior to the detection. By using virulence-associated genes as primers or probes, the presence of pathogenic species can be rapidly determined. However, dead bacterial cells may constitute a problem in basic-PCR-detection in hygiene control. For example, heat-treated samples may contain dead or damaged cells with no relevance to the product safety, however, the dead bacteria may still create positive signals due to the stability of the DNA molecules. In some circumstances (when the RNAse activity of the bacterial population is not destroyed in the sample prior to analysis) RT-PCR can be applicable in assessing the viable and active populations in samples. DNA-based detection methods, especially PCR, may gradually replace traditional methods for assaying microorganisms in food. When applicable (e.g. when no enrichment step is required) real-time PCR, which enables the quantification of target sequences, can prove highly useful for the rapid analysis of food pathogens. However, PCR-detection of pathogens, in food samples is still time-consuming, particularly in the case of large-scale testing. High throughput methods, such as dot blot hybridization using microarrays, have promising future potential for the routine diagnostic and quality control procedures in industrial settings.

One of the challenges for microbial ecology is to gain more information below the bacterial community, genera, and even species level. Subspecies level identification is especially important when a source of contamination is traced in an industrial environment. DNA fingerprinting techniques provide effective molecular tools to

identify and type microorganisms to subspecies level. While typing of the microbial isolates is performed, e.g. to trace a contamination source, the importance of including sufficient numbers of isolates from each sample site should be remembered. Once efficiently integrated, the typing techniques provide precise information on the heterogeneity of target bacterial population at a given time/space combination. However, fingerprinting methods are laborious and time consuming since isolation and cultivation of a large number of bacterial isolates cannot be avoided. Another limitation is that the unculturable strains present in natural ecosystems cannot be reached with typing methods.

In conclusion, bacterial detection, identification, and typing from industrial samples remains a laborious task, mainly due to the fact that frequently large numbers of samples need to be analyzed. Development of automated techniques that allow high throughput analysis of large numbers of samples will greatly facilitate the studies on industrial microbial ecology in the future.

References to the methods described in this abstract can be found in the review article of Maukonen, J., Mättö, J., Wirtanen, G., Raaska, L., Mattila-Sandholm, T. & Saarela, M. 2003. Methodologies for the characterization of microbes in industrial environments: a review. Journal of Industrial Microbiology and Biotechnology, 30, 327–356.

LEGAL ASPECTS OF FOOD SAFETY

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The aim of this paper is to share the Estonian experience in implementation and enforcement of the veterinary and food safety *acquis*. In addition, a broad overview about the EU food safety legislation is given. The paper consists of three parts. The first part describes the existing EU food safety legislation and food control system. The second part describes the creation of food safety legislation and supervision system in Estonia. The third part concentrates on analysing the level of compliance with the EU production and processing requirements in Estonian dairy and meat industry. The amount of investments needed to achieve the fulfilment of the requirements is also evaluated. In addition, the impact of food safety measures on trade is looked at.

In recent years food safety and food quality have been the issues increasingly dominating public and political debate. A major reason for this is the tremendous development that the entire food sector has gone through both in terms of technology and production. At the same time the debate has also questioned whether the consumers' demands and expectations are being met. Food safety and quality are of the greatest importance for the consumers and their sensitivity in that respect is very high. Food safety, nutrition and health considerations, consumer information and environmental policies lead to regular review and amendment of Community legislation with respect to food products, the related production methods and enforcement mechanisms.

The EU food law aims at ensuring a high level of protection of human life and health, taking into account the protection of animal health and welfare, plant health and the environment. This integrated "farm to fork" approach is now considered a general principle for EU food safety policy. Food law, both at national and EU level, establishes the rights of consumers to safe food and to accurate and honest information. The EU food law aims at harmonising existing national requirements in order to ensure the free movement of food and feed in

the EU. The food law recognises the EU's commitment to its international obligations and will be developed and adapted taking international standards into consideration, except where this might undermine the high level of consumer protection pursued by the EU.

There is no doubt that the safety of foodstuffs has the absolutely highest priority for every government but food safety issues are also crucial in trade. International trade in high-value food products has expanded enormously over the last decades, fueled by changing consumer tastes and advances in production, transport, and other supply-chain technologies. The cost of complying with food safety standards has been a major source of concern in the international development and among the countries in transition.

Achieving a standard of food safety is necessary for the development of internal and external markets. The respective Legal framework and appropriate supervising system must be created. Harmonisation of the *acquis communautaire* and particularly meeting the technical requirements demand substantial investments, but as a result the opening of EU internal market is possible. Many aspects of standards compliance do not require large investments or sophisticated technical or administrative capacities. The most significant challenge is often building broad awareness about the need for proper food safety measures and facilitating the broad adoption of good agricultural and manufacturing practices. A coherent regulatory framework and a system to assess compliance and conformity are also needed.

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The regulatory framework in which EU food industry businesses operate is a key determinant of their competitiveness, growth and employment performance. With regard to food trade, formally the development of trade measures has been towards adjustment and transparency, but in practise the agricultural trade has remained a subject to trade restrictions of different kind. Although the main purpose of practising non-tariff measures is the protection of humans, animals, plants and environment, these are often used to restrict trade. Non-tariff barriers are a result of differences in food legislation from one country to another. Examples include diverging labelling requirements, different residue limits or lacking recognition of test results.

The EU food safety policy includes the whole of the animal and human food chain. It provides extensive legislation and outlines the responsiblity of producers and suppliers in helping to ensure a safe quality of the food supply. The EU regulations are amongst the most stringent in the world.

In the scope of the association agreement with the European Union Estonia took an obligation to accept all policies and objectives of the EU without any reservations. That obligation had the main impact on the preparations of legislation and control systems. The accession procedure with the EU has highlighted the fulfilling of the technical requirements set for processing of agricultural products in Estonian food industry. A special feature of Estonia is that all processing facilities are EU fit without a transition period for adapting the EU food safety requirements. Only Cyprus is in the same position. Generally speaking Estonia fulfilled the main goal to join the EU without any transitional period.

Meeting the technical requirements demands substantial investments and as a result the opening of the EU internal market is possible. It is important to achieve optimal level of investments and it is not rational to invest in unutilized processing capacities. Estonia was unfortunately not able to reach this goal, as in some sectors investments were not made in time; in others over-investing

occurred. On one hand, food safety is the main priority of the government and of the food processing enterprises as well. All food operators have to take care of that and make necessary investments. On the other hand, the role of the government is to realize institutional reform and apply the legal framework in the field of food safety. Obligation of compliance with the food safety requirements directly influences the number of operating enterprises. As a matter of fact it is not easy to specify the appropriate period and measures for implementing the requirements in the transition economies.

Today our lifestyles are different from those of the past. There is an increasing number of single-person households, one-parent families and working women that have changed the food preparation and consumption habits. A positive outcome of this has been the development in food technology, processing and packaging techniques to help ensure the safety and wholesomeness of the food supply. The quality and safety of food depends on the efforts of everyone involved in the comlpex chain of agriculture production, processing, transport, food production and consumption. As the EU and World Health Organisation (WHO) formulated it – food safety is a shared responsibility from farm to fork.

Maintaining the quality and safety of food throughout the food chain requires both operating procedures to ensure the wholesomeness of food and monitoring procedures to ensure operations are carried out as intended.

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AIRBORNE BIOCONTAMINATION IN CLEANROOMS AND CONTROLLED ENVIRONMENTS

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In cleanrooms the main source of biocontamination is people. The concentration of airborne biocontamination depends upon the number of people present, their level of activity, and the clothing system used. In controlled areas the supply air may be of the same cleanliness as in the cleanroom but processes and people are not controlled in the same rigid way. Microbiological methods used for monitoring air in controlled areas e.g., during operational or dynamic state should be able to measure both higher and lower concentrations of airborne microorganisms. There are several methods of measuring the airborne contamination and many published reports show that the results – as a number of colony forming units per cubic meter (CFU/m^3) – depend upon the equipment used. The difference in results between microbiological air samplers often depend upon physical parameters of the samplers. These parameters, together with d_{50}-value (cutoff size) are discussed. The d_{50}-value is the aerodynamic particle diameter where 50% of the particles are collected in the sampler and 50% are not collected. In order to evaluate the collection efficiency of impaction samplers, a simplified mathematical model will be presented and examples given.

INTRODUCTION

In cleanrooms the main source of biocontamination is people. The concentration of airborne biocontamination depends upon the number of people present, their level of activity, and the clothing system used. Usually, the processes carried out in cleanrooms and clean zones are well controlled and do not contribute to the airborne biocontamination in the areas. The microbiological monitoring methods are challenged by the task to measure low concentrations of airborne biocontamination.

In controlled areas the supply air may be of the same cleanliness as in the critical areas but processes and people are not controlled in the same rigid way. Microbiological methods used for monitoring air in controlled areas e.g., during

operational or dynamic state should be able to measure both higher and lower concentrations of airborne biocontamination

Monitoring of airborne viable particles can be considered as a specific form of aerosol measurement. The term aerosol means an assembly of liquid or solid particles in a gaseous medium (e.g., air) stabile enough to enable observation and measurement. Generally, the size of aerosol particles is in the range 0.001–100 micron. The measuring devices collect particles from the air and give the collected viable microorganisms a possibility to multiply and be detected as Colony Forming Units (CFUs).

Particle size, shape and density determine the behavior of particles in air. A commonly used term in aerosol science and technology is the aerodynamic particle diameter, which is the diameter of a unit-density sphere (1 g/cm^3) having the same settling velocity as the irregularly shaped particle being studied. The particle diameter is in the literature also called equivalent diameter. Reference to the aerodynamic diameter is useful for describing settling and inertial behavior. Large particles e.g., skin flakes, might have an inertial behavior similar to that of a particle with smaller equivalent diameter. The motion of a particle is of concern for impaction devices (e.g., slit-to-agar samplers, sieve-samplers, centrifugal samplers) and for settling plates.

SAMPLING EFFICIENCY

PHYSICAL EFFICIENCY

The physical sampling efficiency of an aerosol sampler is influenced by inlet or extraction efficiency and by separation efficiency.

- Inlet or extraction efficiency is a function of the inlet design of the sampler and its ability to collect particles from the air in a representative way and transport the particles to the impaction nozzle or the filter.
- Separation efficiency is the ability of the sampling device to separate and collect particles of different sizes from the air stream by impaction onto the collection medium or into the filter medium.

The physical sampling efficiency is the same whether the particles consist of single microorganisms, carry microorganisms, or are nonviable (inanimate). The

physical sampling efficiency is based on physical characteristics of the sampling device such as airflow, orifice shape, and orifice size. The d_{50} (cutoff size) describes the aerodynamic equivalent particle diameter removed by 50% from the air stream and impacted. The d_{50} can be calculated as follows:

$$d_{50} = \sqrt{\frac{9\eta D_h Stk_{50}}{\rho U C}} \quad (1)$$

where η = Viscosity of air (Pa · s)
D_h = Hydralic diameter of the air inlet nozzle (m)
Stk_{50} = Stokes number that gives 50% collection efficiency (non-dimensional)
ρ = Particle density (kg/m^3)
U = Impact velocity (m/s)
C = Cunningham correction factor used for particles smaller than 1 µm (non-dimensional).

Impactor collection data is usually given in terms of an aerodynamic d_{50} (ρ = 1 g/cm^3 = 1000 kg/m^3) and the results of impactor measurements expressed in terms of aerodynamic diameter. The Cunningham correction factor could for particle sizes discussed here mostly is chosen to 1. For smaller particles need more accurate estimations.

It could be mentioned that such a correction for particles with diameters of 1 µm and 0.5 µm a reduction will occur with 8% and 14% respectively. The Stk_{50} number is often chosen to 0.24 to 0.25 for inlet nozzles. Most impaction sampling devices have sharp cutoff characteristics, meaning that almost all particles larger than that of d_{50} are collected. However, it is not yet common for manufacturers of microbiological samplers to present the d_{50}-value of their equipment. Equation (1) can be simplified by using constant factors for air viscosity, particle density, and the Cunningham correction factor. The expression for d_{50} expressed in µm will approximately become

$$d_{50} = \sqrt{\frac{40 \cdot D_h}{U}} \quad (2)$$

where D_h = Hydraulic diameter of the inlet nozzle (mm)
U = Impaction velocity (m/s).

For a round opening the hydraulic diameter D_h is the hole diameter. For a rectangular long slit (length much larger than the width) the hydraulic diameter will approximately be twice the slit width.

EXAMPLE 1

Calculate the d_{50}-value for an impaction sampler (Sieve Sampler) with a sampling air volume flow of 100 liters per minute and a lid with 200 holes of a diameter of 1 mm. The ratio between the airflow and the total area of the holes gives the impaction velocity to 10.6 m/s. With the aid of Equation (2) the d_{50}-value will be estimated to 1.94 µm.

$$d_{50} = \sqrt{\frac{40 \cdot 1}{10.6}} \approx 1.94$$

EXAMPLE 2

Calculate the d_{50}-value for an impaction sampler (Slit Sampler) with a sampling air volume flow of 50 liters per minute and a rectangular inlet slit 1 mm wide and 25 mm long. The ratio between the airflow and the total area of the slit gives the impaction velocity to 33.3 m/s. With the aid of Equation (2) the d_{50}-value will be estimated to 1.55 µm.

$$d_{50} = \sqrt{\frac{40 \cdot 2}{33.3}} \approx 1.55$$

EXAMPLE 3

Calculate the d_{50}-value for an impaction sampler (Sieve Sampler) with a sampling air volume flow of 100 liters per minute and a lid with 12 holes of a diameter of 10 mm. The ratio between the airflow and the total area of the holes gives the impaction velocity to 1.8 m/s. With the aid of Equation (2) the d_{50}-value will be estimated to 14.9 µm.

$$d_{50} = \sqrt{\frac{40 \cdot 10}{1.8}} \approx 14.9$$

Information of the d_{50}-value is an important factor when selecting the appropriate equipment for cleanrooms, clean zones and controlled environments. However, the user should also be aware that in a controlled environment with cleanroom-dressed operators as main contamination source, the aerodynamic equivalent particle size of viable particles usually is smaller than e.g., in operation rooms. A study by Ljungqvist and Reinmüller (Cleanroom Clothing Systems, People as a Contamination Source. Bethesda, MD, PDA. River Growth, IL, DHI Publishing LLC, 2004) of the generation of viable particles from cleanroom dressed operators reported the viable particle size distribution according to results the Andersen® 6-stage Sampler (cascade sampler). The results shown as percentage of airborne CFU separated by the Andersen® 6-stage Sampler are illustrated in Figure 1. The figure shows that approximately one third of the viable aerobic particles recovered by the Andersen® 6-stage Sampler have an aerodynamic diameter below 2.1 μm.

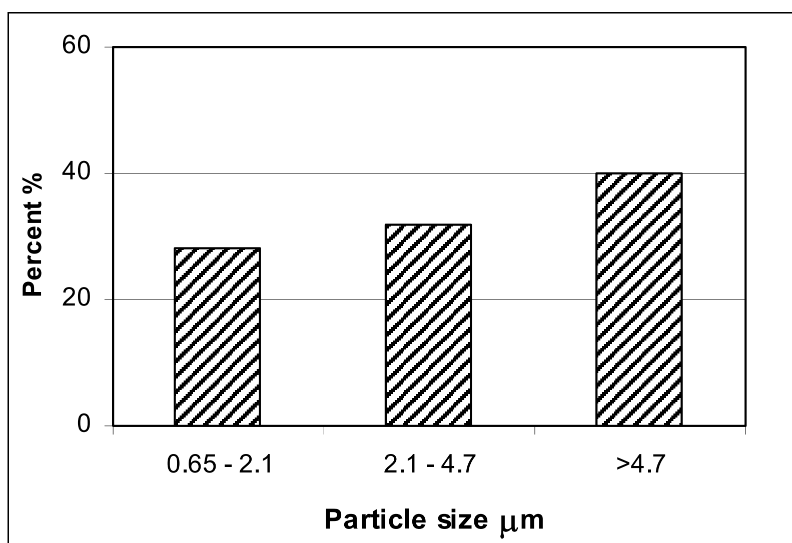

Figure 1. Aerodynamic particle size distribution in percent of airborne aerobic CFU, measured with an Andersen® 6-stage Sampler (cascade sampler), during evaluation studies of operators dressed in new modern cleanroom clothing systems (Ljungqvist and Reinmüller, 2004).

BIOLOGICAL EFFICIENCY

The biological sampling efficiency, mostly below the physical sampling efficiency, is the ability to maintain the viability of the microorganisms during separation and collection in combination with the ability of the collection medium to support growth.

Guidance on the evaluation of biological efficiency is presented in the ISO 14698-1 (ISO 14698-1. 2003. Cleanroom and associated controlled environments – Biocontamination control – Part 1: General Principles and methods. Geneva: International Organization for Standardization) in the informative annex B. The method described is based on a method by Clark, Lach and Lidwell (The performance of the Biotest RCS centrifugal sampler. J. Hosp. Infect., 1981, 2, 181–186) and can not be carried out in common or general microbiological laboratory. The test should preferably be performed in an independent test laboratory specialized for this kind of tests. The results of the tests are expected to be provided by the manufacturer of the air sampler.

The method makes use of airborne particles of different sizes containing spores of *Bacillus subtilis var. niger* NCTC 10073 which survives the sampling conditions. To obtain the concentration of spores in the test chamber a membrane filter method is used. The concentration obtained from the tested sampler is compared to the concentration from the membrane filter method at five particle sizes in the range between 0.8 and 15 µm. For each test at least 10 experiments should be carried out. The efficiency of the tested sampler is calculated using the following equation:

$$Efficiency\ of\ sampler(\%) = \frac{test\ sampler\ count}{total\ count\ (from\ membrane\ sampler)} \times 100 \qquad (3)$$

Measuring the biological efficiency with microorganisms typically found in the cleanroom is suggested as a better method by Whyte (Collection efficiency of microbial methods used to monitor cleanrooms. Eur. J. Parent. Pharmaceut. Sci., 2005, 10:2, 43–50). Whyte also points out the importance of testing the air sampler including the tube extensions if tube extinctions are used.

AIR SAMPLING

There are three main methods for collecting particles that are used for microbiological air monitoring: Impaction, filtration and sedimentation. Impaction and filtration are considered active sampling techniques and require collection of a known volume of air. Sedimentation is the passive collection of particles by "fall out" or settling into an open Petri dish with suitable medium.

The purpose of active air sampling procedure is to collect particles at a representative location, separate the particles from the sampled air, and trap the particles onto a suitable medium without affecting the viability of the microorganisms, and without altering the air flow pattern in the sampling region.

The selection of the most appropriate sampling device for a particular application depends upon the following factors:

- Physical characteristics of the sampling equipment
- The type of viable particle to be sampled (single spores or cells that are carried by non-viable particles)
- The equivalent size of particles to be collected
- The sensitivity of the viable particles to the sampling procedure
- The expected concentration of CFUs in the environments
- The ability to detect low concentrations of CFUs in a reliable way
- The time and duration of the sampling
- The sampling location.

ISO 14698-1 considers air samplers – that collect viable particles by direct impact of particles on nutrient media and filtration samplers that collect particles on special filters – suitable for active sampling in clean zones with low biocontamination.

The impaction velocity should be high enough to separate particles down to approximately 1 micron and low enough to avoid mechanical damage to the cells. For cleanroom applications, 1 cubic meter should be sampled in a reasonable time without drying the collection medium.

SUMMARY

To interpret the results from viable air sampling, the user should understand the dynamics of sampling and collection of particles on the collection medium. Results of 0 CFU per cubic meter in manned cleanrooms could indicate that the sampling process, sampling location or the collection media, incubation temperature and time have not been optimized.

It is important to be aware of the limitations of each sampling method. Results from one sampling method must not be compared with results obtained by another method without careful investigation. To improve the evaluation of controlled environments based on achieved results, the air sampler used has to be specified. An air sampler should be selected based upon a thorough evaluation of the characteristics of the sampler, the sampling conditions and sampling requirements.

Participant Abstracts

Antimicrobial Effects

EMERGENCE OF ANTIMICROBIAL RESISTANCE IN *CAMPYLOBACTER JEJUNI* ISOLATED FROM BROILER CHICKENS IN ESTONIA

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The development of antimicrobial resistance in *Campylobacter jejuni* and *C. coli* is a matter of increasing concern. Since campylobacteriosis is transmitted to humans particularly via food of animal origin, the presence of antimicrobial resistant *Campylobacter* isolates in broiler chickens has important public health impacts. The aim of this study was to determine resistance patterns of *Campylobacter* spp. isolated from Estonian broiler chickens. The strains were isolated from faecal samples collected at a large Estonian chicken slaughterhouse, and from meat samples collected at the retail level in Estonia. A total of 131 *C. jejuni* isolates were collected during 13 month period, and tested by Minimal Inhibitory Concentration (MIC) based broth microdilution method (VetMIC^{TM}). Resistance to one or more antimicrobials was detected in 104 isolates (79.4%). A high proportion of the isolates were found to be resistant to enrofloxacin (96/131, 73.3%) and nalidixic acid (99/131, 75.6%). Multidrug resistance (to three or more unrelated antimicrobials) was detected in 36 (27.5%) isolates and all of those were resistant to enrofloxacin. Our results showed that multidrug resistance was significantly associated with enrofloxacin resistance ($p < 0.01$) and the use of enrofloxacin may select multiresistant strains.

INHIBITION OF *PSEUDOMONAS FLUORESCENS* BY ALGINATE FILMS INCORPORATING LACTOPEROXIDASE SYSTEM

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Growth of microorganisms on food surfaces is a major cause of food spoilage. Especially, microbial contamination of ready-to-eat products is of serious concern to consumer health. Since microbial growth in foods occurs mainly at surface, use of antimicrobial sprays and dips could inhibit growth for a while. Edible films having antimicrobial substances could be a possibility to provide microbial stability for foods if antimicrobial substances in edible films are slowly released to food surface and remain at high concentrations at surface for longer time. Currently, chemicals such as organic acids, inorganic acids, or alcohols, are incorporated into edible films. Health concerns of consumers related to chemical agents caused an increasing demand to use of natural biopreservatives such as antimicrobial enzymes and bacteriocins in antimicrobial packaging. Lactoperoxidase system has been found to exhibit antimicrobial properties and its use has been suggested as a preservative in foods. Lactoperoxide system, with lactoperoxidase, thiocyanate and H_2O_2, is an inhibitory system naturally found in milk. This system inhibits many of the pathogenic and spoilage microorganisms including *Pseudomonas fluorescens*, *Listeria monocytogenes* and *Escherichia coli*. The objective of this research was to assess antimicrobial activity of alginate films incorporated with lactoperoxidase system against *P. fluorescens* at different concentrations of H_2O_2 and thiocyanate. Alginate films were prepared by using 2% (w/v) alginic acid solution and addition of 0.02–0.03 mg lactoperoxidase per g of alginic acid solution. Film solution was spread onto glass Petri dishes and dried at room temperature for 3 d. After drying, 0.3 M $CaCl_2$ was pipetted into plates to cross-link the dried films. Discs (1.3 cm), which were cut aseptically from dried films, were used in antimicrobial activity tests. Sterile nutrient broth (3 ml) was inoculated with *P. luorescens* culture (0.5 ml), grown overnight at 26 °C. Thiocyanate (1 ml, 4 mM) and H_2O_2 (0.1 ml, 0.2, 0.4, 0.8 mM) were also added

to the medium and the test tubes were incubated at 26 °C/24 h. To observe the effect of antimicrobial system at different conditions, varying thiocyanate (1–4 mM) or H_2O_2 (0.2–0.8 mM) concentrations were applied in different sets of experiments. *P. fluorescens* counts were monitored at 0, 6 and 24 h using Nutrient agar and plates were incubated at 26 °C/48 h. The results of the experiments showed that at constant H_2O_2 concentrations an increase in thiocyanate concentration did not correlated well with growth inhibitory effect. In contrast, at constant thiocyanate concentration, increase of H_2O_2 concentration increased the inhibitory effect on *P. fluorescens*. In the first 6 h of the incubation, at constant thiocyanate concentrations levels the *P. fluorescens* inhibition changed between 0.5 and 1.7 log reduction. After 24 h of incubation, counts were increased by 3.0–5.0 log compared to 0 h. At extended periods of incubation of reaction mixtures, inhibitory effect of lactoperoxidase was generally ended due to H_2O_2 and reactive thiocyanate products exhaustion. It is likely that at the studied concentrations, H_2O_2 component of lactoperoxidase system is a critical limiting factor for the obtained antimicrobial effect. These results revealed that lactoperoxidase system could have a potential to be incorporated into alginate films for several food applications. However, further studies are needed to increase antimicrobial activity for longer time.

ANTIMICROBIAL EFFECTS OF CHLORINATED AND OZONATED WATER FOR REDUCING MICROBIOLOGICAL CONTAMINATION DURING BROILER PROCESSING

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In this study, effectiveness of ozonated (1.5 mg/l) and chlorinated (30 mg/l) washing water as antimicrobial agent for killing some harmful microorganisms on broiler carcass, equipment surfaces and personnel hands at pre-determined control points were investigated. On broiler carcass the total aerobic mesophilic bacteria (TAB), *Escherichia coli*, *Staphylococcus* and *Micrococcus* spp. and *Salmonella* spp. were analyzed at fourteen different control points. On equipment surfaces the occurrence of the same bacteria except for *Salmonella* spp. were analysed at forty-two different control points. The hands of fifteen persons from three different production sections were also examined. Furthermore, TAB, *E. coli* and yeast/mould as air contamination source were enumerated in the rooms for hanging living broilers, for evisceration and for portioning and packaging. All microbiological analyses were performed using four replicates. In the broiler carcass treated with ozone and chlorine; the TAB counts were reduced by 81% and 61%, *E. coli* by 98% and 56% and *Staphylococcus* and *Micrococcus* spp. by 85% and 60%, respectively. No viable cells of *Salmonella* were recovered in the samples treated with ozone and chlorine after washing. The following microbial counts were determined on the equipment surfaces in evisceration, chilling and portioning/ packaging during processing: TAB 414–482 colony forming units (cfu)/cm^2, *Staphylococcus* and *Micrococcus* spp. 28–88 cfu/cm^2 and *E. coli* 5–134 cfu/cm^2. The microbial loads on personnel hands from evisceration, portioning and grading/packaging sections were determined 175-306 cfu/cm^2 for TAB, <10 cfu/cm^2 for *E. coli* and 15–80 cfu/cm^2 for *Staphylococcus* and *Micrococcus* spp. TAB, *E. coli* and yeast/mould as of air contamination indicator were determined 800, 588 and <10 cfu/plate on alive chicken hanged, while the same microorganisms were determined 223, 11 and 19 cfu/plate in the broiler eviscerated, respectively.

TAB, *E. coli* and yeast/mould in packaging and portioning were also determined as 30, <10 and <10 cfu/plate. As a conclusion the raw material was determined as the primary contamination source based on the results obtained from the control points examined and the hands of the personnel hands as well as the equipment surfaces were determined as secondary contamination sources. Our study demonstrated that ozonated water was very effective in reducing the populations of TAB, *E. coli*, *Staphylococcus* and *Micrococcus* spp. and *Salmonella* on chicken. It was also a good measure in preventing cross-contamination of process environments.

DETECTION OF *LISTERIA MONOCYTOGENES* IN GROUND TURKEY BY IMMUNOMAGNETIC SEPARATION AND DETERMINATION OF THE ANTIBIOTIC SUSCEPTIBILITY

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The objective of this study was to determine the incidence of *Listeria monocytogenes* in packaged ground turkey from different producers sold in Ankara and to detect the antibiotic susceptibility in isolates obtained. Immunomagnetic separation (IMS) was used for the isolation of *L. onocytogenes* from ground turkey samples and disc diffusion method was used to determine the antibiotic susceptibility of the isolates. In the present study, a total of 180 ground turkey samples were analysed during 1 year period (15 samples each month) and in 32 (17.7%) of the samples tested *L. monocytogenes* were detected. For the incidence of *L. monocytogenes* seasonal differences was not significant. Twenty seven (87.37%) of the 32 isolates were resistant to at least one antibiotic, 21 (65.62%) of them were resistant to more than one antibiotic. It was determined that 22 (68.75%) isolates were resistant to ampicillin and 26 (81.25%) to penicillin G. In addition to these, 13 isolates were intermediately resistant to erythromycin and 6 isolates to streptomycin. In conclusion, the incidence of *L. monocytogenes* in raw ground turkey was found to be high and most of the isolates were resistant to widely used antibiotics. Based on the results, the ground turkey should be produced under suitable hygienic and technological conditions for the prevention of public health. Also, the use of antimicrobials as prophylactic or growth promoter agent should be controlled systematically by the governmental authorities.

ANTAGONISTIC ACTIVITY OF *LACTOBACILLUS PLANTARUM* STRAINS AGAINST ENTEROPATHOGENS

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The foodborne illnesses are the most widespread health problems. The most common bacteria that cause foodborne infections are *Campylobacter, Salmonella* and *Listeria* spp. There are studies showing inhibition of these enteric pathogens by human origin indigenous lactobacilli and probiotic *Lactobacillus* strains. The aim was to investigate the antagonistic activity of eight *Lactobacillus* plantarum strains and one commercial probiotic strain against different enteropathogens. The antagonistic activity of 8 human *L. plantarum* strains and one commercial probiotic strain (*L. plantarum* 299v) was assessed against *Listeria monocytogenes* ATCC 13932, *L. monocytogenes* ATCC 51774, *Salmonella enterica* subsp. *enterica* ATCC 13076, and 3 reference strains of *Campylobacter jejuni*. The antagonistic activity of lactobacilli against selected bacteria was assessed using a streak line method. The media used were: modified MRS medium (MRS medium without triammonium-citrate and sodium-acetate; pH 7.2) for testing *Listeria* and *Salmonella*, Columbia Agar Base supplemented with 7% horse blood and 1% Vitox for C. jejuni. Lactobacilli were seeded on agar plates and incubated under microaerobic environment at 37 °C for 48 h; the growth was inactivated with chloroform gas for 2 h. The following target bacteria *L. monocytogenes* and *S. enterica* grown in peptone water for 18 h (turbidity 10^9 cfu/ml), C. jejuni suspension in Brucella broth adjusted to McFarland density of 3–4. The target bacteria were seeded in duplicate perpendicular to the streak line of lactobacilli on the respective media. The plates cultivated with *L. monocytogenes* and *S. enterica* were incubated in aerobic environment at 37 °C for 18 h. *C. jejuni* was grown in micro-aerobic environment at 37 °C for 48 h. The antagonistic activity of lactobacilli against pathogens was measured in millimetres – the width of the inhibition zone extending from the culture line of lactobacilli. *L. plantarum* 299v

and 4/8 putative probiotic *L. plantarum* strains expressed strong inhibition (mean 24.9 versus 19.2 mm; $p<0.001$) against *Listeria* and *Salmonella* strains. The antagonistic activity of all tested lactobacilli against *C. jejuni* was low (mean 3.5 to 14.0 mm). The inhibition activity of the four stronger putative probiotic *L. plantarum* stains was similar to the commercial *L. plantarum* 299v ones. The inhibition of lactobacilli is strain specific. Due to the antagonistic ability against enteric pathogens the four tested *L. plantarum* strains could be applied as promising probiotic candidates to avoid food-borne illnesses.

THE IMPROVEMENT OF CHEESE QUALITY WITH HUMAN *LACTOBACILLUS PLANTARUM* ADDITIVE

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Secondary flora putatively contaminating the cheese milk plays an important role in the cheese quality. However, contaminating bacteria, entered during manufacture or from milk can cause pasty texture and off-flavors in cheese and could be detrimental to human health. Aim of the present study was to find the best antagonistic adjunct starter from human *Lactobacillus plantarum* strains for the semi-hard Edam-type Estonian cheese in order to develop a cheese with evaluated bioquality. Two *L. plantarum* strains were selected due to their relatively good conjugated linoleic acid (CLA) production from linoleic acid in different media, strong antagonistic activity *in vitro* against various enteropathogens (*Listeria monocytogenes*, *Salmonella enteritidis, Shigella sonnei*) and cheese non-starter microflora. Three batches of Estonian cheese (1.2 tons each) were prepared: a regular cheese (control) and two experimental probiotic cheeses containing human *Lactobacillus sp* additive. The suspension of lactobacilli (inoculation rate 10^7 CFU/g of cheese) was added together with the cheese starter into the pasteurized cow milk before rennet coagulation. Cheese microflora was seeded in decimal dilutions on different culture media. The antimicrobial activity of prepared cheeses against various enteropathogens was measured. The CLA content of cheese was determined by the modified method of Ahotupa *et al.* (1998). The survival of both added *L. plantarum* strains in cheese was excellent (9×10^8 and 2×10^8 CFU/g cheese). Both prototype cheeses were able to suppress non-starter microflora other than lactobacilli. The antimicrobial activity of prepared prototype cheese with strain no 1 remained almost at the same level in comparison with the data of the pure culture *in vitro*. The inhibition of both *Yersinia enterocolitica* and *L. monocytogenes* remained high even at the storage temperature of the cheese at 4 °C. The experimental

cheese with strain no 2 revealed lower antimicrobial activity than the incorporated strain *in vitro*, though significantly higher than the control cheese without probiotic *L. plantarum* additive. The CLA content in experimental cheeses ranged from 1.5 to 3.0 mg per 1 g of cheese. The prototype cheese no 1 contained two times more CLA than the cheese with the strain no 2 and the control cheese. As a conclusion it can be stated that it is possible to control cheese secondary flora by selecting antagonistic *L. plantarum* strains of human origin. Additionally, it may support potential protection against enteric pathogens. Besides the increased the bioquality of cheese, the higher CLA content produced by the *L. plantarum* additive could also contribute to the antimicrobial potential of cheese.

Antibiotic Susceptibility Pattern of *Salmonella* spp., *Listeria monocytogenes* and *Clostridium perfringens* Isolates from Turkey Meat

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The objective of this study was to determine the antibiotic susceptibility profile of the *Salmonella* spp., *Listeria monocytogenes* and *Clostridium perfringens* isolated from brisket, leg and minced turkey meat samples obtained from 3 different producers marketed in Ankara, from June 2005 to January 2006. In this study 55 *Salmonella* spp., 24 *L. monocytogenes* and 78 *C. perfringens* isolates from 180 turkey meat samples were used for antibiotic susceptibility by disc diffusion technique (NCCLS-National Committee for Clinical Laboratory Standards). As a result *Salmonella* spp. isolates were most often found to be resistant to tetracycline (50.9%), nalidixic acid (41.8%), streptomycin and ampicillin (40.0%) and gentamicin (5.45%). Intermediate sensitivity was found in 48 isolates to kanamycin. Multiple antibiotic resistance were observed as followed: 5 isolates were resistant to 2 antibiotics, 17 isolates to 3 antibiotics and 6 isolates to 4 antibiotics. Twenty isolates of *L. monocytogenes* showed resistance against penicillin (83.3%) and 18 isolates to ampicillin (75%). Nine isolates were found intermediate sensitive to erythromycin (37.5%) and 8 isolates to streptomycin (33.3%). All of the *L. monocytogenes* isolates were found susceptible to chloramphenicol, gentamicin, tetracycline and vancomycin. In addition to that 17 isolates were multiple resistant to 2 antibiotics. *C. perfringens* isolates were most often found to be resistant to trimethoprim (100%) and gentamicin (85.8%). Relatively lesser resistance was observed against tetracycline (10.2%) and ciprofloxacin (6.4%). Three isolates were intermediate sensitive to gentamicin (3.8%) and 24 isolates to tetracycline (30.7%). Multiple antibiotic resistance were observed as followed: 58 isolates were resistant to 2 antibiotics and 11 isolates to 3 antibiotics. In conclusion, public health risk of antimicrobial resistance in bacteria as a result of the use of antibiotics in animals for prophylactic and growth promoter agent. Antibiotic resistance associated with

animal origin food has been a global concern. Therefore the Turkish regulation which is in compliance with the EU prohibition regulation for the use of antibiotics in animals for prophylactic and growth promoter agent should apply in national area.

CONJUGATIONAL TRANSFER OF ANTIBIOTIC RESISTANCES IN *SALMONELLA* TYPHIMURIUM

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Salmonella enterica serovar Typhimurium is an important pathogen with a numerous incidences of strains resistant to more than one antimicrobial agent. The genes responsible for multiresistance are mostly located on chromosome as a part of *Salmonella* Genomic Island 1 (SGI1) and code for typical pentadrug-resistant phenotype ACSSuT (ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline). Alternatively, antibiotic resistance genes can be located on high molecular weight plasmids, so-called R-plasmids. The aim of this study was to analyse mobility of antibiotic resistances in a collection of 24 multidrug-resistant *S.* Typhimurium strains with a special interest on resistance markers transferable by conjugation. Plasmid-free recipient *S.* Typhimurium F98 strain and resulting transconjugants were analyzed by pulsed-field gel electrophoresis (PFGE). R-plasmids that were responsible for the transfer of resistance phenotype were determined by comparing plasmid profiles of donor strains with the transconjugants. The conjugational transfer of antibiotic resistance markers was observed in 12 strains. Transfer of complete phenotype was predominant and transfer frequencies were around the value 10^{-3}. In 4 strains (115/0, B71, F8025 and F8475) a transfer of class 1 integrons was recorded. By DNA sequencing, the same type of integron was identified in 2 strains (115/0 and B71). This integron contained *aadA1* and *sul1* gene cassettes and was localized on a plasmid of 190 kb in size. The antibiotic resistance in F8025 strain was encoded by *dfrA1*, *aadA1* and *sul1* gene cassettes that were localized in integron on a large plasmid of about 230 kb in size. In the last strain F8475, a plasmid of about 210 kb was able to conjugate and carried the integron with *dfrA12*, orfF and *aadA2* gene cassettes. In 2 strains (9046 and 9134) an incomplete transfer of antibiotic resistant phenotype was recorded. This happened in approx. 10% of transconjugants and *tetA* and *bla*TEM genes coding for tetracycline and ampicillin resistance were the least stable during the conjugational transfer. By PFGE, we found that the loss of tetracycline resistance and both tetracycline and ampicillin resistance correlated with the loss approx. 10 kb and 25 kb of plasmid DNA, respectively.

PARTICIPANT ABSTRACTS

HYGIENE & QUALITY

DIFFERENTIATION OF *STAPHYLOCOCCUS EPIDERMIDIS* ISOLATES FROM RAW MATERIAL AND FOODSTUFFS AND FOOD CONTACT SURFACES BASED ON THE ABILITY TO FORM BIOFILM

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The aim of this study was to differentiate isolates of *Staphylococcus epidermidis* from raw material and foodstuffs and food contact surfaces by using phenotypic and genotypic methods detecting the ability of the strains to form biofilm. From 2000 to 2006, 197 *S. epidermidis* isolates were obtained from swabs of contact surfaces on the equipment of food processing plants, raw materials and foodstuffs produced from meat and milk. Capability of this microorganism to form a biofilm was investigated by phenotype and multiplex PCR method. Resistance of selected isolates to antimicrobial agents was investigated using the micro dilution method. *Staphylococcus epidermidis* isolates were incubated in brain heart infusion at 35 °C for 72 h in glass tubes. Then the tubes were rinsed with peptone water and suspect biofilm was dried turning the tubes upside down and coloured by 0.1% safranin solution. The intensity of red colour biofilm structure was rated visually. Total genomic DNA was obtained from a bacterial culture, in a brain heart infusion for 24 h. The bacterial suspension was centrifuged and the pellet washed in 500 μl of sterile distilled water. The suspension was then incubated at 80 °C / 20 min and finally centrifuged at 1400 rpm for 1 min. The primers for the detection of *ica* genes (amplicon 546 bp), primers UNB applied as internal control (370 bp) and primers SE705 specific for *S. epidermidis* (124 bp) were used for multiplex PCR method. The amplification mode was 96 °C for 2 min, (96 °C for 10 s, 55 °C for 10 s, 72 °C / 40 s) for 45 cycles with an extension of 72 °C for 2 min. Amplification products were separated by electrophoresis in 2% ethidium bromide-stained agarose gel. A total of 197 *S. epidermidis* isolates were obtained from raw food materials, milk and meat products and from swabs of equipment used in meat and milk processing plants. The production of biofilm was detected in 12 isolates (6%). Most of them originated from raw milk (4) and milk products (4). The *ica*

operon was detected in 9 isolates and the phenotypical ability to produce biofilm was determined in 10 isolates. Different results between the phenotypical and genotypical method were detected in 5 isolates). Resistance to antimicrobial agents was observed in 41 isolates of *S. epidermidis.* The most common resistance was to penicillin. Multiresistant isolates (resistance to 3 and more agents) were detected in 5 cases; one of them was resistant to 8 antimicrobial agents (penicillin, oxacillin, ampicillin-sulbactam, tetracycline, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, and gentamicin). We found virulent strains of *S. epidermidis* with the ability to form biofilm in many food processing environments. It is possible for the microorganisms released from biofilm to carry resistance genes and could represent potential source of food contamination. This may lead to food spoilage or to the transmission of diseases.

MICROBIOLOGICAL QUALITY OF TURKISH CHEESES MANUFACTURED FROM PASTEURIZED MILK PRODUCED IN COMPANIES HAVING THE HACCP SYSTEM

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In Turkey approximately 4–5 million tons (40%) of raw milk are used for cheese production. The production rate of white cheese is 13%, kasar cheese is 3%, and other cheese types are 3% of milk production. The classical white cheese and kaşkaval cheese are the first groups among the other cheese types on export. Milk used in traditional cheese production is not pasteurised in Turkey; however, pasteurized milk is used for industrial scale production. On that point, starter culture is not used in white and kaşkaval cheese producing generally. There are problems in dairy sector with regard to processing and marketing. Producing plants having different capacities of production from the small family plants to modern plants which has European standards and HACCP system, are showing activity together. Most of cheese production is realised in small scale dairies and approximately 10% of cheese is manufactured in modern plants. This research was undertaken to carry out microbiological and chemical analyses in the 316 white and kaşkaval cheese samples produced by 16 different large scale companies having HACCP system in Marmara Region. All cheese samples are produced with pasteurised milk. In these samples, the microbial counts were found as follows: coliform bacteria, 4.3×10^2 cfu/g; yeast, 8.2×10^4 cfu/g; E. coli 3.9×10^2 cfu/g. *Salmonella* and mould were not detected in any of the samples. Mean moisture, salt content, acidity and pH of white cheese samples were 58.7, 7.4, 1.37 and 4.63; and of kaşkaval cheese samples 43.5, 4.12, 3.05, 5.30. When the results were analysed according to the Turkish Food Codex Alimentarius limits for coliform bacteria, *E. coli* and yeasts, 46%, 58% and 22% of samples of the 166 white cheese samples and 4%, 9%, 45% of the 150 kaşkaval cheese samples examined were out of standards, respectively.

INVESTIGATION OF MOULDS IN SALTED YOGHURTS (CONCENTRATED YOGHURT)

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Salted yoghurt is a traditional milk product produced in Hatay region in Turkey. The salted yoghurt has unique properties e.g. saltiness and sour aroma. It can be stored for a year without spoilage and consumed in almost every meal among other yoghurts. Besides this it contains high dry matter, fat, protein and minerals, because it is a concentrated product. Furthermore, it is a highly nutritious product. But, salted yoghurt provides appropriate conditions, e.g. water activity and acidity, for the moulds to grow. Therefore contamination with mould plays an important role in spoilage of the salted yoghurt. In this study, the aim was to determine the type and amount of mould contaminating the production of salted yoghurt produced of cow's and goat's milk. We also looked at the effects of covering it with and without butter and at storage in different temperatures on the type and amount of mould in the salted yoghurt. Firstly the milk was heated (100 °C, 1 min), cooled, inoculated with yoghurt culture and incubated. After fermentation, yoghurt was divided into two batches. The first batch was strained, the other batch was unstrained. The unstrained and strained yoghurts were transferred into boiling pans and cooked for 110 and 50 min, respectively by stirring. The salt was added to both batches 10 min before the end of cooking. The salted yogurt was let to cool down at room temperature and was placed into plastic (rigid–polycitrine) packages. One part of each batch was covered with and without butter and stored at different temperatures (room and refrigerated temperature). After that, the type and amount of mould in the samples were counted according to Onions *et al.* (1981) and Samson and Reenen-Hoekstra (1988). The initial amount of mould in the samples ranged between 1.44 ± 0.16 log cfu/g and 0.46 ± 0.10 log cfu/g. Based on the analysis in the 6th month the amount of moulds in samples covered with butter and stored in refrigerator temperature ranged between 2.60 ± 0.12 cfu/g and 4.46 ± 0.11 log cfu/g and in

samples covered without butter varied from 1.82 ± 0.05 log cfu/g to 2.74 ± 0.49 log cfu/g. The amount of moulds in to samples covered with butter and stored in room temperature ranged, when analysed at the 6th month, between 3.26 ± 0.24 cfu/g and 4.32 ± 0.24 log cfu/g and in samples covered without butter in the 8th week varied from 4.88 ± 0.66 log cfu/g to 5.70 ± 0.20 log cfu/g. The statistical analysis indicates that straining and type of milk did not statistically affect the results of mould contamination in the salted yoghurt stored in refrigerator and room temperature. In addition the amount of mould in the samples stored in refrigerator temperature on the 6th month were not affected significantly if covered with or without butter, but there was statistical difference in the mould amount between samples covered with butter and without butter. Besides that the storage temperature did not make any statistical difference on the amount of mould in the salted yoghurt covered with butter, but according to samples stored at different temperatures there was a statistical difference on the amount of moulds in samples covered without butter. In this study, *Aspergillus* sp. and *Penicillum* sp., were commonly identified. This study showed that if salted yoghurt is stored in the refrigerated temperature it need not be covered with butter. But, if the salted yoghurt is stored in the room temperature, it must be covered with butter, because otherwise, moulds in the salted yoghurt will cause losses.

Effect of Plum Sauce, Pomegranate Sauce and Lemon Juice on Microbiological Quality Parameters of Traditional Raw Meat-ball (Çiğ Köfte)

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Raw meat-ball (çiğ köfte) is consumed in a wide region of Turkey, largely in eastern and south-eastern Anatolia. There are no official regulations on raw meat-ball production and therefore the ingredients, quality and quantity of ingredients differ depending on the consumers' preferences. It is traditionally produced with a mixture of lean veal and "bulgur" (wheat) in a ratio of 2:1 or 1:1, dried red pepper (mostly hot pepper), salt, onion, black pepper, parsley, and tomato and/or red pepper sauce. It is prepared as raw with no cooking process at all. Lately, according to EU regulations, some food products in Turkey are under scrutiny due to the high microbiological risk they carry. Hence, the traditional raw meat-ball is one of these food products to be investigated closely. Firstly in this research, the antibacterial effects of home-made dried plum sauce, pomegranate sauce and lemon juice on *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus* spp. were determined *in vitro* conditions by agar-diffusion method. We found that plum sauce, pomegranate sauce and lemon juice showed high antibacterial activity against the tested bacteria. But, a significant reduction in their antibacterial activity was found when they are used in diluted forms. The antibacterial activity depends on the concentration of antibacterial agent and bacterial species; amongst all the bacteria *E. coli* happens to be the most resistant against all antibacterial agents tested in this research. In the second phase of our research, we looked at the effect of plum sauce, pomegranate sauce and lemon juice on some microbiological quality parameters of raw meat-balls. *Salmonella* spp. and coagulase (+) *S. aureus* were not found in any of raw meat-ball samples. Furthermore, there was no significant reduction in the population of coliforms and *Escherichia coli*. The number of total aerobic mesophilic bacteria in raw meat-ball samples prepared with plum sauce and lemon juice decreased

approximately 1 log cycle when compared to the control sample. It was also found that pomegranate sauce had little antibacterial effect in raw meat-ball.

PARTICIPANT ABSTRACTS

MOLECULAR BIOLOGICAL METHODS

PFGE GENOTYPING AND ANTIMICROBIAL SUSCEPTIBILITY OF *CAMPYLOBACTER* IN RETAIL POULTRY MEAT IN ESTONIA

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In the study, the *Campylobacter* isolates from retail poultry meat in Estonia were sero- and genotyped, and the antimicrobial susceptibility was determined. *Campylobacter* were detected using the NCFA method. The DNA was digested with *Sma*I and *Kpn*I (New England Biolabs, Beverly, Mass.) (20 U per sample), and the restriction fragments were separated with ramped pulses of 1 to 30 s and 1 to 25 s for 19 h, respectively. All *Campylobacter* isolates were tested by disc diffusion method against ampicillin (25 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), nalidixic acid (30 µg), and tetracycline (10 µg) (Oxoid), and by the E-test (AB Biodisk, Solna, Sweden) against ampicillin, ciprofloxacin, erythromycin, and tetracycline. A total of 54 *C. jejuni* isolates were serotyped using commercial *Campylobacter* antisera according to the manufacturer instructions (Denka Seiken, Tokyo, Japan). Before the serotyping test the isolates were cultured on *Brucella* blood agar (Oxoid) plates at 37 ºC for 48 h in microaerobic conditions. We studied 48 chicken (36 Estonian, 12 imported) and 22 turkey (imported) *Campylobacter* isolates from 580 raw broiler chicken (396 Estonian, 184 imported) and 30 turkey (imported) meat samples. Of the isolates, 64 were *C. jejuni*, 4 *C. coli*, and 2 *Campylobacter* spp. Penner serotyping of 54 *C. jejuni* isolates revealed 11 different serotypes, and 22% of the isolates were nontypeable by the commercial antisera. The most common serotypes O:1,44; O:21, and O:55 accounted for 28%, 13%, and 13% of the isolates, respectively. Differences in serotype distribution were seen for chicken and turkey isolates. Genotypic characterization of all *Campylobacter* isolates (n = 70) was performed by pulsed-field gel electrophoresis (PFGE). *Sma*I and *Kpn*I yielded 29 and 34 PFGE types, respectively, revealing high diversity among isolates. The serotype distribution did not show an association with the origin of the sample, but the majority of the isolates sharing a similar

PFGE genotype originated from one country. High level of resistance to ciprofloxacin (66%), nalidixic acid (66%), tetracycline (44%), ampicillin (34%), and erythromycin (14%) were detected among the 70 *Campylobacter* isolates. The simultaneous resistance to two or three antimicrobial agents occurred in 60% of the isolates. The *Campylobacter* isolates from turkey meat had higher resistance to ampicillin, ciprofloxacin, nalidixic acid, and tetracycline than those from chicken meat. None of the chicken isolates were resistant to gentamicin, and no turkey isolates to erythromycin or gentamicin.

IDENTIFICATION OF *ENTEROCOCCUS* SPP. BACTERIA ISOLATED FROM FOOD AND OTHER ENVIRONMENTAL SAMPLES BY PCR METHOD

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The aim of this study, which was supported by the project of Ministry of Education, Youth and Sports of the Czech Republic No. MSM6215712402 Veterinary aspects of food safety and quality and project NAZV 1B53018, was the application of the species-specific PCR method based on the detection of *sodA* genes encoding enzyme mangan-dependent superoxiddismutas for the rapid identification of bacteria *Enterococcus faecalis* and *E. faecium*. It was studied 599 samples isolated from cattle farms in south and north Moravia, human, milk foods as milk, butter and yoghurt. It was identified 247 samples of *Enterococcus* spp. by the help of phenotypic characteristics as production of pyrolidonyl arylamidase (PYRAtest, Pliva–Lachema, a.s., Brno, CZ) and growth and appearance of colonies on blood agar (Blood Agar Base No.2, HiMedia; and sheep blood, Bioveta, a.s., Ivanovice na Hané, CZ), S-B agar and Bile Aesculin agar (Oxoid, Basingstoke, Hampshire, England). To isolate bacterial DNA a 24-h culture from the blood agar incubated aerobically at 37 °C was used. Bacterial DNA was isolated by 20% Chelex 100 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The supernatant was used as template DNA. The species identification of bacteria *E. faecalis* and *E. faecium* was also performed as multiplex-PCR when apart from the species-specific *sodA* genes detection, the internal control (16S rRNA segment) was also included. A product was detected by the gel electrophoresis in 2% agarose (Serva Electrophoresis GmbH, Heidelberg, Germany) with the following visualisation at UV on transilluminator after the staining by ethidium bromide. Among 247 isolated strains the presence of the species-specific *sodA* genes was detected 102 strains *E. faecalis,* 22 *E. faecium*, 51 strains were identified as other kinds *Enterococcus* spp. and 72

strains were no specified as *Enterococcus* spp. The identification of *Enterococccus* spp. using conventional methods and biochemical tests based on their phenotypic characteristics is complicated and time-consuming therefore was designed as multiplex-PCR for the detection of genus-specific *sodA* genes and internal control (16S rRNA). Present of internal control standardizes results of method and makes it possible to reduce number mistaken reading. Simple and rapid isolation bacterial DNA by 20% Chelex 100 is adequate for finish reaction. The above stated PCR method based on detection *sodA* genes can be acceptable for the rapid species identification of bacteria *E. faecalis* and *E. faecium* isolated from samples of environmental, cattle, human and milk foods.

RESISTOPATHOCHIP – TOOL FOR RAPID MOLECULAR CHARACTERIZATION OF *SALMONELLA* STRAINS

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The goal of our study is the development of DNA microarray-based tool for rapid molecular characterization and typing of multidrug resistant and virulent *Salmonella* isolates. DNA microarray contains short oligonucleotide probes (33–35 mers) designed from conservative domains according to thorough sequence alignments. The ResistoPathoChip for Salmonella spp. includes probes for detection of pathogenicity markers localized on the chromosomal (including *Salmonella* Pathogenicity Islands – PAIs) and plasmid DNA, phage-associated genes, fimbriae, integrons and various antimicrobial resistance genetic markers. The chip also contains *Salmonella* species- and serotype-specific probes and a range of external controls for chip analysis. The HPLC-purified oligonucleotide probes were spotted on the surface of Superepoxy slides. The preparation of target DNA, adjustment of suitable hybridization and post-hybridization conditions were properly optimized. The ResistoPathoChip was tested on the 24 samples of multidrug resistant clinical *Salmonella* isolates covering the most prevalent serotypes in Slovakia (Typhimurium, Enteritidis and Kentucky). The results of DNA microarray approach were validated only for antimicrobial resistance markers by PCR as development of multidrug resistant bacteria especially via horizontal gene transfer (HGT) causes some infections untreatable now and antimicrobial resistance represents a major health concern. The majority of PCR results were consistent with the microarray data and also phenotypic susceptibility testing. Moreover, we made a substantial effort in localization of monitored genetic markers either they are fixed on chromosome or localized on transferable elements such as plasmids or integrons. Purified plasmids without any traces of contaminating chromosomal DNA were fluorescently labeled and hybridized to the oligochip. We found that genetic determinant of resistance against cefotaxime (CTX) and ceftriaxone (CRO) as

extended-spectrum beta-lactams *blaSHV* is localized on conjugative plasmid, which was transfered into *E. coli* K-12 3110 recipient strain. Microarray analysis of selected transcojugant reveals transfer of another two resistance genes against aminoglycosides (*aph*) and amphenicols (*catA*) along with *blaSHV*. The ResistoPathoChip described here provides comprehensive information about *Salmonella* genotype, especially focused on pathogenic and antimicrobial resistance potential. Such data are necessary in epidemiological studies as well as for food safety assessment studies with respect to dissemination of antimicrobial resistance and prevalence of multidrug resistant pathogens.

DETECTION OF BACTERIAL TOXINS IN FOODSTUFF

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A wide spectrum of foods is contaminated by low numbers of *Staphylococcus aureus*. In case of incorrect storage of foods these bacteria can multiply and produce enterotoxins. Staphylococcal enterotoxins are heat stable proteins, resistant to proteolytic enzymes and may cause alimentary intoxications. Nowadays 18 types of staphylococcal enterotoxins have been described but only five classical toxins A, B, C, D and E can be detected routinely. Methods using for the detection of enterotoxins in food are reverse passive latex agglutination (RPLA) and ELISA. The sensitivity of these methods is 1 ng/g of food without the use of complicated extraction procedures. ELFA using the automated detection system VIDAS can detect less than 1 ng/g of food. In this study 321 ready-to-eat foods (meat and milk products, delicatessen and confectionary products) from retail market were examined for the presence of *S. aureus* using the EN ISO 6888-1 (1999) guideline. 72 samples (22%) were contaminated by *S. aureus*; the contamination level did not exceed 10^2 colony forming units (CFU)/g or CFU/ml. All the *S. aureus* isolates in our study were screened for the ability of toxin A–E production (using the RPLA test and VIDAS SET 2) and also for the specific genes *sea–sej* encoding the production of enterotoxins (using multiplex PCR method). Out of 72 isolates 42 (58%) harboured one of these specific genes. The most frequently found genes were *seg* and *sei* in 37 (88%), *sec* in 11 (26%), *seh* in 5 (12%), *sea* in 4 (10%), *seb* in 3 (7%) and *sed* in 2 (5%) isolates. Genes *see* and *sej* were not detected. Full agreement was observed between the detection of enterotoxin genes *sea* to *sed* and production of corresponding proteins *in vitro*. As a conclusion we recommend the use of the VIDAS SET 2 method and the PCR method to obtain more objective results in the food analysis. Strains of *S. aureus* isolated from foods are the vehicles of new types of enterotoxin genes more frequently. Some of them have been described as etiological agents of alimentary intoxications.

INCIDENCE OF *LISTERIA MONOCYTOGENES* IN RETAIL CHICKEN MEAT AND ESTABLISHING RELATIONSHIP WITH SOME BACTERIA BY LOGISTIC REGRESSION

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The objective of this study was to determine the contamination of retail poultry meat in Erzurum (Turkey) by *Listeria monocytogenes* and to evaluate its relationship with indicator bacteria using logistic regression. The incidence of *L. monocytogenes* was 32.76%, found in 38 of 116 samples. The application of logistic regression showed that *Enterobacteriaceae, Pseudomonas* spp., and *B. thermosphacta* populations have positive and yeast and mould have negative relationship with *L. monocytogenes* presence. The results of this study demonstrate that there is a serious risk in raw poultry meat for consumer health in Erzurum, because of the high incidence of *L. monocytogenes* in the samples of the present study. Hygienic conditions described in HACCP program should still be enforced in order to minimize *L. monocytogenes* in poultry meat during the processing.

PARTICIPANT ABSTRACTS

PATHOGENES

HYGIENE SURVEY IN ESTONIAN DAIRIES AND PATHOGEN ANALYSIS OF AUTHENTIC SAMPLES

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Hygiene survey was performed in three Estonian dairies. 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*, mycobacterial count, total bacterial count, *Enterobacteriaceae* spp., coliforms, β-glucuronidase positive bacteria, yeast and mould were collected from all dairies and analyzed. Gauze tests, 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 food and non-contact, results showed that food contact samples were less contaminated than non-contact, therefore dairies pay more attention to cleaning process of food contact equipment. Two *L. monocytogenes* positive samples were found from two dairies, both were from raw milk samples, 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 *Mycobacterium porcinum* or *Mycobacterium fortuitum* (both risk group 2), it was found from wastewater. Other was *Mycobacterium phlei* (risk group 1) and it was found from final product. One final product was "very contaminated" with aerobic bacteria and two raw material samples were "contaminated" according to Finnish law. Amounts of "clean", "contaminated" and "very contaminated" samples for each dairy were similar. As contamination from non-contact places (environmental and equipment) may be carried to food contact places and from there to the process line, dairies should pay more attention to cleaning processes of these places. Carriers can be for example employees' hands. Biofilms are problematic in the food industry, because they are protective barrier for microorganisms against sanitizers, and due to it cleaning can be more complicated.

INCIDENCE OF *LISTERIA MONOCYTOGENES* IN DIFFERENT KIND OF MEAT PRODUCTS ON LATVIAN RETAIL MARKET

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The aim of study was to determine incidence and number of *Listeria monocytogenes* in a variety of meat products on the Latvian retail market. A total of 209 samples of sliced, vacuum packed or modified atmosphere packed (MAP) meat products e.g. cold smoked beef, cold smoked pork, fermented meat, hot smoked ham, fermented cold smoked and boiled sausage, pâté and poultry were purchased from retail outlets in Latvian supermarkets. Samples were obtained from 9 meat plants located in Estonia, Latvia and Lithuania. All speciments were investigated 5 d after or before expiry date according to ISO 11290-1:1996 and ISO 11290-2:1996 with minor modifications (Johansson, 1998) during December 2004 – April 2005. *L. monocytogenes* were isolated from cold smoked, vacuum packed and sliced beef at range 56% (30/54) and pork 80% (4/5) samples originated from one Latvian meat producer. One culture of *L.monocytogenes* from pâté 4% (1/24) , fermented meat (basturma) 20% (1/5) and cold smoked, vacuum packed and sliced pork sample from another Latvian meat processing plant 11% (1/9) were isolated. None of hot smoked ham, fermented cold smoked or boiled sausage and poultry meat samples from Estonia, Latvia and Lithuania were found positive. Larger proportion of cold smoked beef, pork samples (32) contains *L. monocytogenes* at low level (<100 cfu/g), in six samples of cold smoked pork, beef and fermented meat samples number of bacteria was between 100 cfu/g and 1000 cfu/g. The number of *L. monocytogenes* did not exceeded 1000 cfu/g in retail meat samples. As expected, cold smoked, vacuum packed meat products should be recognized as high risk products, however prevalence of *L. monocytogenes* at low level (<100 cfu/g) at the time of consumption seems to be of low risk to the consumers.

THE PREVALENCE OF *CAMPYLOBACTER* SPP. AT THE FARM AND RETAIL LEVEL

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The aim of the study was to investigate the *Campylobacter* spp. contamination at the farm and retail level. A total of 102 faecal samples of poultry origin were collected in 2004. A total of 610 raw poultry meat samples were purchased from retail outlets in Estonia. *Campylobacter* spp. was detected using a modification of the NMKL Method no 119, 2nd ed. 1990, which includes the enrichment in Preston or in Bolton broth. Enrichment media was plated out on selective mCCDA (modified Charcoal Cefoperazone Deoxycholate agar, Oxoid). Typical colonies were streaked on Brucella agar, and verified by the Gram-reaction, motility-test, oxidase and catalase test, and hippurate hydrolysis. Fifty-two samples were collected from the farm A and of those 29 (55.8%) were positive. All samples of farm B origin were negative. Farm A samples were collected at the beginning of October and samples from farm B were collected at the beginning of November in order to investigate the presence of seasonal variation. There was a seasonal variation in flock colonization with *Campylobacter* spp. The overall prevalence of *Campylobacter* spp. in raw poultry products was 12%. Of the isolates, 77% were identified as *C. jejuni*. In conclusion, raw poultry products of Estonian origin are contaminated by *Campylobacter* species. The problem appears to be more severe in small-scale operations. The *Campylobacter* contamination observed in present study may indicate that the prevalence of human campylobacteriosis in Estonia is greater than the 124 cases reported by the Estonian Health Protection Inspectorate. An Estonian *Campylobacter* control programme is currently under development, and additional studies of different foods, at farms and processing plants are needed.

DETECTION AND IDENTIFICATION OF *CAMPYLOBACTER* POULTRY CARCASSES

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The aim of the study was the consolidation of the contamination levels of poultry carcasses, procured from the marketplace, with *Campylobacter* bacteria. In 2005 in the Microbiology Laboratory at National Food and Nutrition Institute prepared the monitoring plan to determine the levels of the *Campylobacter* bacteria in poultry carcasses. The monitoring tests were carried out in selected provinces by the laboratories of Provincial Sanitary and Epidemiological Stations, pursuant to the procedure described in the standard PN ISO 10 272:2002 'Food and feed microbiology. Horizontal method for detection of thermotolerant *Campylobacter* spp.'. The monitoring tests covered 240 poultry carcasses in 2005. The number of contaminated carcasses ranged from 0% to 100%. The results of monitoring tests in 2005 with 75.4% of all tested samples contaminated with *Campylobacter* spp., are very close to the results obtained in the previous year. The monitoring tests performed in 2005 allowed to estimate the scale of contamination of poultry carcasses with *Campylobacter* spp. in Poland. Unfortunately the obtained result is much higher than the analogical data collected in most EU countries (2.2–62.2%). Numerous epidemiological data from the Western Europe and the United States confirm the gravity of the foodborne infections caused by thermotolerant *Campylobacter* spp. Unfortunately still the source of contamination remains food of animal origin, primarily poultry carcasses. Therefore, it is legitimate to continue monitoring of the contamination rate of poultry carcasses with *Campylobacter* bacteria. However, it is not necessary to eliminate the contaminated poultry carcasses from the market. Special attention should be paid to educating the society about the ways of *Campylobacter* infection spreading e.g. consumption of raw or undercooked meat and/or improper hygienic habits.

SURVIVAL OF *ESCHERICHIA COLI*, *STAPHYLOCOCCUS AUREUS*, *BACILLUS CEREUS* AND *LISTERIA INNOCUA* DURING REFRIGERATED STORAGE OF 'ŞALGAM' JUICE – A TRADITIONAL FERMENTED TURKISH BEVERAGE

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Şalgam juice (turnip juice) is a popular beverage of southern Turkey, originating from Adana and consumed largely all over the country. Although its Turkish name 'Şalgam suyu' (or shortened, şalgam) does literally mean 'turnip juice', it is, in fact, a product of lactic fermentation. It has a turbid, dark red or purple colour and a very strong soar taste. In the production of Şalgam juice pounded wheat or bulgur flour (setik), yeast, sour dough, salt, black/purple carrot (*Daucus carota*), aromatic turnip (*Brassica rapa* var. *rapa*) and water are used. There is no information on the antimicrobial effect of Şalgam juice. The objective of this study was to investigate survival and growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and *Listeria innocua* during refrigerated storage of Şalgam juice. Şalgam juice samples were also examined for their pH and number of lactic acid bacteria (LAB) during refrigerated storage. In this study *E. coli* ATCC 8739, *S. aureus* (turnip juice isolate), *B. cereus* IAM 6A5 and *L. innocua* NRRL B-33314 were used as test strains. Each test strain of *E. coli* (6.4 log_{10} CFU/ml), *S. aureus* (8.2 log_{10} CFU/ml), *B. cereus* (5 log_{10} CFU/ml) and *L. innocua* (7.8 log_{10} CFU/ml) was inoculated into Şalgam juice obtained from market and their duplicates were kept at refrigeration temperature (± 4ºC). A volume of 1 ml was separately taken from bottles inoculated with the test strain at 4 h intervals and a volume of 0.1 ml of appropriate dilutions was spread plated in duplicate on pre-dried surfaces of Violet Red Bile agar for *E. coli*, Baird Parker agar for *S. aureus*, Tryptone Soya agar for *B. cereus* and *L. innocua*. Counting was done after incubation at 32 ºC for 24–48 h. In order to count LAB a volume of 1 ml of sample was diluted and inoculum from appropriate dilutions was pour plated with double layer in duplicate on MRS agar. LAB were counted after

incubation at 30 ºC for 24–48 h. Results showed that bacteria population inoculated into Şalgam juice decreased rapidly and many of the bacteria were inhibited completely after 24 h. However, the population of *L. innocua* after 24 and 48 h was around 2.6 $\log_{10}$ CFU/g and 2.7 $\log_{10}$ CFU/g, respectively. Survival could be due to adaptation mechanisms used to overcome the stresses (low pH, salinity and lactic acid). Because *L. innocua* may still be viable well after 48 h at refrigeration temperature, there is a food safety risk involved. Additional studies about the relationships between the stresses and the changes in *Listeria* characteristics and acid adapted cells/pathogens are also needed.

ISOLATION AND IDENTIFICATION OF *LISTERIA* SPECIES AND *LISTERIA MONOCYTOGENES* FROM CHICKEN AND CHARACTERIZATION OF AUTOLYSIS OF *LISTERIA MONOCYTOGENES* ISOLATES

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Listeria monocytogenes is an ubiquitous environmental microorganism responsible for human listeriosis. According to the reports of the World Health Organization, the primary mechanism of transmission of *L. monocytogenes* to human is through contaminated foodstuffs. Several outbreaks associated with the consumption of soft cheeses, milk, fish products, meat and poultry products have been reported. In the present study, *Listeria spp*. and *L. monocytogenes* were isolated from raw chicken legs sold in Bursa province between 2001 and 2002 and then the isolates were isolated by biochemical test. By direct and enrichment techniques we have isolated 25 *L. monocytogenes*, 12 *L. innocua*, 10 *L. welchimeri*, 1 *L. seeligeri* and 1 *L. grayi* strain. Most of the isolates demonstrated serotype 4 profiles by serotyping. The second part of the studies was contributed to the characterization of autolysis of *L. monocytogenes* strains isolated from clinical and food specimens under starvation conditions. Late exponential phase harvested bacterial cells were transferred in potassium phosphate buffer (100 mM, pH 7) and incubated at 37 °C to induce lysis. At the end of the 48 h incubation period a heterogeneous autolysis profile was observed among the tested strains. All the clinical strains exhibited high autolysis i.e. above 75%. Meanwhile, the extent of food isolates was not as high as the clinical ones but demonstrated a great variability – ranging between 22 and 88%. An increase in the amount of intracellular markers upon lysis was also measured in the higher autolytic clinical strain – UVF172, compared to low autolytic food strain – UVF114. The results obtained from the study provided evidence that autolysis in *L. monocytogenes* is strain dependent and revealed that tested clinical isolates had a higher level of autolysis than food isolates.

PARTICIPANT ABSTRACTS

PROCESSING & PACKAGING

EFFECTS OF PACKAGING TREATMENT, STORAGE CONDITION AND LENGTH OF STORAGE PERIOD ON EXTERNAL AND INTERNAL TABLE EGG QUALITY

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Packaging is an important stage in providing quality eggs to buyers. It protects the eggs from microorganisms and natural predators; prevents loss of moisture; protects from temperatures that cause deterioration and possible crushing during handling storage and transportation. There are many different types of egg packages, which vary both in design and packaging material used. Cupboard cases are commonly used by the Turkish egg producers and covering these cases by a plastic material before marketing became mandatory after the last avian flu epidemics. But there are no clear findings about the egg quality and shelf life during the marketing process. This study was conducted to determine if packaging system consisted of cardboard with plastic coverings would improve the shelf life of eggs. The second objective was to determine the interactive effect of different storage conditions (room or refrigeration temperature) and length of storage period (5, 10, 15 or 20 d) with packaging treatment (with or without plastic covering) on internal and external table hen egg quality. Freshly laid eggs (n = 480) were collected, randomized, weighed, pointed out numerically and divided into four groups of 120 eggs to test the length of egg storage treatments for 5, 10, 15 or 20 d. Eggs in each egg storage treatment group were randomized and allocated into two batches (with or without plastic covering material) of 60 eggs to test the effect of plastic covering material. Then, eggs were stored at room or refrigeration temperature conditions. Significant differences were observed for the internal and some external egg quality traits related with the length of storage period and storage conditions. All internal and external egg quality were not significantly influenced due to main effects of packaging treatment, except for yolk index. The internal egg quality traits became worst by the prolonged storage particularly when the eggs were stored at room temperature.

THE EFFECT OF VACUUM AND MODIFIED ATMOSPHERE PACKAGING ON GROWTH OF *LISTERIA MONOCYTOGENES* IN RAINBOW TROUT (*ONCORYNCHUS MYKISS*) FILLETS

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Rainbow trout (*Oncorynchus mykiss*) fillets stored at 4 ± 1 ^{o}C were analyzed with respect to microbiological (*Listeria monocytogenes*, total aerobic mesophilic count, phychrotrophic bacteria and *Enterobacteriaceae*) and chemical (pH, Total Volatile Basses Nitrogen) under air (control), vacuum and gas mixture (50% CO_2 : 50% N_2, 80%O_2 : 20% CO_2 and 2.5%O_2 : 7.5% N_2 : 90% CO_2). The minimum *L. monocytogenes* counts were determined form the samples with gas packaged, and no significant differences were found between them. It was determined that the effect of different atmosphere conditions and storage time on total aerobic bacterial count, phychrotrophic bacterial count and *Enterobacteriaceae* count were significantly different ($p<0.01$). The maximum Total Volatile Basses Nitrogen value was determined form the control group. The effects of atmosphere condition on pH values were significant ($P<0.05$).

The Effects of Different Nitrate Levels and Starter Culture on the Reduction of *E. coli* O157:H7 during Sucuk Production

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The objective of this study was to determine the effects of nitrate levels (0, 200 and 400 ppm) and the starter culture (*Staphylococcus carnosus* and *Pediococcus pentosaceus)* on the reduction of *Escherichia coli* O157:H7 (initial level of 10^6 CFU/g) in sucuk (Turkish dry fermented sausage). *Enterobacteriacea*, *E. coli* O157:H7, lactic acid bacteria, *Staphylococcus*/*Micrococcus* counts, moisture, pH, residual nitrite, nitrite/nitrate, salt analysis were also recorded. Microbiological analysis showed that, the number of *E. coli* O157:H7 decreased during the ripening period at a rate of 1.6 log unit in 14th day and a total of 3.3 log units in 21st day. The number of *E. coli* O157:H7 was below the detection limit in all samples at 28th day. But the bacteria were recovered with IMS method in all samples except control. In addition, Verotoxin was found to be positive using PCR. There was no statistically significant difference in the number of *E. coli* O157:H7 between the samples with or without starter culture and nitrate. The effect of starter culture on the pH decrease was very significant; the effect of the nitrate levels x ripening-storage period interaction was significant on *E. coli* O157:H7 counts.

EFFECT OF PROCESSING METHODS ON TOTAL CATECHIN CONTENTS OF TEA (*CAMELLIA SINENSIS*)

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Young leaves of *Camellia sinensis* tea plant are very important sources of catechins belong to the flavan-3-ol class of flavonoids. Tea catechins have been found to have a number of antioxidant activities, including scavenging of such reactive oxygen species as superoxide, hydroxyl and peroxyl radicals, inhibition of lipid peroxidation, inhibition of 2'-deoxyguanosine oxidation in DNA to 8-hydroxy-2'-deoxyguanosine and inhibition of the oxidation of low-density lipoproteins. Also it has been declared that tea catechins had an antibacterial activity against *Helicobacter pylori* and antifungal activity against *Penicillium chrysogenum* and Saccharomyces cerevisiae. Due to the antioxidative and antibacterial effects of tea catechins, it is essential to protect the great amount of tea leaf catechins while they are processing. In this study different kinds of tea processing methods were tried for determining the best tea manufacturing method in terms of protecting tea leaf catechins. Two main kinds of green tea manufacturing methods were tried in this essay; one of them was steaming and the other of them was pan firing. Besides those green tea manufacturing methods, two black tea manufacturing methods were tried as well. One of them was CTC method, the other was CAYKUR method. By using those production methods four tea samples were obtained; two of them were green and two of them were black tea samples. All of those green and black tea samples were analysed by using high performance liquid chromatograpy (HPLC). Not only total amount of catechins but also individual amounts of (+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, (-)-epigallocatechin, and (-)-epigallocatechin gallate were determined using HPLC. As a result of those analyses it was clearly observed that both of produced green tea samples have got high amounts of catechins. Steamed green tea has got the highest amount of catechines with its 13.47% Total Catechin Contents while CAYKUR black tea sample has got the lowest amounts of catechins with its 3.23% Total Catechin Contents. As a result of this study we can say that for obtaining high amount of catechins in dry tea leaves it must be chosen green tea especially steamed green tea.

FOOD SAFETY PROVIDING SYSTEM FOR AUDITING OF PRIVATE LABEL PRODUCER IN 2004–2006

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The aim of the auditing is to develop basic facilities and to evaluate food safety system of the private label producers for supermarket chains in Turkey. For this purpose, food safety providing system auditing has been performed in 272 food producers in the meat, milk, seasoning and tea sectors. Audits have been made in three phases in different producers and cities in Turkey. First phase is on documentation control systems, pest and cleaning files. The system files have more questions about HACCP preliminary necessity conditions. In the auditor controls the files where recorded and found. The second phase is field audit e.g. personal application, engineering, design and cleaning. The auditor looks especially at personal applications and food process surfaces in direct contact with the products produced. The last phase is on hygiene control of personnel and food contact surfaces. The auditor uses swabs for sampling from hands and surfaces. The analyses are performed in the Food Institute laboratories. The micro-organisms examined are *Staphylococcus aureus* and fecal coliforms on hands of the personnel and total aerobic mesophilic bacteria and fecal coliforms on food contact surfaces. All these parameters are evaluated by one or more auditors to finalize the report. According to this evaluation, the report is graded as 1000–901 “Excellent”, 900–750 “Satisfactory”, 750–650 “Acceptable” and <650 “Insufficient”. According to the result of 2005 in which the personal hygiene results were included, 23% were found to be dirty whereas rest of them met the good practices. As a result, 228 of the 272 producers got at least the grade sufficient.

After, third part audits performed by auditors for supermarket chains in Turkey, the topic which got most attention was microbiological contamination and contamination routes. The microbiological results have been evaluated looking at crucial parameters. Most of the audits have been performed mostly in the Marmara Region in Turkey. Cold chain factors were taken into consideration

through samples, which have been taken from the different private label producers, promptly sent to our institute. Furthermore, we separate samples into different groups e.g. dairy, meat and meat products, seasoning and tea groups. The analyses have been performed according to product groups e.g. for dairy products coliforms, *E. coli*, yeast, mould, *S. aureus* and *Salmonella*. For spices the total aerobic mesophilic bacteria, *E. coli*, mould and yeast, *E. coli O157:H7, Salmonella*, *Bacillus cereus* and aflatoxin analyses are carried out. For meat and meat products *E. coli, S. aureus*, mould and yeast, *Salmonella*, *C. perfringens, E. coli* O157:H7 and *Listeria monocytogenes* and for tea total aerobic mesophilic bacteria, coliforms, *E. coli*, yeast and *Salmonella* are analysed In evaluating these parameters limits given in the Turkish Food Codex Alimentarius have been used. Methods published by FDA, Bacteriological Analytical Manual, AOAC International and ISO were applied during the course of study.

FOOD HYGIENE ACTIVITIES AT THE INSTITUTE OF PUBLIC HEALTH IN OSTRAVA

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Institute of Public Health in Ostrava is a health care institute established by the Ministry of Health CR. Institute of Public Health provides services in the field of health promotion and protection. The main activities of Institute comprise determination and measurement of various components of living and working environment, examination of general consumption articles and food characteristics and wide range of investigations in biological and clinical material. The measurements of various components of living and working environment, particularly chemical, microbiological and biological analyses and physical factors measurement are carried out with professional evaluation at the Centre of Hygienic Laboratories. All services are carried out in line with effective legal rules and they are accredited according to the standard CSN EN ISO/IEC 17025. Department of Hygienic Microbiology is a part of the Centre of Hygienic Laboratories and it performs analyses of water, foods, drugs and process hygiene. Basic analyse and presence of pathogens (*Salmonella, Listeria*, incl. *E. coli* O157) in food are carried out.

Shelf Life of Sea Bass during Ice Storage

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In this study sensory and microbiological parameters of sea bass meat on ice were monitored during a 12-day storage period to obtain information about quality and hygiene of stored fish meat. Twenty five sea bass of approximately 300 g were caught for this purpose. The sea bass had been farmed in a Adriatic Sea fish farm. The fish were immediately ice stored in a mobile fridge at the temperature of about 3 ºC. On the storage day 2, 4, 6, 9 and 12, five fishes were subjected to sensor and bacteriological analysis. The sensor quality evaluation was made using the Quality Index Method test (QIM) for sea bass (Icelandic Fisheries Laboratories). The QIM includes sensor indicators of fish freshness and their assessment using the numerical score system. Fresh fish has the minimum (0), while the maximum number of points (20) indicates spoilt fish. In the bacteriological analysis the fish samples were analysed for total viable count (TVC), psychrotrophic count and number of *Pseudomonas* spp. In the microbiological analysis 25 g was diluted in 225 ml of peptone saline and homogenised in the stomacher. After serial dilution, appropriate dilution samples (1 or 0.1 ml) were poured or spread on agar plates. TVC was determined on Plate Count Agar (PCA) at 30 ºC for 72 h, psychrotrophic bacteria on PCA at 6.5 °C for 10 d, and *Pseudomonas* spp. on Pseudomonas agar F at 25 °C for 2 d. After the completion of sensor analysis the results showed that all fish samples taken on the second day of storage were rated first class (QIM – 0–2 points). Changes in the shape and clarity of eye, colour and odour of gills and appearance of gill mucus had occurred already at day 4 of storage (mean value of QIM – 3.5 points). However, during the entire 12 d of storage, the fish showed no substantial sensor changes indicative of spoilage, and on the day 12 they were scored 10-14 points. The TVC at 30 ºC did not increase considerably during the storage of sea bass on ice (from 5.5 $\log_{10}$ cfu/g at day 2 to 6.2 $\log_{10}$ cfu/g at day 12). The psychrotrophic bacterial count increased from 4.3 $\log_{10}$ cfu/g (day 2) to 7.3 $\log_{10}$ cfu/g (day 12). At the same time, an increase in the

number of *Pseudomonas* was recorded i.e. from 3.9 log_{10} cfu/g (day 2) to 5.5 log_{10} cfu/g (day) 12 in fish meat. As conclusion the increased deviation in characteristic appearance and odour of fresh sea bass was simultaneous with the increase of recorded psychrotrophic bacteria and *Pseudomonas* spp. The obtained results are in conformity with the literature data according to which the principal spoilage organisms of the Mediterranean Sea fish belong to bacteria of the genus *Pseudomonas.*

Group Works Based on the Lectures, Participant Abstracts and Literature

BACTERIAL FOODBORNE PATHOGENS OF CONCERN

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INTRODUCTION

A high level of protection of public health is one of the fundamental objectives of food law as laid down in regulations (EC) No 178/2002 and 852/2004. Throughout the European Union (EU) consumers are requiring the food industry to provide them with an increasing range of safe, nutritious and healthy foods of high sensory quality and increased shelf life. To meet the demand for healthier food of high sensory quality, the use of additives and preservatives is being reduced or eliminated and minimal processing techniques introduced. To increase food safety and quality considerable amount of time, effort and money has been spent to food safety control and management (ISO 22000:2005) systems including better packaging methods and improved new pathogen detection methods. Nevertheless there is still little sign within official statistics of significant reductions in the incidence of foodborne illnesses within EU countries. Todd (1997) reported that in the beginning of this decade 73 to 100% of all European outbreaks with known aetiology were caused by bacteria. Particular priority areas are species such as *Listeria monocytogenes, Campylobacter jejuni, Salmonella,* and *Escherichia coli* O157:H7. Biofilm formation and other problems in production environment have been in focus lately. Wirtanen *et al*. (2003) reported that pathogens such as *Listeria*

monocytogenes, *Salmonella* Typhimurium, and *Yersinia enterocolitica* can readily produce biofilms, causing severe disinfection and cleaning problems on surfaces in the food industry. Yeasts belonging to *Saccharomyces*, *Candida*, and *Rhodotorula* have been related with contamination of production environment as well as bacteria like *Legionella pneumophila*, *Pseudomonas* spp., *Gallionella* spp., and fungi of the *Aspergillus*, *Mucor*, and *Penicillium* strains (Wirtanen *et al*., 2003). The aims of the present report are to concisely discuss the most important bacterial pathogen contaminants with respect to associated foods, contamination sources and routes. Results of certain studies in some project related countries and specific aspects of the new European Food Law and the future needs in scientific and industrial research are emphasized.

BACTERIAL FOODBORNE PATHOGENS

LISTERIA MONOCYTOGENES

L. monocytogenes is a Gram-positive and motile bacterium that is commonly present in the environment and occurs in almost all food raw materials from time to time. According to current knowledge the genus *Listeria* contains six clearly distinguishable species (McLauchlin, 2006). The most commonly occurring species in food are *L. monocytogenes and L. innocua,* however *L. monocytogenes* is the only important human pathogen of the genus (Catteau, 1995). Some studies suggest that 1–10% of humans may be intestinal carriers of *L. monocytogenes*. It has been found in at least 37 mammalian species, both domestic and feral, as well as at least 17 species of birds and possibly some species of fish and shellfish. Healthy birds may asymptomatically shed *L. monocytogenes* in faecal material (Skovgaard and Morgen, 1988). However, the poultry meat is contaminated during slaughtering and processing (Rørvik *et al*., 2003). It can be isolated from water, soil, silage, and other environmental sources. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does not form spores. (Johansson, 1999; EuropeAid, 2004). *L. monocytogenes* is transmitted via three main routes: contact with animals, cross-infection of newborn babies in hospital and foodborne infection. The latter two sources result in the majority of cases of listeriosis in humans. Listeriosis is an uncommon but the serious foodborne disease that can be life-threatening to the elderly, people with weakened immune system and pregnant women (Lyytikäinen *et al*., 2000; Frye *et al.,* 2002).

Associated foods: *L. monocytogenes* has been associated with food sources such as raw milk, supposedly pasteurised fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types), and raw and smoked fish (Farber and Peterkin, 1991). Its ability to grow at temperatures as low as 3 °C permits multiplication in refrigerated foods (Roasto, 2004). It can survive or even grow at pH values as low as 4.4 and at salt concentrations of up to 14%.

In the study of Praakle *et al*. (2007) a total of 240 raw broiler legs (120 of Estonian and 120 of foreign origin) from 12 retail stores in two biggest cities (Tallinn and Tartu) of Estonia were investigated from January to December 2002. Of the raw broiler legs, 70% were positive for *L. monocytogenes*. The prevalence of *L. monocytogenes* in broiler legs of Estonian origin (88%) was significantly higher than in broiler legs of foreign origin (53%) ($P < 0.001$). Praakle *et al*. concluded the high prevalence of *L. monocytogenes* showing various PFGE types in the broiler legs could be caused by cross-contamination at retail level.

Ready-to-eat meat products with a long shelf life are associated with risk of transmission of *L. monocytogenes* (Farber and Peterkin, 1991). Prevalence of *L. monocytogenes* in cold smoked, sliced, vacuum packaged pork products during 15-month period from 2003 until 2004 was studied by Bērziņš *et al*. Samples originated from 8 Latvian and 7 Lithuanian manufacturers. The prevalences of *L. monocytogenes* in cold-smoked pork varied from 0 to 67% in Latvian products and 10 to 73% in Lithuanian products (Bērziņš *et al*., 2007). In order to identify the main risk factors associated with *L. monocytogenes* contamination, all production steps were studied separately in each meat processing plant. Bērziņš *et al*. suggested that brining by injection was a significant ($P < 0.05$) factor in contamination. Moreover, long cold-smoking times (12 h) had a significant ($P < 0.014$) predictive value for a sample to test positive for *L. monocytogenes*. The cold-smoking temperatures between 24 and 30 °C can provide an inhibitory effect on presence of *L. monocytogenes*. Low number of *L. monocytogenes* at the end of shell-life (< 100 cfu/g) can be explained by use of starter cultures during processing, which have an antilisterial effect, and affect multiplying of *L. monocytogenes* in pork products.

It is recognised that presence of *L. monocytogenes* in almost all raw foods cannot be completely eliminated, but through the application of effective hygienic measures, it is possible to reduce its incidence and levels in food products. In order to ensure the safety of food products, growing, harvesting handling, storage, processing and food supply systems must be managed by food handlers in such a way as to reliably control the growth of *Listeria monocytogenes* and to prevent from multiplying to potentially harmful levels, >100/g (Commission Regulation, 2005).

CAMPYLOBACTER JEJUNI

Campylobacter jejuni is a Gram-negative slender, curved, non-sporing motile rod. It is a microaerophilic organism, which means it has a requirement for reduced levels of oxygen. It is relatively fragile, and sensitive to environmental stresses (e.g., 21% oxygen, drying, heating, disinfectants, and acidic conditions). Because of its microaerophilic characteristics the organism requires 3 to 5% oxygen and 2 to 10% carbon dioxide for optimal growth conditions. This bacterium is now recognized as an important enteric pathogen. Before 1972, when methods were developed for its isolation from faeces, it was believed to be primarily an animal pathogen causing abortion and enteritis in sheep and cattle. Surveys have shown that *Campylobacter* spp. is the most common registered bacterial causes of human intestinal infections in many developed countries (Hänninen *et al*., 2003). *Campylobacter jejuni* subsp. *jejuni* and *C. coli* are the main cause of *Campylobacter* enteritis in human (Nachamkin and Blaser, 2000). *C. jejuni* is responsible for 80–90% of campylobacteriosis. It causes more disease than *Shigella* spp. and *Salmonella* spp. combined (Nachamkin and Blaser, 2000). Although *C. jejuni* is not carried by healthy individuals in the US or Europe, it is often isolated from healthy cattle, chickens, birds and even flies. It is sometimes present in non-chlorinated water sources such as streams and ponds. In industrialized countries, including Western Europe, US, Canada, Australia and New Zealand, the rate of human *Campylobacter* infections has been increasing steadily. In 2004 a total of 183 961 human cases of campylobacteriosis were reported from 25 Member States of European Union. The Community incidence was 47.6 cases per 100 000 population. (EFSA, 2006.) An estimated 2.5 million cases of *Campylobacter* infection occur each year in the United States, and 80% of these cases have been found to be the result of foodborne transmission (Bhaduri and Cottrell, 2004).

Associated foods and environment: *Campylobacter* spp. is widespread in nature, not only in wildlife but also among food animals such as cattle, sheep, swine, and avian species as commensally organisms (Friedman *et al*., 2000). The avian species are the most common host for *Campylobacter*, probably because of their higher body temperature (Skirrow, 1977). Monitoring studies indicate that most chicken flocks are colonised with *C. jejuni*. Intestinal colonisation usually leads to contamination of the final product, which cannot be prevented in the processing plant.

Studies carried out in slaughterhouses have shown that the main source of the spread of *C. jejuni* on poultry carcasses is their intestinal contents (Stern and Robach, 2003). *Campylobacter* spp. colonization in commercial poultry flocks is widespread in many countries. Studies in Europe indicate flock prevalences ranking from 18 to over 90%, with northern countries showing a lower proportion of positive flocks (Barrios *et al*., 2006). A recent monitoring study in Poland showed that 75.4% of chicken carcasses is contaminated with *Campylobacter* species. It is well established that poultry products are a vehicle for foodborne campylobacteriosis and they are suspected to be an important source of infection (Roasto *et al*., 2005).

Other foods (mainly of animal origin) must be considered as potential sources of infection. *Campylobacter* have also been isolated from such food items as raw milk, pork, beef, lamb, and seafood (Duffy *et al*., 2001). The presence of *Campylobacter* spp. in raw materials and products of animal origin may represent a source of infections, however, a real health hazard exists only when meat consumed is raw or undercooked (Domingues *et al*., 2002). The other major hazard may be a result of improper hygienic habits and disregard of Good Manufacturing Practice (GMP) principles. This is related to the transfer of bacteria from raw meat to other foodstuffs (cross-contamination).

Effective quality-control programme in Estonian large-scale poultry processing plant accounted for the lower contamination levels of fresh chicken meat compare to contamination level with the same type of products of small-scale plant (Roasto *et al*., 2005). Altogether, 279 samples of Estonian raw chicken meat (breasts, carcasses, legs, minced meat, thighs and wings) were analysed during 2000 and 2002 (Roasto *et al*., 2005). Of these, 90 were collected directly from the end of the slaughter line of a small-scale poultry meat plant and 189

from traditional market halls of Tartu town. All chicken meat samples from market halls were sold fresh and unpacked. Of the raw chicken products of Estonian origin, 15.8% were positive for *Campylobacter*. The prevalence of *Campylobacter* in the products (breasts, carcasses, thighs and wings) of the small-scale poultry meat plant (35.6%) was significantly higher than in those originated from the large-scale company (6.3%) ($P<0.001$). In order to reduce the incidence of campylobacteriosis in humans a number of preventive measures are needed throughout the way from farm to table.

SALMONELLA

Salmonella spp. are facultatively anaerobic, Gram-negative, straight, non-spore-forming small rods, which are usually motile with peritrichous flagella. *Salmonella* is a genus within the family *Enterobacteriaceae* in which approximately 2200 serotypes are recognised. Some of these strains are specifically adapted to hosts and largely restricted to them, e.g. *S.* Typhi in man and *S.* Dublin in cattle. The growth range for salmonellae is 5–47 °C at pH 4.0–9.0, with optimum growth at 35–37 °C and pH 6.5–7.5. Salmonellae are not particularly salt-tolerant, although growth can occur in the presence of 4% sodium chloride. The lower limit of water activity (a_w) permitting growth is 0.93 (Mead, 1993).

Associated foods: A wide variety of foods have been implicated in outbreaks of illness caused by many different serotypes of *Salmonella*: raw meats, poultry, eggs, milk and dairy products, fish, shrimp, frog legs, yeast, coconut, sauces and salad dressing, cake mixes, cream-filled desserts and toppings, dried gelatine, peanut butter, cocoa, and chocolate. Various *Salmonella* serotypes have long been isolated from the outside of eggshells. The present situation with *S.* Enteritidis is complicated by the presence of the organism inside the egg, in the yolk. This and other information strongly suggest vertical transmission, i.e., deposition of the organism in the yolk by an infected layer hen prior to shell deposition. Foods other than eggs have also caused outbreaks of *S.* Enteritidis disease. *Salmonella* still is the most frequently recorded pathogen in the production chain of food of animal origin. At present the predominant serotypes are *S*. Enteritidis and *S*. Typhimurium. This is true especially considering the most important meats from pig and poultry. In areas such as Scandinavia measures against this pathogen have been traditionally more thoroughly endeavoured, finally resulting in a lower prevalence of *Salmonella* in these

countries compared to Continental Europe (EuropeAid, 2004). Whatever the *Salmonella* serotype, effective controls for minimising/eliminating the hazard of *Salmonella* from foods involve control of the following steps: raw materials, personal and environmental hygiene, process conditions, post-process contamination, retail and catering practices, consumer handling.

ESCHERICHIA COLI O157:H7

E. coli is a facultatively anaerobic, non-spore forming, Gram-negative rod within the family *Enterobacteriaceae.* They form part of the natural gastro-intestinal microflora of man and warm-blooded animals. Because many microbes from faeces are pathogenic in animals and humans, the presence of the intestinal bacterium *E. coli* in water and foods indicates a potential hygiene hazard. Normally *E. coli* serves a useful function in the body by suppressing the growth of harmful bacterial species and by synthesizing appreciable amounts of vitamins. Although most *E. coli* are harmless commensal organisms, there are many pathogenic strains capable cause a variety of illness in humans. There are six recognized groups of pathogenic *E. coli* (EPEC, ETEC, EIEC, EaggEC, EHEC, NTEC). Each group has different virulence traits and mechanisms of pathogenity (Duffy, 2006). Currently, there are four recognized classes of enterovirulent *E. coli* (collectively referred to as the EEC group) that cause gastroenteritis in humans. Among these is the enterohemorrhagic (EHEC) strain designated *E. coli* O157:H7. *E. coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine. These toxins (verotoxin (VT), shiga-like toxin) are closely related or identical to the toxin produced by *Shigella dysenteriae*.

Associated Foods: Undercooked or raw hamburger (ground beef) has been implicated in many of the documented outbreaks, however *E. coli* O157:H7 outbreaks have implicated alfalfa sprouts, unpasteurized fruit juices, dry-cured salami, lettuce, game meat, and cheese curds. Raw milk was the vehicle in a school outbreak in Canada.

LEGISLATION

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs constitutes that foodstuffs should not contain micro-organisms or their toxins or metabolites in quantities that present an unacceptable risk for human health. Regulation (EC) No 178/2002 lays down general food safety requirements, according to which food must not be placed on the market if it is unsafe. The use of microbiological criteria should form an integral part of the implementation of HACCP (hazard analysis and critical control points) based procedures and other hygiene control measures. According to Article 4 of Regulation (EC) No 852/2004, food business operators are to comply with microbiological criteria. This should include testing against the values set for the criteria through the taking of samples, the conduct of analyses and the implementation of corrective actions, in accordance with food law and the instructions given by the competent authority. Article 5 of Regulation (EC) No 2073/2005 is laying down specific rules for testing and sampling, according to which the ISO standard 18593 shall be used as a reference method. Food business operators manufacturing ready-to-eat foods, which may pose a *L. monocytogenes* risk for public health, shall sample the processing areas and equipment for *L. monocytogenes* as part of their sampling scheme. Food safety and process hygiene criteria are given in chapter 1 and 2 of the Commission Regulation (EC) No 2073/2005 for microbiological criteria for foodstuff.

FUTURE NEEDS

There is research need for more sensitive, reliable, and cost-effective tools, particularly sampling methodologies, for analysing food and environmental samples (*e.g.,* high priority commodities include produce, eggs, and seafood) for microbial pathogens, where frequency and extent of contamination are expected to be low, for identification and evaluation of relevant characteristics of different forms of product packing and handling on the safety of a variety of foods. Developing modelling techniques to assess microbial behaviour in various foods, human exposure and dose-response relations to certain foodborne pathogens (*e.g.,* enumerative detection methods for pathogens), potential risk of those pathogens causing human illness, and the setting of safety performance standards to regulate microbial content of food, determining the population trends with respect to food safety knowledge, attitudes, and practices, especially

behaviours that may be significant risk factors for foodborne illness (*e.g.,* food consumption, in-home food preparation and handling) are the other aspects needed research. The microbiological safety of food has been advanced substantially by the introduction and implementation of HACCP. HACCP provides a systematic conceptual framework for identifying hazards and focusing efforts on the proper functioning of key food production, processing, and marketing steps. HACCP cannot be expected to control unknown hazards, such as emerging foodborne pathogens. There is a need to re-examine how food is produced, processed, marketed, and prepared to identify conditions that contribute to emergence. For example, organic acids are used extensively throughout the food industry to control spoilage and pathogenic microorganisms (Baysal and Unluturk, in press).

The changing epidemiology of foodborne disease calls for improved surveillance including rapid sub-typing methods, cluster identification, and collaborative epidemiological investigation (including case-control studies). Also examined was the need for better integrated, coordinated, and standardized animal disease surveillance and health monitoring programs. The new problems of foodborne disease require new control and prevention strategies to ensure that food in both domestic and international trade is safe. Topics included a need for multidisciplinary teams that can provide "just in time" research; for basic research to explain factors associated with food production and processing that contribute to new foodborne microbial threats; for prompt evaluation and implementation of innovative preservation methods (e.g., food irradiation) to meet consumer demand for fresh foods; for the use of emerging molecular methods (e.g., DNA hybridisation and polymerase chain reaction) to examine emerging foodborne disease organisms; and for models to predict the probability of a particular microbial event (e.g., growth and death), which may be useful in the design of HACCP programs and in defining processes, formulations, and storage conditions to yield foods with acceptable shelf life and safety characteristics.

CONCLUSION

It is a long way from the new borne food animal to the consumer's table. On one hand, there exist several stages independent from each other, where the persons involved do not always contact. On the other hand, there are a lot of

circumstances and hazards, which may or may not constitute a risk to humans. As a consequence, measures should be taken especially, where the prevalence of pathogens has been high, i.e. hygiene in the primary production, immunisation, logistic slaughter or measures in cleaning and disinfection the site. The horizontal and vertical transfer of pathogens must become under tighter control: the routes of the agent via transport to the abattoir are not at all safe. There is not point, where *Salmonella* or other pathogens would be safely prohibited to invade the human food chain. It is obvious, that the inspection service by the authorities cannot afford the total of surveillance in every production process. The hygienic status of intermediate products and end products is particularly dependent on the circumstances of previous stages of production. In consequence hygiene is an issue of day-to-day practice and checks must be carried out frequently. As a consequence the authorities have to rely more in the responsibility of the plant. So the role of the authorities is presently in reconsideration in order to focus the available resources on the essentials of surveillance. This is true also with respect to future additional tasks of surveillance in husbandry, which possibly demands more personnel in the future. It should be emphasized that the producer is responsible for the product and should do everything to guarantee it.

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FOOD PROCESS HYGIENE, EFFECTIVE CLEANING AND SAFETY IN THE FOOD INDUSTRY

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INTRODUCTION

Understanding the term "food safety" along the food chain has various aspects. One can start with technology integrating regulation and continuing with nutrition and finishing with consumer. In the food safety program we should be able to identify all hazards, analyze them, evaluate the likelihood of their occurrence and identify measures for their control. A hazard is a biological, chemical, physical agent or condition with the potential to cause an adverse health effect or spoilage. Today one can manage food safety based on HACCP through good agriculture, good manufacturing practice, good hygiene practice, good transport practice, good storage practice, good catering practice and good laboratory practice. The objective of this group work is to review the different approaches to assure good hygiene and consequently the safety of the food.

FOOD PROCESS HYGIENE

CROSS-CONTAMINATION

In the early days, sources of contamination included insects, rodents, sticks, stones, straw, wood, sand and dirt. People have learned to avoid these contamination sources; they can be eliminated by maintaining physical cleanliness in and around the food plant. Looking beyond these sources of gross contamination, today one is concerned with sources of microbiological contamination. Microorganisms may come from people working in the plant or handling the food, from raw products, from the food materials or ingredients or from lack of proper cleaning of the food plant equipment or the food plant. A third problem area is potential chemical contamination. This source of contamination can be controlled by proper usage of pesticides. That is, using the right pesticides on the right time, and in the right amount. This same principle applies to chemicals used in the manufacturing of the food, that is, the proper use and application of food additives and/or chemicals used in the cleaning and sanitizing of the food plant.

Preventing cross-contamination is one step to eliminate food borne illness. Cross-contamination of food is a common factor in the cause of food borne illness. Foods can become contaminated by microorganisms (bacteria and viruses) from many different sources during the food preparation and storage process. What is cross-contamination? Cross-contamination is the contamination of a food product from another source. There are three main ways cross-contamination can occur: from food to food, from people to food or from equipment to food.

Food can become contaminated by bacteria from other foods. This type of cross-contamination is especially hazardous if raw foods come into contact with cooked foods. Here are some examples of food-to-food cross-contamination:

- In a refrigerator, meat drippings from raw meat stored on a top shelf might drip onto cooked vegetables placed on lower shelf.
- Raw chicken placed on a grill touching a steak that is being cooked.

People can also be a source of cross-contamination to foods. Some examples are:

- Handling foods after visiting the toilet without properly hand washing.
- Touching raw meats and then preparing vegetables without washing hands between tasks.
- Using an apron to wipe your hands between handling different foods, or wiping a counter with a towel and then using it to dry your hands.

Contamination can also be passed from kitchen equipment and utensils to food. This type of contamination occurs because the equipment or utensils were not properly cleaned and sanitized between each use. Some examples are:

- Using unclean equipment, e.g. slicers, can openers, utensils to prepare food.
- Using a cutting board and the same knife, when cutting different types of foods, such as cutting raw chicken followed by salad preparation.
- Storing a cooked product, such as a sauce, in an unsanitized container that previously stored raw meat.

Cross-contamination is the transfer of biological or chemical contaminants to food products from raw foods, food handlers, or the food handling environment. The type of cross-contamination most frequently implicated in food borne illness occurs when pathogenic bacteria or viruses are transferred to ready-to-eat foods. Follow these steps to prevent cross-contamination and reduce hazards to food:

- Wash your hands thoroughly between handling different foods or after using the toilet.
- Wash and sanitize all equipment and utensils that come in contact with food.
- Avoid touching your face, skin, and hair, or wiping your hands on cleaning cloths.
- Store foods properly by separating washed or prepared foods from unwashed or raw foods.
- Try preparing each type of food at different times, and then clean and sanitize food contact surfaces between each task.

- Many cross-contaminations can be avoided through better employee ‘practices’, separation of raw and ready-to-eat foods, and plant design to prevent cross-contamination.

AIRBORNE MICROFLORA

Bacteria, fungi, algae, protozoa, and viruses float in air currents. The microbial flora of air is not constant and is highly variable or transient. The numbers of microorganisms in the air range from 10 to 10,000 per cubic meter. Microorganisms do not multiply in the air; their reproduction is uncommon in air because of lack of moisture and nutrients. The presence of micro-organisms in air is almost entirely accidental. Indoor air is generally dry and contains no available microbial nutrients; microorganisms cannot grow in air but can survive in association with dust particles. Dust particles in air come from clothing, bedding and similar textiles human and animal skin scales and hair dried soil and similar contaminating sources. Dust particles are dry and highly charged on the outside but may be moist in the centre. The different types of micro-organisms that are normally detected in air include bacteria, fungal spores and some yeast:

- Bacterial endospores and exospores of e.g. *Bacillus*, *Clostridium*, *Streptomyces*.
- Vegetative cells of many bacterial species mainly gram positive bacteria which are more resistant to drying. Bacteria from human and animal skin, hair, respiratory aerosols etc. mainly Gram positive bacteria.
- Fungal spores of many species.

Airborne microorganisms in food processing plants are extremely hazardous because of the economic and health problems they may cause. Because of the rich nutrients and moisture, microorganisms can reproduce at an incredible rate in the foods. Beside this atmospheric micro flora, sneezing, talking, laughing, falling hair, using soiled laboratory coats, as well as shedding from hands and arms contributed to the microbial contamination of air in food processing plants. Immediate washing of floors containing materials that may support microbial growth has been observed to be critical in reducing the airborne counts in food processing plants. The raw materials in processing plants contribute a major part of the microbial air contamination. From these raw materials, microorganisms may be deposited into the air during handling and processing of the raw materials.

Virtually all micro-organisms can be transmitted from air to food surface due to their bioaerosols form. The pathogens are extremely sensitive to the environment and cannot be outside of the host for any length of time. Only a few pathogens may be transmitted by air. Some airborne pathogenic bacteria, such as *Staphylococcus aureus*, spore form the antrax-causing bacterium *Bacillus anthraci* are well known to exist. But, no data are available to contaminated food by air-borne pathogens. But there is a potential risk for foods produced in livestock farms. Contamination from airborne yeasts, moulds and other microorganisms can result in shortened shelf life of food. A few researches about air micro flora in different food production are as follow:

- Donnelly (1977) researched airborne microbial contamination in a winery bottling room. He isolated *Acetobacter*, *Lactobacillus*, *Bacillus*, *Saccharomyces*, *Rhodotorula*, and *Serratia* species from the air.
- Ström and Blomquist (1986) monitored concentrations of airborne fungal particles in the sorting departments of two fruit warehouses during the handling of moldy citrus fruits. They found that concentrations of airborne moulds varied between 10^5 and 10^8 colony-forming units per cubic metre (CFU m^{-3}). They counted that the highest mean value recorded 7.7×10^8 spores m^{-3} and showed that 85–95% of the micro-organisms were <3.5µm. They indicated that the dominant flora consisted of *Penicillium italicum* and *Penicillium venucosum.*
- Ellerbroek (1997) examined bacterial loads in the air at different locations of a poultry slaughtering and processing plant and found that relatively high mean counts of airborne *Enterobacteriaceae* (log 3.2 cfu/m^3) were recovered in the bird reception area and at the entry to the evisceration room (log 2.6 cfu/m^3), while low mean counts of *Enterobacteriaceae* were recovered in the cutting and deboning area (log 1.0 cfu/m^3). He found that the microbes near evisceration were predominantly *Staphylococcus* spp., *Moraxella* spp., *Micrococcus* spp., *Acinetobacter* spp., and *Klebsiella* spp. In the reception area mainly *Micrococcus* spp., *Staphylococcus* spp., *Corynebacterium* spp., yeasts and *Flavobacterium* spp. were found and in the chilling rooms *Corynebacterium* spp., *Acinetobacter* spp., *Moraxella* spp., and yeasts.
- Salustiano *et al*. (2003), researched the microbiological air quality at processing areas in a dairy plant. For all processing areas, they

determined that the numbers of mesophilic aerobic bacteria, yeast and moulds were higher than 90 CFU·m^{-3}, which is the maximum value recommended by American Public Health Association. They stated that the results showed a difference ($p<0.05$) for the *Staphylococcus aureus* numbers (from <1.0 to 4.3 CFU·m^{-3}) at processing areas.

- Northcutt *et al*. (2004) researched total aerobic bacteria, moulds/yeasts, coliforms and *Pseudomonas* in the air in three shell egg processing operations. They found that highest counts for total aerobic bacteria (5.9 log cfu/ml air), moulds/yeasts (4.0 log cfu/ml air) and coliforms (2.5 log cfu/ml air) were found in the hen house. Highest counts for *Pseudomonas* were found in the hen house (3.2 log cfu/ml air) and behind the egg washer (3.5 log cfu/ml air). Lowest counts for total aerobic bacteria (2.5 log cfu/ml air) and moulds/yeast (2.7 log cfu/ml air) were found in the post-processing cooler.
- Soldatou *et al.* (2006) isolated primarily Gram-positive, catalase negative cocci, micrococci and bacilli from the air of cheese factories. They also stated that airborne contaminants exhibit acidifying and proteolytic activities and may thus contribute to cheese ripening and flavour. However, they indicated that room air quality may be the cause of unsatisfactory shelf life.

Contamination of microorganisms from air may be reduced practically by good ventilation, use of face masks, controlled temperature and RH and establishing pressure differentials between rooms. In current, different technical methods are used for prevention of air-borne microorganisms:

- Air filtration – a typical HEPA (High Efficiency Particulate Air) filter will filter micron sized particles at about 95% efficiency. In theory, HEPA filters should be highly effective against bacteria and fairly effective against viruses.
- Ultraviolet irradiation – Microorganisms are uniquely vulnerable to the effects of light at wavelengths at or near 253.7 nm due to the resonance of this wavelength with molecular structures. Ultraviolet light possesses the right amount of energy to break organic molecular bonds and damages the microorganisms. The outdoor die-off rate, which is due to the ultraviolet component in sunlight, varies from one pathogen to

another, but can be anywhere from a few seconds to a few minutes for a 90–99% killing of viruses or contagious bacteria. Spores, and some environmental bacteria, tend to be resistant and can survive much longer exposures.

- Outdoor air purging – Airborne microorganisms can be removed by purging with outside air, which is naturally sterilized.
- Electrostatic precipitation is commonly used to remove particles including microorganisms from airstreams.
- Negative air ionization has the potential to reduce the concentration of airborne microorganisms. The effect results from the ionization of bioaerosols and dust particles that may carry microorganisms.
- In addition, photocatalytic oxidation, air ozonation, carbon adsorption, passive solar exposure, ultrasonic atomization, microwave atomization, pulsed light have been researched for control of airborne microorganisms.

SURFACE HYGIENE AND BIOFILMS

A biofilm is a complex aggregation of microorganisms marked by the excretion of a protective and adhesive polysaccharide and glycoprotein matrix. Biofilms may contain spoilage and pathogenic microorganisms. All this microorganisms within biofilm are protected from sanitizers increasing the likelihood of survival and subsequent contamination of food. Biofilms also play an important role in the spreading of resistant microbes. Planktonic bacteria are sensitive to the antimicrobial and disinfectants, but biofilm bacteria are more resistive. Within the high dense bacterial population, efficient horizontal transfer of resistance and virulence genes takes place. This increases the risk of reduced shelf and disease transmission. The ability to form biofilm is therefore considered to be an important virulence factor. Microbes inhabiting contact and environmental sites in food processing are mostly harmful, because these microbial communities are formed in critical process places.

Some microbes naturally have a higher tendency to produce biofilm than others, but biofilm can generally be produced by any microbe suitable conditions. Once a biofilm has been formed, it can be a source of contamination for foods passing through the same processing line. The attachment of bacteria with subsequent

development of biofilms in food processing environments is a potential source of contamination of finished product. This is the case especially if the process equipment is not hygienically integrated in the process line or the cleaning and disinfection procedures are not properly designed to remove the organic soil from the process surfaces. The surfaces of equipment used for food handling, storage, or processing are recognized as major sources of microbial contamination. Areas that are more prone to biofilm development include dead ends, joints, valves, and gaskets. In such places bacteria can remain on equipment surfaces even with acceptable cleaning systems in place. The microbes in thin biofilms can be removed from the surface only by the combination of the physical and mechanical cleaning methods. These microorganisms can not be removed only by spraying or washing methods and the decontamination can not be obtained. This point causes the cross contamination and recontamination.

In the food industry, it is very hard to find the best food grade cleaning chemical or disinfectants. Is has to be taken into consideration if the concentration of the chemical is not strong enough the effectiveness of it will be passive and the insufficient cleaning of the surface will not display an antimicrobiological effect. Besides, the equivalent chemical of the food grade chemicals are considerable cheap and do not has a legal necessity. For that reason manufacturers prefers these chemicals.

The investment cost of the chemical and physical cleaning applications is an additional cost for the manufacturers. Beside, the tolls used in mechanical cleaning (brush, cloth ext.) are as important as the contact time with the surface of the chemical and it increases the effect of the cleaning. This information's is determined detailed in the chemical usage instructions. The need of ambience ventilation for the heavy cleaning chemicals or evaporation attributed acid and base applications prove us that mechanical cleaning is the easiest and the safest solution for every condition. At the food manufacture production lines, even it is installed with the new technology designed machines, the line tendency to dirty it self and the food hidden to the dead points can be removed only by the mechanical cleaning.

TRAINING OF PERSONNEL IN HYGIENE AND FOOD SAFETY

An important way to prevent food contamination is to maintain a high standard of personal hygiene and cleanliness. Even healthy people carry food poisoning bacteria on their bodies. By touching parts of the body, such as nose, mouth, hair and including clothes, you can spread bacteria from hands to the food. It is essential that personnel are well informed and educated about hygiene rules and recommendations in the food industry. Training of employees and managers needs to be performed on a continuous basis.

A major reason for improved personal hygiene is the dreaded of foodborne illness outbreak. When this occurs, the reputation and life of a company can be severely harmed. The management must ensure that their hygiene procedures are adequate to safeguard the product quality and hinder or at least minimise outbreaks to few isolated cases of low severity. The HACCP system helps in prioritising process steps focusing on elimination or at least reduction of hazards.

Most infectious intestinal diseases are spread through contaminated hands via the fecal-oral transmission route. It has been estimated that between 25 and 40 percent of all foodborne illnesses are caused by poor personal hygiene. This includes outbreaks traced to food processing and service establishments as well as in the home environment. Specific outbreak-causing organisms have varying degrees of personal hygiene involvement as contributing causes. Therefore, the food industry personal hygiene program is a prerequisite in most food processing environments. If food handling step is deemed to be a critical control point (CCP), then hand contact should be avoided/eliminated or hand washing should be monitored, documented and verified (MDV). In addition to monitoring the employees' hand washing behaviour through use of documentation forms filled out by managers, there are a number of newly introduced hand wash monitoring systems, which provides automatic documentation satisfying the MDV requirement. It must be remembered that the personal hygiene must not be neglected, even though the proportion of foodborne illness caused by products produced in the food processing is low. With current large-scale production and distribution channels for food products, a single flawed practice in a processing plant or lapse of hygiene by a single individual can cause national or international product recalls. Sometimes bare hand contact with food under quite exaggerated circumstances has been described to occur. Even low level of hand

contact e.g. handling sliced pineapple, tomato or ice cubes has been shown to initiate outbreaks. Also extremely hazardous activities have been documented e.g. plunging hands into milk containers to change malfunctioning spigot, repeated and repeated tasting food with fingers, using bare hands to remove meat from bones or mixing salad by hand. Such hand contact should not be tolerated and alternatives should be sought e.g. gloves, food handling utensils, tongs or bakery papers.

EFFECTIVE CLEANING IN THE FOOD INDUSTRY

MECHANICAL CLEANING

Mechanical cleaning processes are good alternatives to traditional solvent-based cleaning operations. They reduce waste production and eliminate potential safety problems with the handling and usage of toxic, ozone-depleting, and often-flammable solvents. These cleaning processes are many and varied. Cleaning of almost any piece of equipment, surface, or component is possible if it is study enough to withstand the friction and force produced by the mechanical work of cleaning operations such as sanding, grinding, polishing, brushing, and scraping. These methods might not be appropriate for precision or delicate parts and the stripping rate varies with the type of paint, coating condition and coating thickness. The following benefits have been seen:

- Significant reduction in the amount of hazardous waste generated compared to chemical stripping.
- Reduces hazardous waste disposal costs.
- Provides solvent cost savings.
- Reduces the number of hours required for paint stripping in comparison to chemical stripping.
- Reduces worker exposure to solvent, hazardous waste and hazardous air emissions.

DRY AND WET CLEANING

In food industry the efficient cleaning is very important part of the whole production. The whole point of cleaning is to remove all dirt (residues of food,

biofilms of microorganisms). Because of the different types of food industries, there are different types of cleaning e.g. dry cleaning, wet cleaning and cleaning with enhanced mechanical – physical forces. The dry cleaning is used in bakery industry and wet cleaning is in production of beverage and in all kind of industry where we are dealing with moisture and grease. Mechanical – physical cleaning is used mostly to prepare the working desk or other working tools before using the wet cleaning. The chemical cleaning is mostly used is places where all other ways of cleanings are not suitable.

The dry cleaning is mostly used in bakery industry because of the specific characteristics and types of ingredients. The flour is present in the air all the time and because of that the water can cause a lot of damage especially in cauldron. On the other hand with water you can easily flush away all the dirt. In bakery industry the compromise between dry and wet cleaning and the whole production most is done. On daily and weekly bases the dry cleaning is used and the wet cleaning is used on monthly bases. In the process of dry cleaning the broom, grater and the special types of vacuum cleaners are the most important help. But the same broom or grater most not be used for working desk and for the floor for instance. In that purpose the brooms are marked with labels in different colours (green colour for working desks, red colour for toilettes, yellow colour for floor, etc.).

The wet cleaning on daily bases is useable only for some parts of the machines which have become in contact with wet or greasy ingredients. Those cleanings if possible most be performed in special places marked only for that. In production of fresh cakes-patisserie the dry and wet cleaning are used. There is very important to separate facilities where the sponge cakes are baked and where the crèmes are prepared. In first part of the production only the dry cleaning is used and in second part of the production only the wet cleaning in used. In good hygiene practice those two ways of cleaning most never mixed. In baking tin the water can do a lot of damage, on other hand only the dry cleaning is useless in production of crèmes. Only the thorough hygiene practice in those facilities is sufficient. Regardless of the type of the cleaning, cleaning is necessary at home as in production facilities at work.

CIP CLEANING

Cleaning-in-place (CIP) is a method for cleaning equipment quickly and efficiently without needing to dismantle the equipment. The cleaning solution are taken from a storage tank, circulated in the process tanks and pipelines, and returned to the storage tank (www.worldpumps.com). Typically a CIP system would include a solution reservoir, a supply pump, and a return circuit in the case of a tank or other similar vessel, a spray device and some type of return pump may also be included. Any closed vessel (tanker, tank, vat, chamber, hopper, or bin) can be CIP cleaned using one of the following spray devices: disc, stick, ball, ring or tanker spray. Also, any closed circuit, including piping, pumps, valves, and plate heat exchangers can be cleaned in place. The success in cleaning any vessel or pipeline circuit in place depends on five considerations: time, temperature, concentration and mechanics.

Considering the disinfection technology you have to distinguish between appropriate procedures of closed piping systems (CIP – cleaning in place) and containers with large quantities of circulated solutions and good turbulence, good microbial suspension, simple temperature application between cold (4 to 10 °C) to heated (40–60 °C) and generally good pre-rinsing. In closed cleaning systems pre-rinsing with cold water is carried out to remove loose soil, and the CIP treatment in normally performed using a hot cleaning solution, but a cold solution can also be used in handling fat-free product. In dairy CIP cleaning the warm alkaline cleaning solutions, normally of 1–1,5% sodium hydroxide (NaOH), is heated to 75–80 °C and the cleaning time is 15–20 min. Cleaning step before disinfection in CIP program must be effective enough to affect biofilm.

The separate disinfection, carried out after the cleaning operation, deals with less soiling, but is an additional step. If the extent of soiling is not too excessive one would therefore normally carry out a combined cleaning and disinfection agents. In this context, the choice of cleaning and disinfecting agents must depend among other parameters on the type and intensity of soiling and contamination considering also the material quality of the process equipment. The CIP disinfection technology can be generally applied with low concentration of active ingredients. Disinfectants used in the food processing industry include oxidizing agents, hypochlorite, hydrogen peroxide, ozone and peracetic acid,

denaturizing agents, alcohol-based products, non-oxidizing and surface tension diminishing agents, and enzyme based compounds.

DISINFECTANTS

Disinfectants are applied as the final step in the cleaning program and disinfection should be performed again if time between completion of disinfectant program and startup exceeds four hours. Different microorganisms vary considerably in their sensitivity or resistance to disinfectants. In order to be effective, a disinfectant must:

- provide a broad spectrum of proven activity
- demonstrate a reduction in viable organisms for which it comes in contact
- work in a broad temperature range and in a variety of pH conditions
- work in the presence of organic material (such as urine, feces, or blood).

Before deciding to use a disinfectant, consider whether a more appropriate method is available. The main use of chemical disinfection is for heat-labile equipment where single use is not cost effective. Some of these items (e.g. bronchoscopes) require high-level disinfection. A limited number of disinfectants (e.g. glutaraldehyde 2%, 6% hydrogen peroxide, 0.2–0.3% peracetic acid) can be used for this purpose. If a sporicidal action is required, immersion in 2% glutaraldehyde for at least 3 hours is required.

Chemical disinfectants must be made up freshly to the correct concentrations according to the manufactures' instructions and discarded after the correct period of time or number of uses. They should be stored in clean bottles with plastic stoppers. When the bottle is empty it should be thoroughly cleaned before re-filling. Partially empty bottles should not be topped up since this will encourage contamination with and multiplication of disinfectant resistant organisms. The object must be thoroughly rinsed with sterile water after disinfection. If sterile water is not available, freshly boiled water can be used. After rinsing, items must be kept dry and well protected from being recontaminated.

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SAFE PROCESSING AND PACKAGING OF FOODS

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Pure, safe, wholesome food is the minimum expectation of today's consumer. The food industry respect different approaches to assure safety food and to gain consumer trusts with implementation of different standards (ISO 9001-2000, ISO 22000, HACCP, IFS, BRC, etc.). The EU legislation put into force a several requirements to assure safety food for protection consumers' health and to help the producers. Food processors must have enough knowledge of safe processing methods and a range of management skills to assure that this knowledge is continuously applied. Properly controlled production, packaging, storage and distribution practices minimize the risk of product spoilage and health risk. In order to provide the required assurance of product safety and stability each food industry must develop procedures, criteria and control system appropriate to their situation. Therefore, the risk of food poisoning is minimized only by designing and implementing appropriate preventive measures. This means understanding its causes, determining the required preventive and remedial measures and managing food handling so that these measures are always used.

The basis for a positive assurance of product safety and stability in each food processing company is a Hazard Analysis Critical Control Point (HACCP) concept. Before any HACCP principles are taken into consideration the processing plant have to be aware of good manufacturing and good hygienic practices – to facilitate in controlled production conditions and in good sanitary manners.

The scope of food processors should be minimal deterioration of raw ingredients, which means to use the minimal thermal degradation of food and mechanical forces during processing and minimum usage of preservation chemicals with choosing suitable packaging materials and methods. Today consumers prefer minimally processed food with no preservatives and a long shelf life. It is very important to know some aspects of microbiological safety in food preservation technologies as pathogens as well as spoilage organisms should always be assumed to be present in every stage of processing and packaging.

RAW MATERIALS

Any unprocessed (raw) ingredients may be expected to contain pathogens or spoilage microbes, e.g. milk, vegetables, meat, dried herbs, etc. For certain ingredients, e.g. which will receive no "kill" step during processing, microbiological monitoring of becoming incoming batches may be carried out more regularly than those undertaken thermal processing to conform that they conform to the specification for that ingredient. So it is very important to obtain high quality raw materials and establish quick methods to determine raw material quality. It depends on the further processing but in many cases microbiological parameters should not only be according to regulation guidelines but many times below them. It is to emphasize that ingredients must be segregated from finished products. Ingredients must be stored so that microbiological growth is either prevented or minimized. Chilling or freezing is a stage which inhibits growth of microbes. It must be strictly controlled as germination and outgrowth of surviving spores should be prevented. For example, for chill ingredients, storage should be at no more than 5 °C and preferably less than 3 °C and the storage time needs to be limited.

When we discuss about HACCP programmes which determine safe food processing from farm to fork, we may not forget about on farm hygiene programmes, when the animals becoming a food are reared. The new legislation (Regulation EC 852/2004) has not declared the principles of HACCP approach into the primary production, but the producers have to establish very closed rules to those that HACCP are, especially from the part of hygienic preprogrammes. Disease challenge by viruses, bacteria and fungi presents a mayor threat to profitable production for instance in poultry production. Biosecurity, in other words reducing the number of infectious organisms in the environment, is the

most effective form of protection. It means that on farm HACCP system, we should establish CCP's which determine control points at which pathogen reduction can take place as a part of a biosecurity programme:

- site security: transport sanitation, wheel dips and foot dips,
- personnel hygiene: protecting clothing, hand hygiene and showering in and out,
- water system: sanitize the drinking water,
- aerial: fogging the house to control airborne pathogens,
- litter: clean litter can be sprayed to reduce infection,
- rodent control: integrated pest management programme and
- terminal disinfection programme of broiler house.

The great challenge for control of hazardous bacteria e.g. thermotolerant *campylobacter* in poultry industry is in water monitoring and proper use of sanitizers for decontamination of biofilm, which has been identified as a possible source for transmission of *campylobacter.* Also other means of controlling this bacterium have been developed and should be implemented into the broiler house in the future. The food processes records must be kept to show that the biosecurity programme is in place and implemented correctly and continuously. Records should be kept of products used, critical limits, cleaning schedules and any corrective action provide documentation for control and for monitoring. Such means show that the control of animals as raw materials entering the slaughtering process is taken care of.

PROCESSING

Since so many processing methods may be applied to foods it would be impractical to detail each in this abstract. The objective during manufacture is to maintain the safety of a food by preventing microbial contamination during packaging. All equipment must be of suitable design and construction and thoroughly cleaned and sanitized before use, which means rigorous application of cleaning schedules as part of GMP. Some food processing requires a manual process. To minimize contamination good personnel hygienic practices must be enforced, but environmental contamination must not be forgotten and these operations should be take place in a "high care" area.

Each process used must have its appropriate controls applied during manufacture. It means that through risk assessment potential hazards are identified and suitable preventive measures undertaken and any critical control point (CCP) taken into control. For example, drying of salami will need the temperature and time of drying to be specified, and the allowed tolerances for that process stage. Pasteurization of liquids will be regulated in the usual way by specified temperature being achieved, correct flow-rate insuring that the liquid is held for the specific time and a flow rate diversion valve fitted to reject under-processed liquid. Pasteurization (cooking) of meat, preserves, etc. requires temperature monitoring of the slowest heating portion of the product batch to insure that the pasteurization temperature is reached. Both cooking and cooling of products must be monitored for temperature and time. Whatever control is applied, instruments used for measurement must be accurately calibrated. Process parameters must be recorded and verified.

Today's customers have become more and more aware of quality in foods and have an increased expectation of consistency in all quality parameters. With the large scale of modern food industry and nationwide distribution of products, a small number of viable organisms have a potential to contaminate large volumes of products, resulting in severe financial losses for the producers affected.

Fruit juices were until recently considered to only be susceptible to spoilage by yeasts, moulds and lactic bacteria. Spoilage by these organisms was prevented by the acidic pH of fruit juices and heat treatment applied during the hot-fill-hold process. Despite these control measures an increasing number of spoilage causes of fruit juices and fruit juices products due to contamination by thermophilic acidophilic bacteria have been reported.

The thermophilic and acidophilic characteristics of *Alicyclobacillus* spp. allow resistence to current pasteurization process and the ability to produce off flavors unacceptable in juices. The first *Alicyclobacillus* spp. was isolated in 1982 from spoiled apple juice and was implied as the causative agent for spoilage. It is currently one of the microorganisms of concern in the fruit juice industry. The most likely source of contamination of fruit juices is from fruit contaminated by soil during harvest or introduced into the manufacturing process by unwashed or poorly washed raw fruit. Water and sugar can also be a source of contamination. Till now acidophilic spore formers were isolated from various environmental

sources including garden soil, forest soil, apple, pear and orange juices. The major off-flavors associated with the spoilage caused by *Alicyclobacillus* spp. is guaiacol which is generally accepted as the predominant metabolite associated with the smoky taints in fruit juice. Spores of *Alicyclobacillus* survive pasteurization conditions of 95 °C for 2 min and grow within a pH range of 2.5 to 6.0. During the heat treatment of foods pathogens and most non-spore forming microorganisms are killed but a heat process sufficient to destroy all the microbial spores will have a harmful effect on the organoleptic quality of the product. Procedures that requires attention is the optimization of the membrane filtration which can assist in recovering low numbers of *Alicyclobacillus* spp. spores and many fruit juice manufactures are currently utilizing this method. The quality of membrane filters differ greatly among different manufactures and this should be carefully assessed for their ability to retain *Alicyclobacillus* spores.

PACKAGING

Packaging materials are of many types, but they serve as a major defence against external hazards. However undesirable interactions between packaging material and food can give rise to potential problems e.g. migration of packaging components or penetration of micro-organism through packages. The importance of hygiene and suitable packaging material and method is very important.

One of the safest methods of packaging is aseptically filling. In aseptic packaging, a product is sterilized, cooled and transported under sterile conditions to a container which has also been sterilized. The product is filled and sealed under sterile conditions into the container. The end product is hermetically sealed for prolonged periods of time at ambient conditions. Depending upon the material, packaging sterilization may be accomplished by heating, use of chemical or radiation. The sterility of filling can be maintained by air filters, positive pressure, vaporized H_2O_2, nitrogen and heat.

The other very used method for liquid product is hot filling. The product is pasteurized, kept hot in holding tank, filled hot into the container or other kind of packaging and sealed/closed. Hot filling of products must be monitored for temperature.

FINAL PRODUCT

It is important to emphasize that once processed and packed food must be avoided to any cross-contamination which might lead to spoilage or health risk. Finished products must be undertaken to some monitoring to verify the appropriate processing and to determine their quality and complying with regulation. It is important that methods should be quick and reliable.

CONCLUSIONS

Despite all activities for food protections the industry meets many problems connected with lack of knowledge in complex food matrix as well as in the cooperation with stakeholders. Based on our discussion we found that there are some very interesting research topics for industry e.g. *Alicyclobacillus* detections in soft drinks, better detection of *Campylobacter* in drinking water in broiler houses and improving microbiological quality of water, biofilms in processing, comparison detections methods for catechins in tea, which can be used to inhibit spoilage bacteria and pathogens, improving safety and shelf-life of egg products in different packaging materials and spore formers e.g. *Bacillus cereus* in complex matrix such as liquid chocolate syrup.

The industry needs better communication with research and scientific institutions to get better knowledge on e.g. unknown or less known microorganisms, package materials and improved risk assessment. Furthermore, the industry needs also better cooperation with food chain stakeholders especially in the primary production to improve trust and reduce costs in controlling materials entering the process plants. The big challenge in the controlling area are quick tests results using reliable method of checking the hygiene and therefore money and knowledge should be put into development of quick detection methods e.g. biochemical methods.

UTILIZATION OF MOLECULAR TECHNIQUES FOR THE IDENTIFICATION OF SALMONELLA FROM FOODSTUFFS

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INTRODUCTION

Detection and identification of pathogens on live food animals and plants as well as in processed food and water is essential for ensuring the safety of food products for human consumption. Foodborne illnesses are defined as diseases, usually either infectious or toxic in nature, resulting from the consumption of food contaminated with pathogenic bacteria, viruses, parasites or their toxins. Such contamination usually arises from improper handling, preparation or food storage. Good hygiene practices before, during, and after food preparation can reduce the chances of contracting an illness. The action of monitoring food to ensure that it will not cause foodborne illness is known as food safety. Recently, reductions in the incidence of some foodborne infections in the USA and European countries have occurred, in part caused by implementation of Hazard Analysis Critical Control Point (HACCP) program aimed at improving food safety through the improved science-based regulation of food production and quality control. HACCP programs are designed to identify and prevent biological (i.e. pathogenic bacteria), chemical (i.e. toxins) or physical (i.e. metals) risks posed by contaminated food.

Most common bacterial foodborne pathogens are *Salmonella spp*., *Listeria monocytogenes*, *Campylobacter spp*. and *Escherichia coli* O157:H7. From further foodborne bacterial threats we should mention *Bacillus cereus, Coxiella burnetii, Shigella spp., Streptococcus spp., Vibrio spp*. and *Yersinia enterocolitica*.

Salmonella is a rod-shaped, Gram-negative enterobacteria that causes typhoid fever, paratyphoid fever and foodborne illness. They are motile and produce hydrogen sulfide. At least 2400 different serovars of Salmonella exist in the nature among these nearly 20 types are responsible from human salmonellosis. They are spread in intestines of different animals including mammals, birds, snakes, turtles and lizards. Hens, chickens, gooses, ducks, pheasants, pigeons, cattle and swine are often infected with salmonella. People becomes infected directly with eggs, meat (poultry, pork, beef), milk and dairy products made from raw milk or from milk that was thermal treated with low temperatures (below pasteurization temperature), ice cream, chocolate, waters, vegetables, fruits, sprout seeds, mussel, shrimp and mollusc. *Salmonella* was named after Daniel Elmer Salmon, an American veterinary pathologist who, together with Theobald Smith first discovered the bacterium in 1885. The scientific classification of *Salmonella* was:

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gamma Proteobacteria
Order:	Enterobacteriales
Family:	Enterobacteriaceae
Genus:	*Salmonella.*

Every year, approximately 40,000 cases of salmonellosis are reported in the United States. Because many milder cases are not diagnosed or reported, the actual number of infections may be thirty or more times greater. Salmonellosis is more common in the summer than winter. Children are the most likely to get salmonellosis. Young children, the elderly, and the immunocompromised are the most likely to have severe infections. It is estimated that approximately 600 persons die each year with acute salmonellosis.

According to EC regulation for safety foods (Commission regulation (EC) No. 2073/2005 of 15. November 2005 on microbiological criteria for foodstuffs)

Salmonella must be absent in 25 g except by minced meat and meat preparations made from poultry meat intended to be eaten cooked, minced meat and meat preparations made from other species than poultry intended to be eaten cooked, mechanically separated meat. In this case Salmonella must be absence in 10 g.

BIOFILM FORMATION BY *SALMONELLA*

Salmonella Enterica, a cause of bacterial gastroenteritis, has reached world wide distribution. As other inhabitants of human and animal gastrointestinal tract have evolved special mechanism for persisting outside of host, surviving the environment and disseminate along the food chain. This benefit consists in the ability to form specialized surfaces structures with adhesives abilities which leads to the formation of highly organized bacterial community, a biofilm. A biofilm is a community of microbes embedded in an organic polymer matrix, adhering to a surface. Its formation is an organized multi-step process which leads to the formation of multi-cellular structure and is a main virulence determinant in many bacterial infections. In general, the specific physiology of biofilms especially slow growth and the barrier function of the extracellular biofilm matrix determine resistance to antibacterial compounds.

Structures involving in multi-cellular behavior and subsequent biofilm formation are composed of protein or of polysaccharides. The most important are adhesives structures; thin aggregative fimbriae (or curlifimbriae), voluminous exopolymer cellulose and newly described capsular polysaccharide. Those structures contribute to the three dimensional structure of biofilm in cooperation with many regulators and biofilm associated proteins. Curli fimbriae and cellulose are the most important component of extra-cellular matrix with protective function. Increasing resistance to antibiotics is an important event in bacterial species especially in bacteria causing infectious diseases in humans and animals. Genes of antibiotic resistance may influence changes in the cell surface properties, such as hydrophobicity and charge, which are relevant to the initial stages of biofilm formation and may contribute to the multi-cellularity behavior.

ISOLATION AND IDENTIFICATION OF *SALMONELLA*

Salmonella is usually isolated on Mac Conkey agar, XLD agar or DCA agar. Because they cause intestinal infections and are greatly outnumbered by the

bacteria normally found in the healthy bowel, primary isolation requires the use of a selective medium, so use of a relatively non-selective medium such as CLED agar is not often practiced. Numbers of *Salmonella* may be so low in clinical samples that stools are routinely also subjected to "enrichment culture" where a small volume of stool is incubated in a selective broth medium, such as selenite broth or Rappaprt Vassiliadis soya peptone broth overnight. These media are inhibitory to the growth of the microbes normally found in the healthy human bowel, while allowing salmonellae to become enriched in numbers. *Salmonella* may then be recovered by inoculating the enrichment broth on one or more of the primary selective media. On blood agar, they form moist colonies about 2 to 3 mm in diameter. They usually do not ferment lactose.

Traditional methods, though reliable and efficient, require several days to weeks before results are obtained. Furthermore, phenotypic properties by which the bacteria are identified may not always be expressed; and when expressed, they may be difficult to interpret and classify. Another disadvantage of traditional methods is that cells which are viable, but otherwise non-culturable cannot be detected, e.g. some stressed *Campylobacter* spp. These methods therefore do not meet requirements of making rapid assessments of microbiological safety

Classical methods of isolation and identification of Salmonella in foodstuffs samples are chosen in accordance with ISO 6579 standard and can require up to 7 and at least 4 d. Method includes primary and secondary selective enrichment of foodstuffs, isolation and identification of isolates with morphological, biochemical and serological tests. Types of Salmonella tests available:

- Conventional culture: Reference method v. optimum method for sample type/purpose
- Rapid Methods: IMS, ELISA, PCR, Conductance Impedance – not fully optimised for faeces and environmental samples in all labs
- Serology: Eg. ELISA for SE, STM, Mix ELISA.

Meanwhile since the conventional methods are time consuming and results can be obtained in 4 to 7, studies are conducted on the development of new and quicker methods. PCR-based methods offer theoretically many advantages over traditional microbiological methods but they should be evaluated prior implementation in routine testing. Appropriate procedure of PCR method is

faster than standard microbiological methods, it has the same relative accuracy, relative sensitivity and relative specificity and means good basis for further inter-laboratory validation.

SEROTYPING AND PHAGE TYPING

Historically, the classification of *Salmonella enterica* into strains has been based on methods such as biotyping, serotyping, antibiotic resistance profiles phage typing, and plasmid typing. *Salmonella* serotypes are defined on the basis of somatic (O) antigens which define the serogroup and flagellar (H) factor antigens, both are present in the cell wall of *Salmonella*. More than 2,400 serovars of *Salmonella* exist and the prevalence of the different serovars changes over time but *S*. Enteritidis and *S*. Typhimurium are the most frequently reported serovars.

The combination of three PCRs enables to completely serotype organisms belonging to the *Salmonella* species. Two multiplex PCRs distinguish the most common first- and second-phase antigens. A third multiplex PCR identify the most common serogroups (O:B; O:C1; O:C2; O:D and O:E). This method was found to be very helpful in the laboratory as an alternative method for typing strains causing outbreaks, and it can be used to supplement conventional serotyping, since it is also applicable to motionless and rough strains.

Phage typing of salmonella has the longest tradition. The first system was established in 1943. The basis of Anderson phage typing system is a collection of *Salmonella* phages which were propagated on particular hosts of the species *S*. Typhimurium. The Anderson typing phages used worldwide today are aliquots of phage stocks prepared decades ago. In 1987, it was described a phage typing scheme for *S*. Enteritidis. The most common phage types of *S*. Enteritidis varies between countries; while phage type (PT) 4 is reported to be dominant in most countries in Western Europe, PT 8 is common in North America and also a few European countries.

IDENTIFICATION OF *SALMONELLA* BY MOLECULAR TECHNIQUES

Alternative rapid methods have been developed to overcome problems such as time- and labor- intensiveness of the traditional approach. Based on varying analysis principles and targets, these methods can be divided into a number of

categories, i.e. modified and automated conventional methods, bioluminescence, cell counting using flow cytometry and immunological methods. The most promising are those methods based on direct analysis of nucleic acid from foodborne pathogens. However, their common disadvantage is inability to distinguish between vital and dead cells. Several methodologies with different underlying techniques are based on nucleic acids (mainly DNA) detection:

- **Nucleic acid hybridization –** Hybridization techniques are based on the formation of double stranded complexes between complementary molecules of either DNA or RNA. DNA or RNA probes in a single stranded form are used for the analysis. The specificity of a hybridization assay is completely controlled by the nucleotide sequence of the detection probe. The first step in these genetic methods is the isolation of nucleic acids, usually followed by labeling (mainly fluorescent). Following hybridization step allows the labeled sample DNA to hybridize to probe DNA. Washing and analysis follow. Microarray technology is the most sophisticated form of nucleic acids hybridization principle.
- **Amplification methods** – Because of the greater sensitivity, DNA-based methods which include an amplification step have become increasingly popular. The most popular method of amplification is the polymerase chain reaction (PCR) technique. In this method, numerous thermal cycles are applied and special thermostable DNA synthesizing enzymes are used, allowing exponential growth of target DNA molecules in each cycle. Starting from a single target DNA or RNA sequence, more than one billion product sequences can routinely be synthesized within few hours. This quantity of DNA can be either visualized on special gel or used in other downstream procedures. Many PCR protocols for the detection of foodborne bacteria and viruses have been described
- **Molecular subtyping methods** can identify different strains within a species, generating data useful for taxonomic or epidemiologic purposes. Nucleic acid-based methods have the advantage over phenotypic identification methods of not being influenced by environmental conditions of the cells, because the nucleotide sequence of the DNA does not change during growth. In the restriction fragment length polymorphism – RFLP technique, DNA is cleaved by restriction

enzymes and the resulting fragments are separated by gel electrophoresis. In Pulsed-field gel electrophoresis (PFGE) restriction enzymes digest the complete genome and large DNA molecules are resolved by continuous reorientation of the electric field during gel electrophoresis. This technique is considered to have superior reproducibility and resolving power when compared to previous and some other similar techniques.

The microarray technology currently dominates gene expression profiling studies, but begins to play an increasingly important role in genomic studies, toxicology research or drug discovery. Additionally, this technology provides valuable attributes for the needs of multiple organisms' identification. The potential of determining several thousands of unique and precisely defined genetic features (such as distinctive features of individual species or organisms) in a single analysis within relatively short time period offers an excellent solution for the needs of simultaneous detection of several pathogens with their varying forms. This potential has not escaped the interest of several research groups and several works have been published on this topic.

Two main types of DNA arrays can be applied in detection of multiple organisms: genomic microarrays and oligochips. Genomic microarrays comprise whole genome, genomic fragments from a cDNA library or ORFs from a strain of microorganism. Genomic microarrays can also be adapted to include genomic DNA from several related strains of a microorganism, referred to as a mixed genomic microarray. Genomic microarrays are useful for pathogen identification based on fingerprinting. Oligonucleotide arrays generally involve oligonucleotides between 18 and 70 nucleotides in length. They are often used for pathogen detection and genomic analysis because these arrays can consist of multiple sequence variants of a target gene allowing for more sensitive detection. Therefore, DNA microarrays can be adapted to combine effective strategies for detecting and identifying pathogenic isolates from food samples, while providing information for the characterization and potential determination of the pathogenicity of the isolate.

The most severe challenge of the detection of pathogens in food systems using microarray analysis is the sample preparation step. Particularly, DNA or RNA extraction from pathogens present in complex food samples as well as detection

of foodborne pathogens present in low levels could be extremely challenging. DNA microarray detection of amplified PCR products was 32 times more sensitive than traditional end-point detection: agarose gel electrophoresis. Thus oligochip combined with PCR amplification could be a sensitive method for detecting foodborne pathogens. For the purpose of food safety surveillance, researchers were able to detect 55 CFU/ml of enterohemorrhagic *E. coli* isolated from chicken rinse.

Despite significant improvements in speed, sensitivity, complexity of obtained information advantage over traditional methods, one of the aspects of microarray analysis with optical detection is its relatively intense procedural complexity. However, this complexity can be optimized. Several works addressing the use of biosensors based on non-optical detection principle have been published. The greatest advantage of biosensor is the omission of labeling step in sample preparation, significantly reducing cost and labeling-associated problems. Furthermore such biochips can be used as simple portable micro-devices for convenient real-time pathogen detection.

ANTIMICROBIAL EFFECTS IN FOOD AND PREVENTION OF CONTAMINATION IN FOOD INDUSTRY

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INTRODUCTION

The most commonly recognized food-borne infections are caused by *Campylobacter jejuni* and *Salmonella* spp. In the past 25 years, *Listeria monocytogenes* has become increasingly important as a food-associated pathogen. Though, an annual incidence of human listeriosis is between two and ten reported cases per million in EU countries. Because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to food-borne illness. *L. monocytogenes* and *C. jejuni* has been shown to adapt to different environmental stress factors, including disinfectants. *Salmonella enteritidis*, *C. jejuni* and *L. monocytogenes* may also form a biolfim which helps to survive in the environment. For that reason, the food industrial hygiene has become important to avoid contamination of raw food and food products with these pathogens.

The aims of the present report are to describe and discuss different antimicrobial methods that prevent the contamination and transmission of food-borne pathogens. The antimicrobial methods could be divided such as physical activities (agents) and chemical agents (e.g. sanitizers, antibiotics).

PHYSICAL ACTIVITIES (AGENTS)

CLEANING OF EQUIPMENT

Since cleaning and sanitizing may be the most important aspect of a sanitation program, sufficient time should be given to outline proper procedures, and parameters. Detailed procedures must be developed for all food-product contact surfaces (equipment, utensils, etc.) as well as for non-product surfaces such as: non-product portions of equipment, overhead structures, shield, walls, ceilings, lighting devices, refrigeration units, heating, ventilation and air conditioning (HVAC) systems, and anything else which could impact food safety.

Equipment cleaning methods can be categorized with regard to cleaning method as follows: a) Mechanical cleaning often referred to as cleaning-in-place (CIP) requires no disassembly or partial disassembly; b) Cleaning-out-of-place (COP) can be partially disassembled and cleaned in specialized COP pressure tanks & c) Manual cleaning requires total disassembly for cleaning and inspection.

THERMAL SANITIZATION

Thermal sanitization involves the use of hot water or steam for a specified temperature and contact time. On the other hand, the effectiveness of an antimicrobial agent depends on its chemical and physical state, treatment conditions (such as water temperature).

Hot-water sanitizing, through immersion (small parts, knives, etc.), spray (dishwashers), or circulating systems, is commonly used. The time required is determined by the temperature of the water. Typical industrial requirements for use of hot water in dishwashing and utensil sanitizing applications specify: immersion for at least 30 sec. at 77 °C for manual operations; a final rinse temperature of 74 °C in single tank, single temperature machines and 82 °C for other machines.

The primary advantages of hot-water sanitization are: relatively inexpensive, easy to apply and readily available, generally effective over a broad range of microorganisms, relatively non-corrosive, and penetrates into cracks and crevices. Hot-water sanitization is a slow process which requires come-up and cool-down time; can have high energy costs; and has certain safety concerns for

employees. The process also has the disadvantages of forming or contributing to film formations, and shortening the life of certain equipment or parts thereof (gaskets, etc.).

FILTRATION

The guarantee of food quality, and particularly food safety, places an onerous responsibility on all food processors. EU Regulations (EN 29000–29004) define this responsibility not only in terms of the quality of the end produce, but also the precautions which must be taken in the preparation and processing of foods to minimize the risk of contamination.

For all food products, and especially for those which are heat sensitive (e.g. milk or egg-based) and subject to minimal heat treatment, all aspects of the process must be rigorously controlled to prevent microbial contamination. This includes not only the processing equipment, but also the processing environment including the air, which may reduce product shelf life, and in certain circumstances, pose a serious hazard to food safety.

While air filtration is now standard practice in food processing environments, significant increases in aerial microbial counts may occur intermittently due to failure to adhere to Good Manufacturing Practices and/or failure of the air filtration system due to design, inadequate maintenance, malfunction, etc.

Frequent and effective monitoring of the air sterility, in the process environment is therefore essential to alert processors of the potential risks, and the possible need for corrective action. However, the lack of reliable quantitative air sampling techniques, coupled to the uncertainty about the behaviour or control of micro-organisms within air filtration systems, present serious obstacles to effective control of processing environments.

FREEZING IN FOOD PRESERVATION

According to EU Directive 89/108, the temperature of quick-frozen foods must be stable and maintained at -18 °C or colder at all points. The temperature of frozen foods must be -12 °C or colder, and this temperature must be maintained. Directive 89/108 requires that after quick-freezing the product temperature must

be -18 °C or colder after thermal stabilization. This is useful wording, as it means that the freezing process can be stopped before the core temperature is -18°C. It is sometimes recommended that for practical purposes the freezing process can be stopped when the core temperature is -10 °C or colder. For most products, this would mean that the average temperature is the same or colder than the stipulated storage temperature.

The freezing process can be considered ended when all points in the food are -18 °C or colder. The length of this stabilization period depends on the type of food (thermal conductivity), how it is packaged, and how the temperature distribution in the food is at the beginning of stabilization. In some countries, there has been legislation requiring that a core temperature of -18 °C must be reached in less than 1 hour for small pieces (steaks), 2 to 6 hours for medium sized pieces, e.g. poultry and roasts, and 24 hours for large packs, e.g. cartons with boned meat. Frozen foods must be stored at -12 °C or colder, quick-frozen foods at -18 °C or colder. Some frozen foods, e.g. beef, broilers, butter, have a fairly long storage life even at -12 °C, while foods such as lean fish require storage temperatures around -28 °C in order to reduce the quality loss and have a long storage life.

The EU Directive 92/1 requires that storage facilities must have installed a temperature recording device. The refrigerating capacity must be sufficient to obtain a maximum difference between evaporator temperature and room temperature of 7 °C. The QFF Directive requires that the temperature of quick-frozen foods must be maintained at -18 °C or colder at all points in the product, with possibly brief upward fluctuations of no more than 3 °C during transport. It is often recommended, e.g. given in the cooking instructions on the label, that if frozen foods must be thawed before cooking, this should be done in a refrigerator.

PASTEURIZATION

Pasteurization is achieved by a treatment involving (i) a high temperature for a short time (at least 72 °C for 15 s); (ii) a low temperature for a long time (at least 63 °C for 30 min); or (iii) any other combination of time and temperature conditions to obtain an equivalent effect, such that the products show, where applicable, a negative reaction to an alkaline phosphatase test immediately after such treatment.

Ultrahigh temperature (UHT) treatment is achieved by a treatment (i) involving a continuous flow of heat at a high temperature for a short time (not less than 135 °C in combination with a suitable holding time) such that there are no viable micro-organisms or spores capable of growing in the treated product when kept in an aseptic closed container at ambient temperature; and (ii) sufficient to ensure that the products remain microbiologically stable after incubating for 15 days at 30 °C in closed containers, or for 7 days at 55 °C in closed containers, or after any other method demonstrating that the appropriate heat treatment has been applied.

PRESSURE

Food preservation using high pressure is a promising technique in food industry as it offers numerous opportunities for developing new foods with extended shelf-life, high nutritional value and excellent organoleptic characteristics. High pressure is an alternative to thermal processing. The resistance of microorganisms to pressure varies considerably depending on the pressure range applied, temperature and treatment duration, and type of microorganism. Generally, gram-positive bacteria are more resistant to pressure than Gram-negative bacteria, moulds and yeasts; the most resistant are bacterial spores. The nature of the food is also important, as it may contain substances which protect the microorganism from high pressure. Despite the introduction of food standards obligatory in EU countries, epidemiologists believe that 75% of food-borne diseases are caused by bacteria.

High pressure is an alternative to thermal processing. The resistance of microorganisms to pressure varies considerably depending on the pressure range applied, temperature and treatment duration, and type of microorganism. The first high pressure processed food products appeared in Japan in the early 1990s. In Europe, high pressure processing (HPP) of foods was rather at the stage of research or pilot production in the last decade. EU legislation included HPP foods in the "novel food" category. EC Novel Food regulation (EC 258/97) has introduced a statutory pre-market approval system for novel foods across the whole of the European Union. Recently, rapid progress of HPP toward commercial exploitation has been achieved, but still the process requires close collaboration between researchers, food and equipment manufacturers, as well as proper financial support.

SONICATION

Ultrasound is energy generated by sound waves of 20,000 or more vibrations per second. Presently, most developments of ultrasonic (sonication) for food applications are nonmicrobial in nature. High frequencies in the range of 0.1 to 20 MHz, pulsed operation and low power levels (100 mW) are used for non-destructive testing. Industrial applications include texture, viscosity and concentration measurements of many solid or fluid foods; composition determination of eggs, meats, fruits and vegetables, dairy and other products; thickness, flow level and temperature measurements for monitoring and control of several processes; and non-destructive inspection of egg shells and food packages. Researchers also listed direct process improvements such as cleaning surfaces, enhancement of dewatering, drying and filtration, inactivation of microorganisms and enzymes, disruption of cells, degassing of liquids, acceleration of heat transfer and extraction processes and enhancement of any process dependent upon diffusion. It is evident that ultrasound technology has a wide range of current and future applications in the food industry.

The bactericidal effect of ultrasound is generally attributed to intracellular cavitation. It is proposed that micro-mechanical shocks are created by making and breaking microscopic bubbles induced by fluctuating pressures under the ultrasonication process. These shocks disrupt cellular structural and functional components up to the point of cell lysis.

PULSED ELECTRIC FIELD

Pulsed electric field (PEF) processing is a non-thermal method of food preservation that uses short bursts of electricity for microbial inactivation and causes minimal or no detrimental effect on food quality. PEF can be used for processing liquid and semi-liquid food products. PEF processing offers high quality fresh-like liquid foods with excellent flavour, nutritional value, and shelf-life. Since it preserves foods without using heat, foods treated this way retain their fresh aroma, taste, and appearance. PEF processing involves treating foods placed between electrodes by high voltage pulses in the order of 20–80 kV (usually for a couple of microseconds). The applied high voltage results in an electric field that causes microbial inactivation.

ELECTROPORATION

This method is used to transform a wide variety of microorganisms and requires a brief exposure to a high-voltage electric field to introduce genetic material into a microorganism. Electroporation is the most popular technique for introducing genetic material in microorganisms because of its simplicity, efficacy and versatility. Electroporation is the phenomenon in which a cell exposed to high voltage electric field pulses temporarily destabilizes the lipid bilayer and proteins of cell membranes. The plasma membranes of cells become permeable to small molecules after being exposed to an electric field, and permeation then causes swelling and eventual rupture of the cell membrane. The main effect of an electric field on a microorganism cell is to increase membrane permeability due to membrane compression and poration.

OHMIC HEATING

Ohmic heating is an advanced thermal processing method wherein the food material, which serves as an electrical resistor, is heated by passing electricity through it. Electrical energy is dissipated into heat, which results in rapid and uniform heating. Ohmic heating is also called electrical resistance heating, Joule heating, or electro-heating, and may be used for a variety of applications in the food industry. Like thermal processing, ohmic heating inactivates microorganisms by heat. Additional non-thermal electroporation type effects have been reported at low-frequency (50–60 Hz), when electrical charges can build up and form pores across microbial cells however, it is not necessary to claim such effects since heating is the main mechanism. The shelf life of ohmically processed foods is comparable to that of canned and sterile, aseptically processed products. In various countries, including Italy, Greece, France, ohmic heating has been used to produce a low-acid particulate product in a can, as well as pasteurized liquid egg.

CHEMICAL AGENTS

SANITIZERS

The selection of a sanitizer depends on the type of equipment to be sanitized, the hardness of the water, the application equipment available, the effectiveness of the sanitizer under site conditions, and cost. Sanitizing compounds which

contain phenols impart strong undesirable odors and flavors to foods and should not be used. Thorough cleaning is essential before using a sanitizer. Sanitizers are less effective when food particles or dirt are present on equipment surfaces. Typical disinfecting agents used in the food industry include quaternary ammonium compounds (QACs), chlorine, alcohols, hydrogen peroxide, peracetic acid and iodofors.

Legislation concerning disinfectants include Directive 98/8/EC of the European Parliament and of the Council on the placing on the market of biocidal products. Member States had to transpose the rules before 14 May 2000 into national law. The Biocidal Product Directive aims to harmonise the European market for biocidal products and their active substances and to protect humans, animals and the environment (http://ec.europa.eu/environment/biocides/index.htm).The new REACH Regulation (EC) No 1907/2006 of the European Parliament and of the Council and Directive 2006/121/EC of the European Parliament and of the Council were published in the Official Journal on 30 December 2006. The aims of the proposed new Regulation are to improve the protection of human health and the environment while maintaining the competitiveness and enhancing the innovative capability of the EU chemicals industry. REACH will also give greater responsibility to industry to manage the risks from chemicals and to provide safety information on the substances. REACH will enter into force on 1 June 2007 (http://ec.europa.eu/enterprise/reach/index_en.htm).

Many commonly used disinfectants have been shown to be effective against *L. monocytogenes* in suspension. Other factors affecting on efficacy of disinfectants against *L. monocytogenes* include e.g. the concentration and effect time as well as temperature and pH of the use solution. *L. monocytogenes* strains can vary in resistance against disinfectants. *L. monocytogenes* has been shown to adapt to different environmental stress factors, including disinfectants. The adaptation may occur especially when the disinfectant is present in the environment in sub lethal amounts. Differences in the susceptibility and adaptive response of strains to disinfectants have been observed, and it has been suggested that such differences could influence the survival of the strains in the food processing plants. Generally, the recommendations of use-concentration of disinfectants should be followed. However, these may not always be sufficient against *L. monocytogenes*.

Future investigations include further studies on mechanisms of adaptation and differences between strains. Besides disinfectants, other technologies for reducing use of water and chemicals in disinfection in food plants should be investigated.

ANTIBIOTICS

Antibiotics are chemical substances, either produced naturally by micro-organisms or manufactured synthetically, that are lethal to other bacteria. Antibiotics are used for the treatment of bacterial infections in both humans and animals. The emergence of antibiotic resistance as a serious problem in human medicine has prompted concerns about the public health implications of antibiotic use in agriculture. Antimicrobial resistance has emerged among *Campylobacter* mainly as a consequence of the use of antimicrobial agents, especially fluoroquinolones, macrolides, and tetracyclines in food animal production. In the recent study a high number of *Campylobacter* isolates from Estonian poultry had increased antimicrobial resistance to ciprofloxacin, tetracycline. The use of the antibiotics as growth promoting agents is be forbidden in EU countries since January 2006 (Regulation (EC) No 1831/2003 of the European Parliament and of the Council).

FUTURE NEEDS

New technologies are needed for surveillance of food-borne disease and food monitoring. These include typing pathogens, different *in vitro*, animal and clinical testing. New research and development are required in food industry such as application of antimicrobial surface materials and green technologies (enzymes).

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APPENDIX 2: SEMINAR PROGRAMME

Monday, January 22, 2007

9.00–9.15	Coffee
9.15–9.30	Welcome
9.30–10.00	Biofilm formation in food processes; Gun Wirtanen, VTT, Finland
10.00–10.30	Microbial ecology in manufacturing paper-based packaging materials for use in food industry; Laura Raaska, VTT, Finland
10.30–11.15	The relevance of psychrotropic spoilage microbes in food hygiene; Elina Vihavainen, University of Helsinki, Finland
11.15–12.15	Lunch
12.15–13.00	Enteric pathogens prevalence in food products and mechanisms of suppression by probiotic lactobacilli; Marika Mikelsaar, University of Tartu, Estonia
13.00–13.45	Classification and differentiation of Salmonella sp. using various DNA based techniques; Ivan Rychlik, VRI, Czech Republic
13.45–14.15	Coffee and tea
14.15–15.00	Composition of intestinal microflora in health and disease and its association with food-borne diseases; Marika Mikelsaar, University of Tartu, Estonia
15.00–17.30	Group works incl at least 30 min for preparation of presentations
16.00–17.30	Coffee and tea
17.30–18.30	Group work presentations
19.00–21.00	Dinner

Tuesday, January 23, 2007

9.00–9.30	Effect of maintenance routines in food processing on production hygiene especially on Listeria contamination routes; Kaarina Aarnisalo, VTT, Finland
9.30–10.00	Hygienically integrated systems in food processing to avoid microbiological contamination; Lotte Dock Steenstrup, BioCentrum-DTU, Denmark
10.00–10.30	Coffee
10.30–11.00	Methods for evaluation of process hygiene; Satu Salo, VTT, Finland
11.00–12.00	Molecular techniques and microscopy in bacterial detection and typing; Johanna Maukonen, VTT, Finland
12.00–13.00	Lunch
13.00–14.00	Food safety legislation and food control systems according to the new EU food law; Tiina Saron, Estonian Dairy Association, Estonia
14.00–14.30	Coffee
14.30–15.30	Air microbiology; Berit Reinmüller, KTH, Sweden
15.30–15.50	Concluding remarks

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Author(s)
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Title

MICROBIAL CONTAMINANTS & CONTAMINATION ROUTES IN FOOD INDUSTRY

Abstract

Microbes able to establish and develop in the processing environment and on food products pose a potential problem in terms of food safety and quality. Initially it is the capability to adhere to a surface that gives an advantage towards the majority of microbes being present. Other important capabilities are competitive growth and long survival. Although pathogenic microbes rarely cause the spoilage of foodstuffs (due to their slow or non-existent growth in foods at low temperatures) they and their toxic secondary metabolites naturally form a health risk to the consumer. To guarantee the safety of foodstuffs microbial contamination must be detected and traced as early as possible and also at low levels. In addition, the safety risks related to the microbial contamination must be evaluated as accurately as possible. To establish corrective (and for the future also preventive) actions exact knowledge on the process hygiene level of the production plant is needed. Characteristics of important contaminants, contamination routes and factors enhancing microbial contamination of process line are important items covered in this *Microbial contaminants and contamination routes in food industry* -seminar by international lecturers and participants who have provided research studies and group work material published in this seminar proceedings.

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