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3rd Seminar arranged by SAFOODNET – Food Safety and Hygiene Networking within New Member States and Associated Candidate Countries

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SAFOODNET – FOOD SAFETY AND HYGIENE NETWORKING WITHIN NEW MEMBER STATES AND ASSOCIATED CANDIDATE COUNTRIES; FP6-022808-2006

TALLINN, ESTONIA, MAY 4–6, 2009

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tel. växel 020 722 111, fax 020 722 4374

VTT Technical Research Centre of Finland
Vuorimiehentie 5, P.O. Box 1000, FI-02044 VTT, Finland
phone internat. +358 20 722 111, fax + 358 20 722 4374

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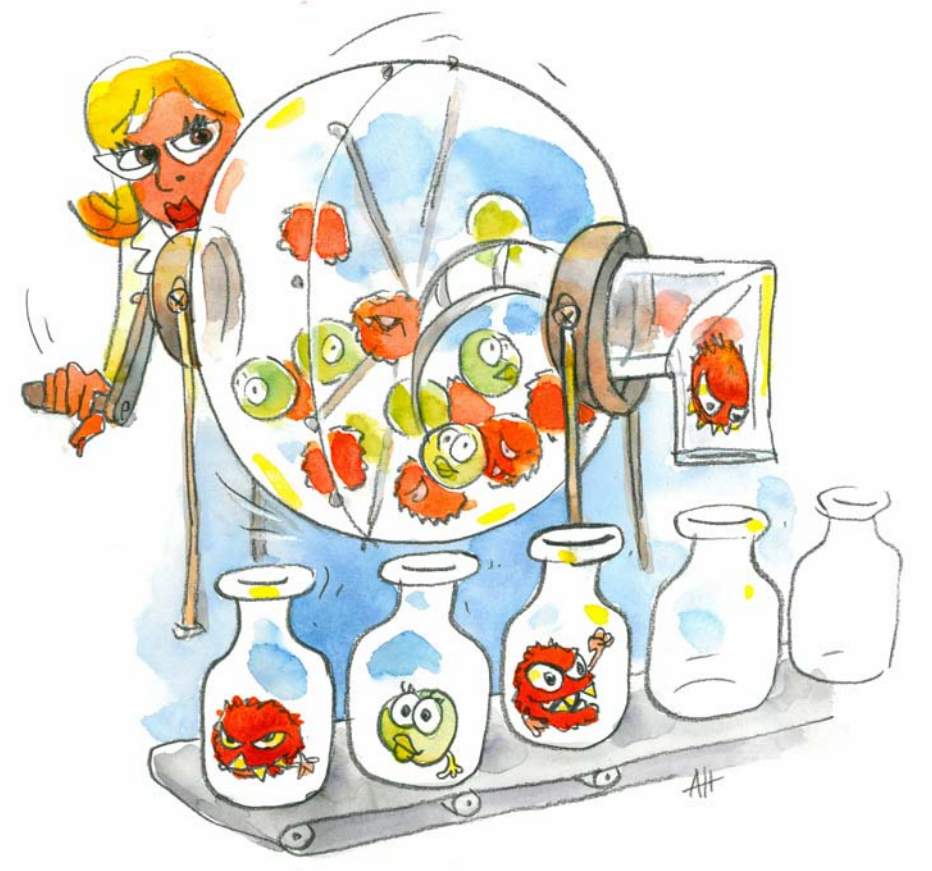
PREFACE

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 pilot actions, seminars, and workshops on process hygiene and product safety were carried out in Cyprus, Czech Republic, Denmark, Estonia, Finland, Romania Slovenia and Turkey. Interested researchers and SME representatives from other new EU countries and ACCs are encouraged to participate in the activities. The objectives of SAFOODNET were to: 1) disseminate knowledge from national and international food safety projects in open seminars, workshops, practical exercises, RTD activities and pilot actions possibly resulting in new research projects especially with SMEs; 2) establish an expert group in which authorities, scientists, industrial representatives strengthen existing networks and identify specific needs for future food safety RTD activities and 3) bridge food safety networks within the new EU, fostering scientific co-operation and knowledge transfer.

The final seminar on *Risk management by hygienic design and efficient sanitation programs* focused on preventive activities e.g. factory layout, equipment design, choice of surface materials and sanitation programmes. The present most efficient means for limiting the growth of microbes and microbial biofilm formation are equipment design, choice of surface materials and use of suitable cleaning and disinfectant programmes. Poorly designed sampling valves can destroy entire processes or give incorrect information due to biofilm formation at measuring points. Dead ends, corners, cracks, crevices, gaskets, valves and joints are vulnerable points for biofilm accumulation. Cleaning should be based on systematic planning, because accumulation of particulates and also cells occurs where cleaning for any reason is inappropriate. Inadequately cleaned and sanitized surfaces can act as the source of contamination within the process. Disinfection is also required in food plants where wet surfaces provide favourable conditions for microbial growth. These aspects including EU legislation and standardisation work were covered in *this third open seminar held in Tallinn (Estonia) May 4–6, 2009.* An overview of prior achievements in the project was given on the third day. More information, please, see the project homepage http://safoodnet.vtt.fi.

INVITED LECTURES



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FOOD LEGISLATION IN THE EU

Külli Rae
Food and Veterinary Department, Ministry of Agriculture of Estonia, Estonia

Over the past few decades, food crises and scientific or technical progress, coupled with the globalization of markets has put food safety assurance systems to the test and presented them with new challenges. For example, increased use of food additives, novel combinations and foods which have not traditionally been used as food have now reached the market. Our diet is enriched by more and more exotic foods imported from third countries. However, due to the contamination of the environment, more attention should be paid to the contaminants level in food. Therefore, agreements in the form of legislation must be put in place in order to ensure that safe and healthy food reaches consumers. Food safety in the European Union is mostly regulated at Community level.

European legislation is made up of Directives and Regulations which are mandatory legal acts for every Member State and must be implemented at the Member State level. Directives define the result that must be achieved but leave to each Member State the choice of form and methods to transpose the directive into national laws, usually within 2–3 years after adoption. Regulations are binding in their entirety and automatically enter into force on a set date in all Member States. Decisions are binding on those parties to whom they are addressed. Recommendations and opinions have no binding force. Amendments to existing EU legislation are usually published in new and separate Directives and Regulations. However, food law can also come into force as a result of European Union Case-Law (Cassis de Dijon case in 1979). Case-law includes judgments of the European Court of Justice and of the European Court of First Instance in response to referrals from the Commission, national courts of the Member States or individuals. In addition, certain aspects which are not regulated in detail at EU level may be handled differently in different Member States in form of national law. The first legislative instruments covering food safety issues at the Community level were the Commission Directive in 1962 on food colourings, in 1963 one on preserving agents and in 1964 on intra-Community trade in cattle and pigs and in fresh meat. The legal basis for these directives was article 100 of the 1957 Treaty of Rome concerning establishment or functioning of the common market. Other possible articles as a legal basis are article 37, 95, 133 and 152(4)(b) of the Treaty.

Today the EU food law is following the integrated approach “from farm to table” and legislation covers all aspects of the food chain: primary production, processing, and transport, distribution through to the sale or supply as well. The main areas are the food safety general provisions, product labelling and packaging, veterinary checks and animal health rules, hygiene of food, animal nutrition, plant health checks, contamination and environmental factors, international market, specific themes as GMOs, BSE. Although the EU legislative Acts cannot be ranked in importance, the following two Acts can be regarded as most important and widely applicable:

- Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety and
- Regulation (EC) No 882/2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules.

These Regulations establish legal framework common definitions, including definitions of food and official control, and lay down business operators and competent authority’s responsibilities and rights. In contrast to the earlier approach, all business operators must ensure the safety of food within their business at all time and not market food that is unsafe. Although primary responsibility for food safety lies with business operators, national competent authorities and control bodies have the task of ensuring that law is respected by operators properly. At the same time, consumers have the right to safe food and to information which is usable by diet choices. The Regulation 178/2002 establish first time in the Community food law the principles of risk analysis and European Food Safety Authority as an independent scientific risk assessment and communication body. The European Food Safety Authority works in close cooperation with national scientific agencies and institutions (Scientific Panels, Art 36 Cooperation) providing scientific advice on know or emerging risk issues to the European Parliament, Commission Member States and public. In case, the scientific information concerning risk is incomplete or data are nor sufficient for risk assessment, may do the Commission and Member States as risk managers use precautionary principle as an option to the risk assessment. The last key issue in this regulation is crisis management, including network of contact points of the Commission and the Member States in case of to human health deriving from food (Rapid Alert System, RASFF). Other legal Acts regulate more specific areas such as the hygiene or contaminants of food of animal origin.

Over the last fifty years European food legislation has been moved from the single legal acts to the modern legal framework covering various aspects food chain. Legislation must be based on risk assessment prioritizing safety issues. In addition to the health and environmental aspects, elements such as the producers, processors and trade operators must also be taken into account. National scientists and competent authorities, as well as the European Food Safety Authority are involved in the assessment of known or emerging risks. The aim of the food safety assurance system is to ensure that the food produced in one Member State is safe for consumers in all Member States. The same approach is relevant for imported food. It should meet health requirements at least equivalent those set by the European Community for its own production. Following general principles described above the Community legislation comprises a set of rules whose purpose is to ensure that food in the EU is wholesome and meets a high level of food safety. More detailed summaries of EU legislation, including food law are available in the EU homepage http://europa.eu/scadplus/leg/en/s80000.htm. All legal acts are published in the Official Journal of the European Union and are freely accessible in EURLEX portal http://eur-lex.europa.eu/en/index.htm.

FOOD CHAIN MANAGEMENT FROM THE PERSPECTIVE OF SUSTAINABILITY, PRODUCT SAFETY AND QUALITY

Tiina Saron
Piimaliit, Tallinn, Estonia

This paper aims to present a short overview about Quality Assurance Systems in food chain and food chain management from the perspective of food safety and quality. The objective is to describe that besides food safety issues the quality of agricultural products has been brought up to the focus of society. On one hand the industry has to fulfil consumer expectations, on the other hand increasing globalization and concentration of retail sector affects the food sector as well. The food companies try to differentiate the products by stressing the value of attributes such as tradition, origin, culture and culinary heritage. It leads to an increase of interest to introduction of quality assurance schemes and implementation of different quality marks. In addition, the European Commission has initiated a discussion on agricultural product quality policy, which means that these issues are important from the perspective of agricultural policy as well. The paper is structured as follows: a short overview of quality assurance schemes, benefits, disadvantages and costs of QAS and the Estonian case.

INTRODUCTION

The production of food has expanded over the frontier of one particular country. More often we speak about global village and common environment. The main issues are healthy and safe food, environmental-friendly food packaging and hygiene systems, animal welfare, sustainable production under minimized impact on soil, air and water. Food safety and quality have the greatest importance for the consumers and their sensibility in that respect is very high. The safety of foodstuffs has the absolutely highest priority for food producers but the demands of the market are diverse and multiplying. Consumers with growing incomes are demanding taste, tradition and authenticity in their food as well as the application of higher animal welfare and environmental standards.

From the industry side it is a very big challenge and very much related to the cost of production. The industry must implement a new food safety requirement and keep food safety risks under the control. Increasing globalization and concentration process in retail sector affect the food processing enterprises as well. In addition to the food safety, the retailers require implementation of their own quality schemes. To cope with these developments food companies try to differentiate by stressing the value of attributes such as tradition, origin, culture and culinary heritage. It leads to an increase of interest to introduction quality assurance schemes and implementation of different quality marks.

SHORT OVERVIEW OF QUALITY ASSURANCE SCHEMES

In recent years the concept on food quality has changed and these changes will continue. Quality consists of two dimensions: objective and subjective dimension. The objective quality includes the physical and chemical characteristics integrated in the product and is naturally in the responsibility of food technologists. Subjective quality is based on the consumer's perception. Food producers will be competitive when being able to successfully link the two dimensions and to translate consumer's quality requirements (subjective quality) into physical product characteristics (objective quality).Most quality assurance systems are based on the quality management principles of ISO 9000/ISO 22 000 and the HACCP-concept. Some are following the Good Agricultural Practice.

Worldwide QAS. The Codex Alimentarius is enclosing all quality systems and standards. The Codex Alimentarius Commission aims the harmonization of all national food laws in order to reduce trade barriers and to improve the free and fair trade between all nations.

European QAS. The International Organization for Standardization (ISO) and the Codex Alimentarius Commission developed the ISO 22 000 standard, which was implemented in September 2005. ISO 22 000 incorporating the ISO 9000 standard and the HACCP concept in one standard and it meant to be applied to all types of organizations within the food supply chain, independently or integrated into other management systems.

On the enterprise level, both horizontally and vertically oriented quality systems are applied. Horizontally oriented quality systems are developed through retailer initiatives such as British Retail Consortium (BRC). The main focus of that type of schemes is on process quality. Vertically oriented quality systems focus on product liability. (Label Rouge, Little Red Tractor, Approved Estonian Taste, etc.).

The HACCP-concept, Good Manufacturing Practice (GMP) and Good Hygiene Practice form the centre of the quality assurance schemes. The main focus of the HACCP-concept is on product safety, whereas the main focus of the ISO standards is indirectly on the process quality. The same difference is between horizontally oriented QAS and vertically oriented QAS.

Good Agricultural Practices and cross-compliance

Dairy farmers are in the business of producing food. The concept of Good Agricultural Practices has evolved in the recent years in the context of a rapidly changing and globalizing food economy. Cross -compliance is a central part of reformed CAP. Cross -compliance means that farmers have to respect a set of standards to avoid cuts in payments from the European Union. These standards cover protection of the environment, public, animal and plant health, animal welfare and the maintenance of the land in good agricultural and environmental condition. Cross -compliance has the dual aims of helping to make farming more sustainable and making the CAP more compatible with the expectations of consumers and taxpayers. It is made up of two components, the "statutory management requirements" (SMRs) and "good agricultural and environmental condition" (GAEC). The SMRs are made up of 19 laws, while Member States have to define minimum standards for GAEC based on an EU framework. Generally speaking the quality issues are going to be an important one in the agricultural sector as well.

Benefits, disadvantages and costs of QAS

The main aim of a QAS is the assurance of the quality of the food product through improved process and product quality. The results are reduced costs because of optimizing the process organization and better understanding and controlling of production process. Increased traceability over the food chain, product liability, easier fulfilment of the EU- regulations are important results as well. The disadvantages for implementing even basic quality standards are high costs of external certification and high administrative efforts. There is no guarantee that these costs will be refunded through higher product prices.

According to the study carried out in Germany in 2003 the main costs were related to documentation of the quality management (23%), followed by process analyses of quality assurance requirements (20%) and inspections of raw materials (20%). In that study 80% of the responding enterprises followed the HACCP-system and more than

60% applied the ISO 9000 standards. Unfortunately there are no studies concerning quality assurance costs in Estonian food processing enterprises.

Estonian case

Until 1992 Soviet quality standards were applied in Estonia. These standards contained both quality and safety requirements which were mandatory for food companies. In practice it was almost impossible to introduce companies' own standards and the product development was very weak. After Estonia regained independence in 1991 the system has been changed and as a result, Estonia introduced the standards laid down by the international standard setting organizations (FAO (Codex Alimentarius). Food safety issues arose rapidly and the first food safety regulations were drafted. In the beginning it was extremely difficult to explain, what is the HACCP-concept and what are the differences between ISO standards and the risk analyses. Eventually, from the state point of view we skipped the quality issues and focused only on food safety issues. As a result the food companies dealt with quality schemes by themselves, there were no support (including attention) from the state. In the open market conditions Estonian food companies operated in conditions of unfair situation.

Estonian quality labels

The Estonian government, respectively the Estonian Chamber of Agriculture and Commerce, established the first Approved Estonian Taste quality label in 1997 – the clover leaf label. The clover leaf label is given to food produced in Estonia, which successfully passed laboratory and sensory evaluation. The origin of the raw material is not considered for the awarding of the label.

In 2000, a second Approved Estonian Taste label was introduced, denoting the Estonian origin and high quality (swallow-mark). This label is granted to products which are made of raw material of 100% Estonian origin and which have passed the respective laboratory and sensory evaluation. The producers fulfilling the requirements of the labels are allowed to use the label for two years, during which random after-control is conducted. After two years the firms can renew the contract of the use of the labels. According to the EU-rules, the clover label was re-designed for universal use, so that is not solely open for Estonian producers. Today 126 food products can bear the "swallow-label", out of that 67 are milk products. Clover label is not so popular and the enterprises can use it for 58 food products, including only 4 dairy products.

Estonian consumer's point of view

Over the years the Estonian Market Research Institute has carried out different market research studies, including consumer's awareness of quality marks, their interest and willingness to buy these quality labelled products. According to a study, the awareness of the quality labels has risen and the Estonian labels are more known compared to the EU-labels. The most known label is the "swallow-mark" (known to 95% of the respondents), followed by the "clover-label" (known to 71% of the respondents). In 2008 awareness of swallow-label has risen most.

The awareness of the EU quality labels is very low, the most known label is the EU organic label (known to 13% of the respondents). The PDO and PGI are almost unknown. One of the reasons would be the fact that Estonian products are not classified as EU- quality products. In 2004 two Estonian dairies submitted the application to a PGI but it has been very difficult to fulfil the respective conditions. Comparing our country with the countries in Southern- Europe, there are very different natural conditions and food culture is also different.

Consumer's recognition of the quality of the quality-labelled products and willingness to pay for these products were also studied. According to the study 47% of respondents considered the quality of special labelled products were better, 22% said that there was no difference and 31% didn't have any opinion. For conclusion, we might say that there is a difference in the quality of products and the consumers can recognise it. Concerning the consumers' willingness to pay more for quality labelled products, 32% of respondents were ready to do so and 68% were not. 44% of the consumers are ready to pay more for the Estonian products, but only 14% for the EU quality products. These figures correspond rather well with the figures for awareness of the respective labels.

Functional foods

The interest in the food we eat has never been greater than it is today. Many consumers are interested in knowing how food might affect their health. The relationship between diet, health and lifestyles has become a top priority issue for many EU governments. According to the study made by the Estonian Economic Research Institute 23% of the respondents have consumed functional food very often, 31% rarely, 13% never and 33% maybe, but they can't differentiate the origin of the product.

Concerning the consumer's opinion on functional foods, 47% of the respondents don't have any opinion about the issue, 20% respectively don't recognize difference compared to ordinary products, and only 12% of the respondents regard functional foods as tasty and healthy products. Based on the results of the survey we can say that Estonian consumers' knowledge on functional foods is quite low. This subject needs to be addressed as soon as possible. There is no negative mentality which needs to be changed. It had better to increase the knowledge of consumers' right now.

Further developments

The European Commission has launched the document "Communication from the Commission to the Council, the European Parliament and the European Economic and Social Committee on agricultural product quality policy". By today the consultation process of stakeholders is over. In the light of these consultations and examinations of the current measures, the Commission has identified three main issues to be addressed in developing agricultural product quality policy, namely to improve communication in food chain about the qualities of agricultural products, to increase the coherence of EU agricultural product quality policy instruments and to make it easier for farmers, producers and consumers to use and understand the various schemes and labelling terms.

The Commission's next steps are to develop the guidelines for the good functioning of certification schemes, including specific criteria for any new EU schemes and develop the EU marketing standards within the single Common Market Organisation. In addition, existing schemes and marketing standards should be simplified and clarified.

European Food Sustainable Consumption and Production Round Table (Food-SCP)

The official launch of the Food-SCP will be on May 6th 2009. The main founding organizations in the Food-SCP are the Confederation of the Food and Drink Industries in the EU (CIAA) and the European farmers' organization (COPA/Cogeca). The objective of Food-SCP is to make the European food chain sustainable. Its activities will address not only to the EU policy initiatives but also to the international efforts (UNEP)

There are three main topics in the management of environmental sustainability along the European food chain – identification of scientifically reliable and uniform environmental assessment methodologies for food and drink products categories,

identification of suitable communication tools to consumers and other stakeholders and promoting and reporting on continuous environmental improvement along the entire food supply chain.

CONCLUSIONS

The food chain is a complex infrastructure of networks involving supplies, farmers, processors and retailers as well as customers. Food chain management deals with the organisation, management and coordination of the complex processes and interactions throughout the food value chain. The concept of the food quality has changed and these changes are going to continue. In addition to food security, safety and availability the consumers are demanding taste, tradition and authenticity in their food as well as the application of higher animal welfare and environmental standards. In coming years the wholesomeness and nutrition are going to be in the focus of public interest and the food industry has to be prepared for that.

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INTERNATIONAL (MICROBIOLOGICAL) STANDARDIZATION

Pauline Kalkman* & Rijkelt Beumer
WUR, Wageningen, The Netherlands
* NEN, Delft, The Netherlands

The International Standardization Organization (ISO) is a network of the national standards institutes of 159 countries (e.g. NEN (NL), BSI (UK), AFNOR (F), and DIN (D)) one member per country, with a Central Secretariat in Geneva, Switzerland, that coordinates the system. ISO is a non-governmental organization that forms a bridge between the public and private sectors. On the one hand, many of its member institutes are part of the governmental structure of their countries, or are mandated by their government. On the other hand, other members have their roots uniquely in the private sector, having been set up by national partnerships of industry associations. Therefore, ISO enables a consensus to be reached on solutions that meet both the requirements of business and the broader needs of society.

WHY CONSENSUS

Because ISO standards are voluntary agreements, they need to be based on a solid consensus of international expert opinion. Consensus, which requires the resolution of substantial objections, is an essential procedural principle. Although it is necessary for the technical work to progress speedily, sufficient time is required before the approval stage for the discussion, negotiation and resolution of significant technical disagreements. “Consensus” is officially defined as “general agreement, characterized by the absence of sustained opposition to substantial issues by any important part of the concerned interests and by a process that involves seeking to take into account the views of all parties concerned and to reconcile any conflicting arguments”. The definition notes, “Consensus need not imply unanimity”. An International Standard is the result of an agreement between the member bodies of ISO. It may be used as such, or may be implemented through incorporation in national standards of different countries.

DEVELOPMENT OF ISO STANDARDS

ISO launches the development of new standards in response to sectors and stakeholders that express a clearly established need for them. An industry sector or other stakeholder group communicates its requirement for a standard to one of ISO's national members. The latter then proposes the new work item to the relevant ISO technical committee developing standards (Figure 1) in that area. New work items may also be proposed by organizations in liaison with such committees. When work items do not relate to existing committees, proposals may also be made by ISO members to set up new technical committees to cover new fields of activity.

To be accepted for development, a proposed work item must receive the majority support of the participating members of the ISO technical committee which, amongst other criteria, verifies the "global relevance" of the proposed item – this means that it indeed responds to an international need and will eventually be suitable for implementation on as broad a basis as possible worldwide.

DETAILED STAGES IN INTERNATIONAL STANDARD DEVELOPMENT

International Standards are developed by ISO technical committees (TC) and subcommittees (SC) by a six-step process (Figure 1):

Stage 1: Proposal stage
Stage 2: Preparatory stage
Stage 3: Committee stage
Stage 4: Enquiry stage
Stage 5: Approval stage
Stage 6: Publication stage.

Stage 1: Proposal stage – The first step in the development of an International Standard is to confirm that a particular International Standard is needed. A new work item proposal (NP) is submitted for vote by the members of the relevant TC or SC to determine the inclusion of the work item in the programme of work. The proposal is accepted if a majority of the P-members of the TC/SC votes in favour and if at least five P-members declare their commitment to participate actively in the project. At this stage a project leader responsible for the work item is normally appointed.

Stage 2: Preparatory stage – Usually, a working group of experts, the chairman (convener) of which is the project leader, is set up by the TC/SC for the preparation of a working draft. Successive working drafts may be considered until the working group is satisfied that it has developed the best technical solution to the problem being addressed. At this stage, the draft is forwarded to the working group's parent committee for the consensus-building phase.

Stage 3: Committee stage – As soon as a first committee draft is available, it is registered by the ISO Central Secretariat. It is distributed for comment and, if required, voting, by the P-members of the TC/SC. Successive committee drafts may be considered until consensus is reached on the technical content. Once consensus has been attained, the text is finalized for submission as a draft International Standard (DIS).

Stage 4: Enquiry stage – The draft International Standard (DIS) is circulated to all ISO member bodies by the ISO Central Secretariat for voting and comment within a period of five months. It is approved for submission as a final draft International Standard (FDIS) if a two-thirds majority of the P-members of the TC/SC are in favour and not more than one-quarter of the total number of votes cast are negative. If the approval criteria are not met, the text is returned to the originating TC/SC for further study and a revised document will again be circulated for voting and comment as a draft International Standard.

Stage 5: Approval stage – The final draft International Standard (FDIS) is circulated to all ISO member bodies by the ISO Central Secretariat for a final Yes/No vote within a period of two months. If technical comments are received during this period, they are no longer considered at this stage, but registered for consideration during a future revision of the International Standard. The text is approved as an International Standard if a two-thirds majority of the P-members of the TC/SC is in favour and not more than one-quarter of the total number of votes cast are negative. If these approval criteria are not met, the standard is referred back to the originating TC/SC for reconsideration in light of the technical reasons submitted in support of the negative votes received.

Stage 6: Publication stage – Once a final draft International Standard has been approved, only minor editorial changes, if and where necessary, are introduced into the final text. The final text is sent to the ISO Central Secretariat which publishes the International Standard.

VOTING

For a document to be accepted as an ISO International Standard, it must be approved by at least two-thirds of the ISO national members that participated in its development and not be disapproved by more than a quarter of all ISO members who vote on it.

ISO TECHNICAL COMMITTEE 'FOOD PRODUCTS'

There are different subcommittees within the Technical committee Food products (Figure 2). The secretariat of this Technical Committee has since a couple of years been allocated to AFNOR (France) and ABNT (Brazil).

SUBCOMMITTEE (SC) 9 – MICROBIOLOGY

The scope of SC9: Standardization in the field of human and animal foodstuffs as well as animal and vegetable propagation materials, in particular terminology, sampling, methods of test and analysis, product specifications and requirements for packaging, storage and transportation. Excluded from its scope are products covered by ISO/TC 54, *Essential oils* and ISO/TC 93, *Starch (including derivatives and by-products)*. SC 9 has 7 Working Groups (WG):

SC9 WG1: Meat and meat products,
SC9 WG2: Statistics
SC9 WG3: Method validation
SC9 WG4: Proficiency testing
SC9 WG5: Culture media (Joint WG with ISO 147/SC 4 (water microbiology)
SC9 WG6: *Cryptosporidium* and *Giardia*
SC9 WG7: General requirements & guidance for microbial examinations (ISO 7218).

CEN – EUROPEAN STANDARDIZATION

CEN's 30 National Members work together to develop voluntary European Standards (ENs), with a CEN Management Centre (CMC) in Brussels, Belgium, that coordinates the system. These standards have a unique status, since they also are national standards in each of its 30 Member countries. With one common standard in all these countries, and every conflicting national standard withdrawn, a product can reach a far wider

market with much lower development and testing costs. ENs help build a European Internal Market for goods and services and to position Europe in the global economy.

COOPERATION BETWEEN ISO AND CEN

The so-called Vienna agreement is a technical cooperation between ISO and CEN. The main objective of the Vienna Agreement is to provide a framework for the optimal use of resources available for standardization work, and to provide a mechanism for information exchange between ISO and CEN to increase transparency of work ongoing in CEN to ISO members and to avoid an overlap of the work.

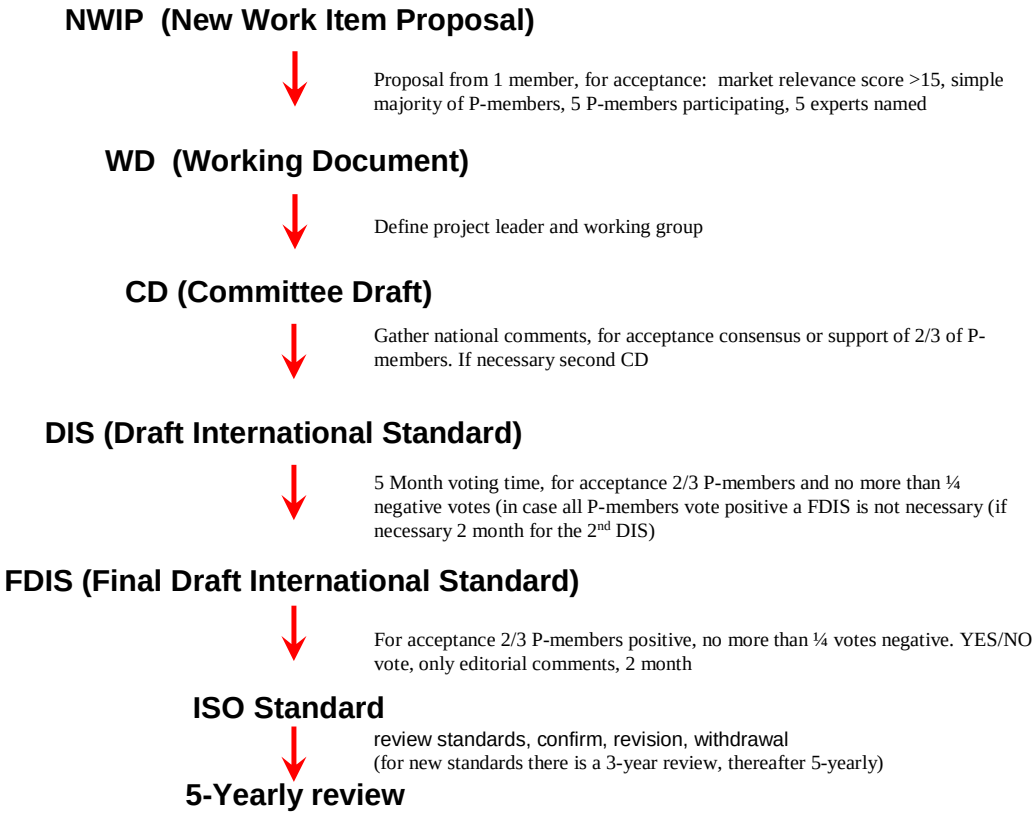

Figure 1. Detailed stages of the development of International standards.

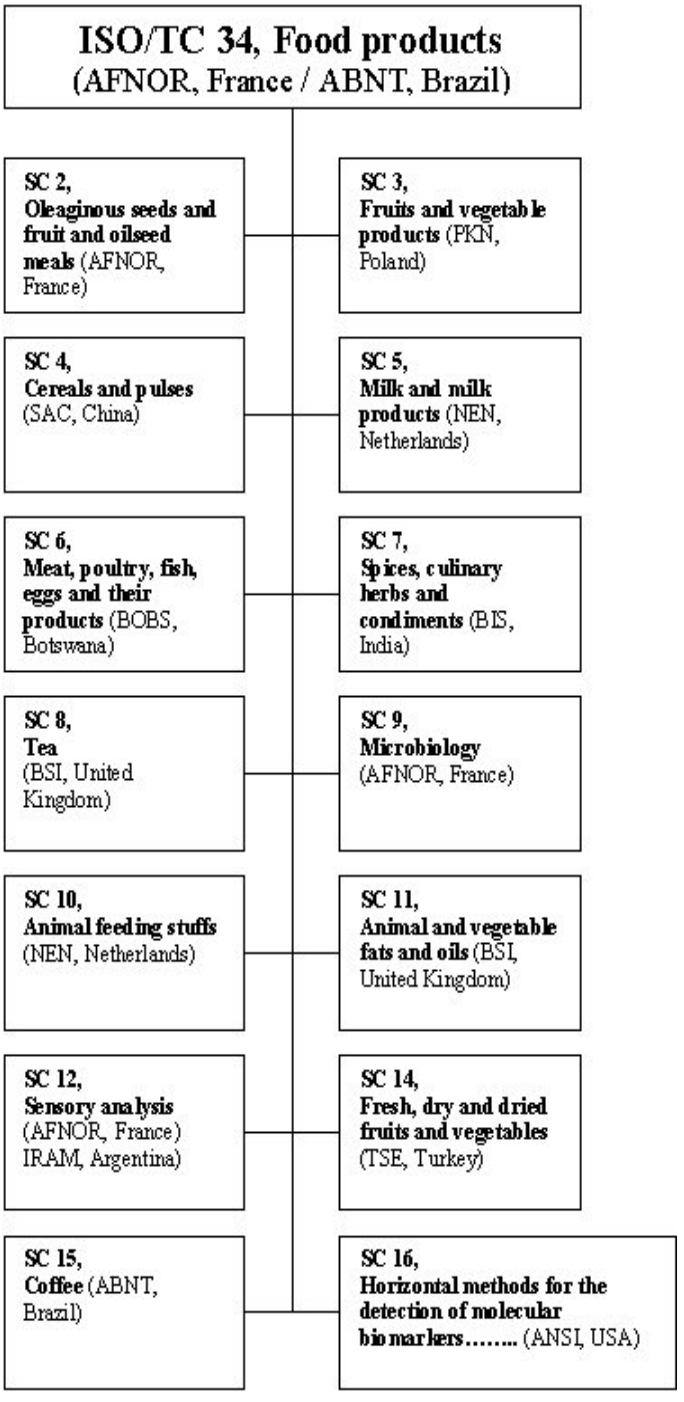

Figure 2. Subcommittees within ISO Technical committee 34 (Food products).

DOES EVIDENCE BASED RESEARCH IN FUNCTIONAL FOOD AREA AVOID RISKS FOR HEALTH: DIFFERENT REGULATIONS

Marika Mikelsaar
University of Tartu, Tartu, Estonia

In the wealthy societies the high stress of life, the increasing number of elderly people and reduced physical activity are considered the reasons for large spread of civilization-associated chronic diseases like atherosclerosis, hypertonia, tumour, diabetes, peptic ulcer, neurodegenerative diseases and different syndromes as adipositas, fatigue and depression. The crucial role of the impaired host functions is attributed to processed foods very rich in sucrose, saturated fat and sodium. At the same time, a lot of foods are characterized with deficiency of a number of human nutrients like omega-3 fatty acids, arginine, glutamine, taurine, nucleic acids, vitamins and antioxidants (Brit. J. Nutr. 1999; 81:1S-27S9). The deprivation of lactic acid bacteria has been demonstrated in communities with different degree of industrialization. The too hygenic environment, free of commensal bacteria contribute to development of allergies. There is abundant epidemiological and experimental evidence that dietary intake of functional food can decrease the risk of civilization-associated chronic diseases. Consequently, the search for new effective components of food, relevant to improvement of human health, is a vital and expanding process. In biomedical research the new approaches for influencing different functions of host are expected and applied.

FUNCTIONAL FOOD AND PROBIOTIC

Functional (medicinal) food is any fresh or processed food, including probiotic food and dietary supplements claimed to have the ability beneficially influence some body functions in order to improve the state of well-being and health and/or reduce the risk of disease. Probiotic is defined as a live microorganism which when administered in adequate amounts confers health benefit on the host (www.who.int/foodsafety/fs_management/en/probiotic_guidelines.pdf). Widely accepted probiotic strains contain lactic acid producing bacteria of human origin: bifidobacteria, lactobacilli or enterococci. The area of commensal, non-harmful bacteria of human origin serving as

probiotic is rapidly expanding. Usually the probiotic research starts with an attempt to find basic solution to some important medical problem: either prevention of infection, immunological defence to the treatment of infections or influence some metabolic pathway of host. The review of literature and also the acquaintance with selected patents help open the new fields of investigation. At University of Tartu the large culture collection of *Lactobacillus* sp. strains has been developed. Additionally to the everyday cultivation and characterizing the isolates obtained from different industrial experiments and clinical trials, the application of methods for transcriptomic and metabolomic studies on lactobacilli has been introduced. To date, the microbial activity can be measured using culture with different biomarkers, assessing the mRNAs, proteins and different metabolites. In our laboratory together with Department of Biochemistry several novel strains of human origin with newly discovered properties like production of wide range of polyamines, conjugated linoleic acid, nitrogen monooxide etc. have been described and licensed to Bio-competence Centre of Healthy Dairy Products of Estonia. The centre is working hand in hand with University of Tartu and University of Life Sciences together with cattle breeding and dairy enterprises. The essential outcome of the basic and applied research has been directed to some invention towards new biotechnological platforms which could be delivered to enterprises.

PATENTING OF NOVEL *LACTOBACILLUS* SP STRAINS

In the field of intestinal microbiota. numerous patents are filed world wide. The patenting of the novel *Lactobacillus* strains has been widely used in development of some new technological platforms for milk and cheese processing. The disclosure of invention of the novel microbial strain comprise characterization of its origin, cultivation media and conditions, its proper cultural-morphological and biochemical characterization, molecular identification and multilevel characterization of the novel component or some metabolic properties or a product of the strain. Further, it is very important to describe some best mode for carrying out the invention either *in vitro*, animal experiments or in volunteers (Mikelsaar et al. US Patent, 2007; priority date 2001).

DEVELOPMENT OF PROBIOTIC

However, the patenting process did not grant the application of the novel *Lactobacillus* strain as a probiotic delivered by some food product to consumers for improvement of health. The widespread agreement is that the probiotic strains should be safe, effective and stable in the final product. The internationally accepted criteria have been proposed

to consider the selected microbes as probiotics (Food Agriculture Organization, 2002; FEMS Immunol Med Microbiol. 2006, 46:149–157). The evidence based research in probiotic area includes different *in vitro* and *in vivo* assays for testing the functional properties and the putative effectivity of the candidate probiotic strains. Several supporting and confounding intrinsic, ecological and technological factors may be of importance in selection of suitable candidates for probiotics: properties of the strain, metabolic capacity of the strain during passage through GI tract, acid, bile and heat tolerance, the ability to grow in milk and to metabolize different substrates, including prebiotics.

Guidelines for the safety assessment suggest that probiotic safety should be assessed by measuring properties related to systemic infections, deleterious metabolic activity, excessive immune stimulation and gene transfer (FAO/WHO, 2002; Food Microbiol., 2003, 116:325–331). The translocation of probiotic candidates into blood and mesenteric lymph nodes assessed in different animal experimental models can serve as a warning marker for systemic infections. The hazard of production of biogenic amines by probiotic candidates is the important issue if planning to use probiotic strains in products with long ripening (Int.J. Food Microbiol, 1998, 44:15–20). More epidemiological data are needed, still. Use of integrons carrying resistance determinants as markers for detection of putative gene transfer from indigenous microbiota of intestinal tract to probiotics has not widely explored yet. However, EFSA (2005) has recently put forward for consideration the Qualified Presumption of Safety (QPS) status to lactic acid bacteria which seemingly reduces the need for multiple safety testing systems.

The general health benefits gained by applying probiotics were recently revised by a project group Joint IDF/ISO Action Team on Probiotics (Bull Intern Dairy Fed. 2008; 429:2–6). The provisional regulations of International Life science Institute (ILSI) and EU Research Commission require sound evidence by using probiotic a) of general balancing and enhancing the human particular functions or b) reducing the risk of certain diseases. The new possibilities for linking the microbial and host metabolic activities have been evolved with development of new molecular/biochemical technologies (Curr Opin Biotechnol. 2006; 17:204–210). The ILSI symposium together with the IDF has in 2008 drafted three different levels of probiotic action. These comprise 1) direct interactions with gut microbiota, including pathogens, relying on colonization resistance mechanisms; 2) fortification of the gut barrier function by influencing the tight junction quality; 3) modulation of the mucosal immune cells amount and activity and the systemic immune system. Thus, probiotic normalize the composition of the intestinal microbiota and modulate the immune functions of the

host. Emerging evidence has revealed that the prevention of GI tract colonization by variety of pathogens is a primary mechanism of beneficial effects mediated by probiotic (FEMS Immunol Med Microbiol. 2006 (46):149–157).

Besides infection control, several gut microbes are elaborated for use as probiotic in functional food, which aims to prevent and treat various other health problems such as allergy, neoplastic growth and inflammatory bowel diseases. The newer area includes the influence of probiotics on the metabolism of dietary components, like lactose digestion, lipid metabolism, proteins and indigestible dietary compounds. To explore the impact of different probiotics to cardio-vascular system and the lipid metabolism the important biomarkers have been the blood cholesterol and triglycerides and the modulation of defense against high-grade oxidative stress, conducted only in a few well-designed clinical studies (Nutr J. 2005, 4:22; Microbial Ecology in Health and Disease, 2009, 21:1–27).

Today, to define the health claims of a new probiotic, mainly two independent double-blinded placebo-controlled studies are recommended (Bull Intern Dairy Fed. 2008, 429:2–6). However, as different components of food could influence in complex way the metabolism and functions of the host, the sound information is hard to achieve with different products containing the particular probiotic. Also, the individuality of persons involved into clinical trials makes it difficult to assess the proper doses and duration of consumption of functional food necessary for getting expected results.

The EFSA regulations of European Parliament (1924/2006) have started the registration of the health claims made on foods referred either in Article 13 or Article 14. The proposed health relationship, the conditions applying to them and references for scientific substantiation should have been included for claims of Article 13 referring mainly on improved function of some organ-system of host. More complicated is the issue with claims of Article 14 related to the reduction of risk of some diseases where the target population of the intended claim, food quantity and pattern of consumption to obtain the claimed effect and restrictions to use it in different populations should be addressed. The weighing of the scientific evidence for substantiation of the health claims seemingly will be a very hard task for EFSA yet important to the expected success for enterprises that have put large resources into the research and developmental area.

HYGIENIC ENGINEERING GUIDELINES IN CLOSED EQUIPMENT

Alan Friis
DTU National Food Institute, Kgs. Lyngby, Denmark

Computational Fluid Dynamics (CFD) was found to be applicable to evaluate the design of closed process equipment with respect to cleanability. Specifically CFD was used to simulate hydrodynamic characteristics relevant for cleaning. The characteristics were compared to cleaning trials using the certified test method for closed equipment established by the European Hygienic Engineering and Design Group (EHEDG). Simulations show zones in process equipment, which were difficult to clean and thus undesired flow patterns were identified. The results obtained can be used for validation of cleaning as well as for design and redesign of process equipment and will set new standards for hygienic design of equipment. The effect of flow and promotion of desired flow patterns were discussed especially in relation to fully three-dimensional flow, turbulence, unsteady flows, recirculation zones, and other relevant aspects. Cleaning-In-Place (CIP) procedures are used throughout the food industry as the only practical way to clean closed process equipment. However, investigations concerning the influence of the hydrodynamics of flow on the cleaning of surfaces in the food industry exposed to real life conditions still lack attention. Furthermore validation techniques are limited to non-invasive methods mainly based on analysis of the rinse water from the cleaning procedure. Such tests do not yield much information about the final hygienic state of the inside of process equipment. In reality we know what we have removed but not what's left in side. The interest in investigating the influence of fluid flow on hygienic aspects of closed processing equipment is evident when reviewing the literature. Many study flow parallel to surfaces in order to quantify the effect of hydrodynamics on biofilm formation and mechanisms of microbial adhesion and removal. The studies employ laminar flow under uni-axial flow conditions. The conclusion is that the wall shear stress is a controlling factor and hence so-called critical wall shear stresses are reported for specific microorganisms on selected surfaces.

The flow patterns observed in industrial applications are seldom similar to uni-axial flows. Normally a higher degree of complexity is found and in many cases flow patterns need to be considered three-dimensional due to turbulence and build-up and break down of flow patterns. Furthermore time effects can play a role yet other flow patterns are unsteady either on a bulk or local level. Examples of poor cleaning are reported due to insufficient fluid exchange and recirculation zones in up-stands, dead-ends, heat exchangers, expansions or contractions. These examples can all be considered complex geometries, however a complex geometry is not necessarily causing problems with respect to cleaning. In the housing of a mix-proof valve recirculation zones are found along with very low wall shear stresses, however cleaning is found to be efficient in practical applications. A validatation method for cleaning-in-place of process equipment using test methods has been developed by the European Hygienic Engineering & Design Group (EHEDG). Other methods are in-house tests with equipment manufactures for example utilising removal of visible residues from transparent equipment, which allows visualisation of both the flow pattern and the removal process on a surface. The advantage of a simulation over experimental testing is that time can be saved and the number of prototypes can be reduced. The application of flow simulation to assist in design of process equipment and prediction of cleaning efficiency has been suggested in several publications. Flow simulation using computational fluid dynamics (CFD) has been used for decades to describe flows in process plants. However, describing hydrodynamic parameters close to or at walls is not the traditional intended application. This is due to the fact that the resolution near the wall requires very much attention. Development of the CFD model near the wall was explained by Jensen (2003) in his dissertation entitled ‘Hygienic design of closed processing equipment by use of computational fluid dynamics’. These results showed that CFD could be a qualitative tool for evaluation of cleaning efficiency in closed process systems. It was demonstrated that complex equipment was not necessarily difficult to clean. A set of hydrodynamic parameters was identified as the major controlling factors in cleaning of closed processes. The wall shear stress play a role but cannot explain the whole picture by it self. This is due to the fact that fully three-dimensional turbulent flows proved to clean better than expected based on uni-axial flow investigations. Concerning the nature of real flows full 3-D considerations must often be included since swirl zones can only be discovered this way. Furthermore transient simulations can provide additional information on fluctuations in turbulent flows.

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HYGIENIC ENGINEERING GUIDELINES IN OPEN EQUIPMENT

Satu Salo & Gun Wirtanen
VTT Technical Research Centre of Finland, Espoo, Finland

The European Commission (EC) Regulations 852/2004, 853/2004 and 854/2004 cover the principal objective of the new general and specific hygiene rules to ensure a high level of consumer protection with regard to food safety. Legislative demands set the basic requirements for the manufacturing of safe food products whereas food safety management systems and food safety guidelines and standards based on given legislation help the food industry to keep up with current food safety requirements.

The hygienic design of process equipment has a significant impact on reducing the risks of contamination of food during production. Food processing equipment has been shown to contaminate food products. The hygienic design of process equipment and components should be based on a sound combination of process and mechanical engineering and knowledge of microbiology. Poor hygienic design of process equipment and components used in the food processing industry can result in food contamination, because such equipment is difficult to clean. Having a good hygienic design the lifetime of the equipment will increase, the maintenance and the manufacturing costs will also be reduced. The process equipment is easy to clean if the surface materials are smooth and in good condition. Dead ends, corners, cracks, crevices, gaskets, valves and joints are vulnerable points for biofilm accumulation. Hygienic requirements should be adopted at the initial stage of developing process equipment and components because upgrading existing designs to meet hygienic requirements is often both expensive and unsuccessful.

The European Hygienic Engineering & Design Group (EHEDG) has made guidelines about design criteria for hygienic equipment. Guidelines and methods on design of new hygienic equipment published by 3-A Sanitary Standards Inc. and NSF International are also available. National standards and/or directives applicable to the hygienic design of food machinery are also available, but only few international standards exist and they are mainly directed to dairy industry. The food safety of products produced

with equipment that meets the requirements of EU legislation and regulations, e.g. the EU Machinery Directive 98/37/EEC, is increasing. If the manufacturer has assigned the ‘CE mark’ to its equipment and process lines then the production safety improves even more. However, the CE mark, which is granted according to the EU Machinery Directive, clearly falls short in terms of hygiene.

EHEDG has a guideline for hygienic design of equipment for open processing (doc. 13). Open processes include very different types of equipment, e.g. machines for bakery products, meat and fish. The EHEDG doc 13 deals with principal hygienic requirements for equipment used in open processing. It describes methods of construction and fabrication and gives examples of how the principal design criteria can be met in open process equipment.

ZONING AND HYGIENIC INTEGRATION

Alan Friis
DTU National Food Institute, Kgs. Lyngby, Denmark

New approaches to application of zoning and integration procedures in assurance of plant hygiene and sanitation will be presented. The goal is to assure that an installation which is hygienic at the design state will remain hygienic through maintenance, re-design and of cause the intended use. The presentation will pertain to application of known principles such as zoning, construction of plant master plans and the relatively new topic of hygienic integration. Applying proper integration, focus will remain on the most important issues allowing process engineers to plan and re-design plants in a safe manner. Also product developers can include issues of hygienic requirements early in their development processes. The proposed principles will allow for easy and effective communication inside companies as well as externally to public bodies and costumers. The work build partly on the proposed integration guideline from European Hygienic Engineering & Design Group (EHEDG) partly on discussions and inspiration form a large amount presentations made by people in the hygienic design and cleaning community.

HYGIENIC ENGINEERING

Poor decisions are often made during the sequence of designing, fabricating, installing, contracting and making design changes, or when maintaining a production assembly, a line, or a facility, because the sequential approach to problem-solving is adopted. This way, hazards may be unintentionally created in the process line, such as leaving a valve on a branch closed, thus creating a dead end, or simply placing equipment inexpediently, making cleaning very difficult.

Another important issue for obtaining a line that runs optimally is to make sure it is operated systematically. One way to ensure high performance is to implement HACCP and GMP, which primarily deal with hygiene, cleaning and critical control point monitoring. Furthermore, high performance is ensured by employing changes in management, by establishing and maintaining documentation with regard to installation,

automation, operation, maintenance, and cleaning as well as by testing the operation and performance of the equipment before routine use. The ideas presented here are part of the imminent EHEDG guideline on Hygienic Systems Integration (HSI). The EHEDG guideline has the task of linking and supporting current guidelines on hygienic design regarding specific equipment and hygienic tests, and which can be viewed as vertical guidelines. The HSI guideline, on the other hand, is classed as a horizontal guideline, which is a completely new approach. Neither the EN1672-2 nor the HACCP standards are replaced by the HSI guideline.

HYGIENIC INTEGRATION

The integrated approach to hygienic design is a systematic way of combining hygienic entities into a hygienic facility. This may be a new design or reassignment of existing entities. An entity is a component, which is part of a hygienic system, and can be a part, an assembly, a module, a line, or a factory. Part of the scope of the HSI guideline is:

- ⇨ to describe the integration of entities, including the manufacture and supply of goods, in order to produce safe food or related products cost effectively, and
- ⇨ to describe integration topics that can affect hygienic design, including installation, operation, automation, cleaning and maintenance, especially those that are common or a frequent cause of failure.

The guideline defines ‘hygienic integration’ as a process of combining or arranging two or more entities to work together while eliminating or minimizing hygiene risks. While the focus is on the hygienic standard of the equipment, there are many surrounding issues that must be controlled in order to complete ‘hygienic integration’. For example, a facility must conform with all specified requirements, which may originate from legislation, users, product quality or safety. The integrated approach also involves determining specifications for product flow, control strategy, automation, maintenance, change management and training of personnel. Furthermore, implementation of HACCP and GMP is a necessity. 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 parts of a process may also be carried out.

The integration process comprises a set of actions, which are given in Figure 1. Each step is carried out by following a flow diagram, which takes the user through the necessary steps in order to complete each particular action properly. Examples of such flow diagrams are given in Figures 2 and 3.

Each integration-action 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 passed the functional requirement for integration. Instructions must cover: installation, operation, cleaning, sterilisation (if applicable) and maintenance. Concurrency with design and validation activities other than those concerned with hygiene is naturally a prerequisite. For an unassigned module or assembly, the provisionally intended process or processes and product(s) must be defined in a prospective list.

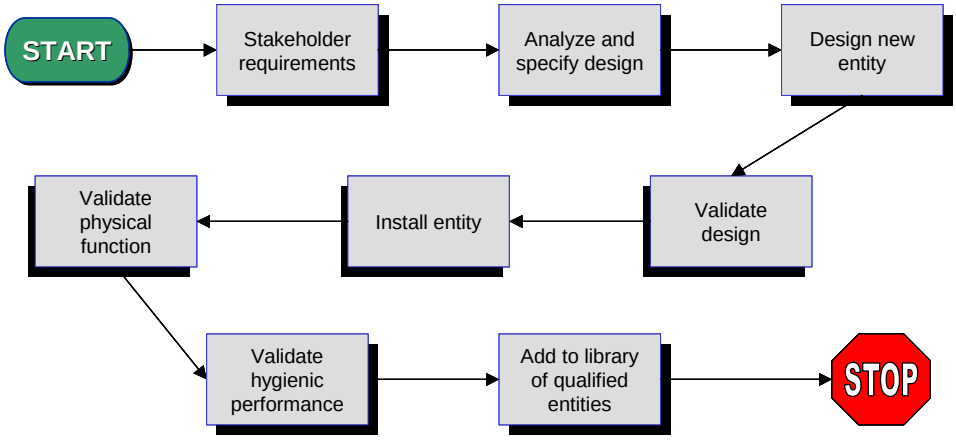


Figure 1. The figure shows a single integration displaying the required integration actions.

The first action is to determine the stakeholders' requirements, which can originate from customer, food safety, environmental legislation, or some other type of constraint. After listing the stakeholders' requirements the user goes through the first flowchart: 'Analyse and specify the design', given in Figure 2. Going through the stakeholders' list of requirements should produce a conceptual design for the entity or entities under examination. Every time such a stage is completed, the flow diagram takes the user through a confirmation step, making sure there is compliance between the information obtained and the outcome of the analysis. For example, if the user forgot to take some legislation issues into consideration in the conceptual design, the user should be able to notice this before going on to specify the design in more detail. The flow chart also asks to record data produced during the decision process and to record the decision itself (Figure 2). The user then continues through the integration 'snake' (Figure 1), and goes on to design the new entity, validate physical function, install the entity, validate the design, and the hygienic performance. There is a separate flowchart for

each of these actions taking the user through the necessary steps to complete a particular action.

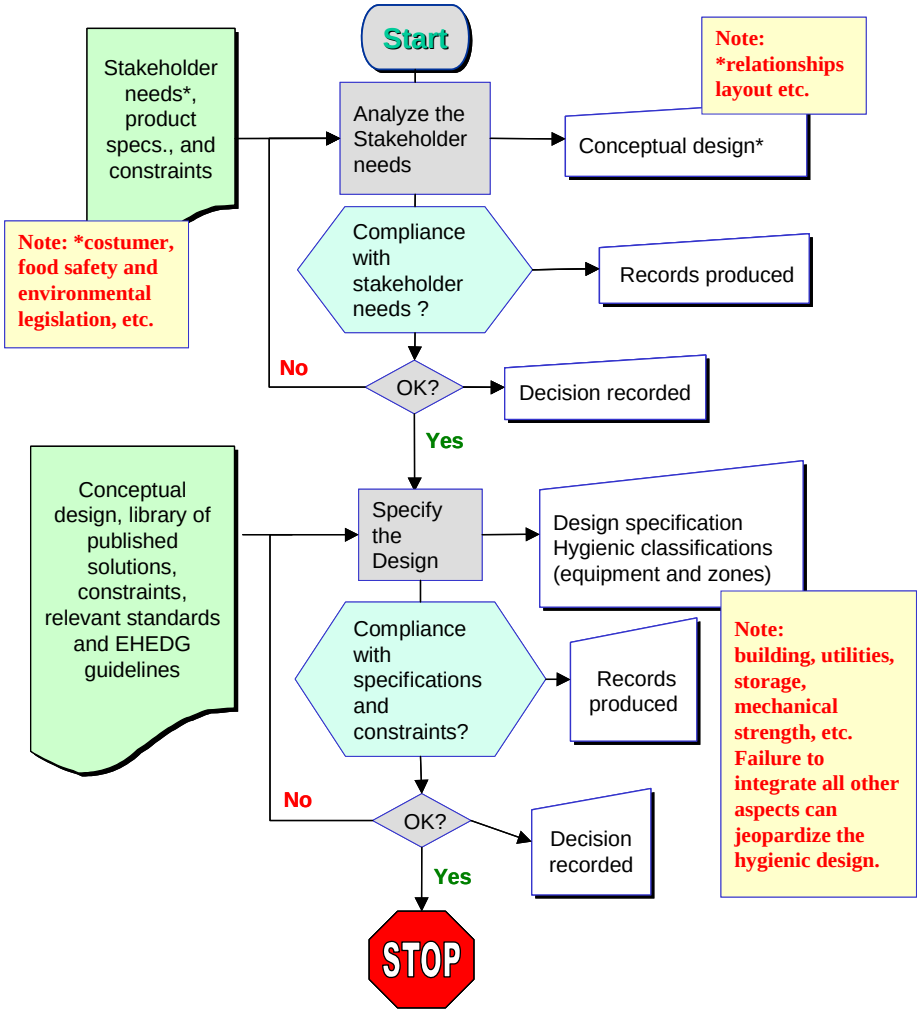


Figure 2. Flow Diagram for the integration action: 'Analyse and specify the design'.

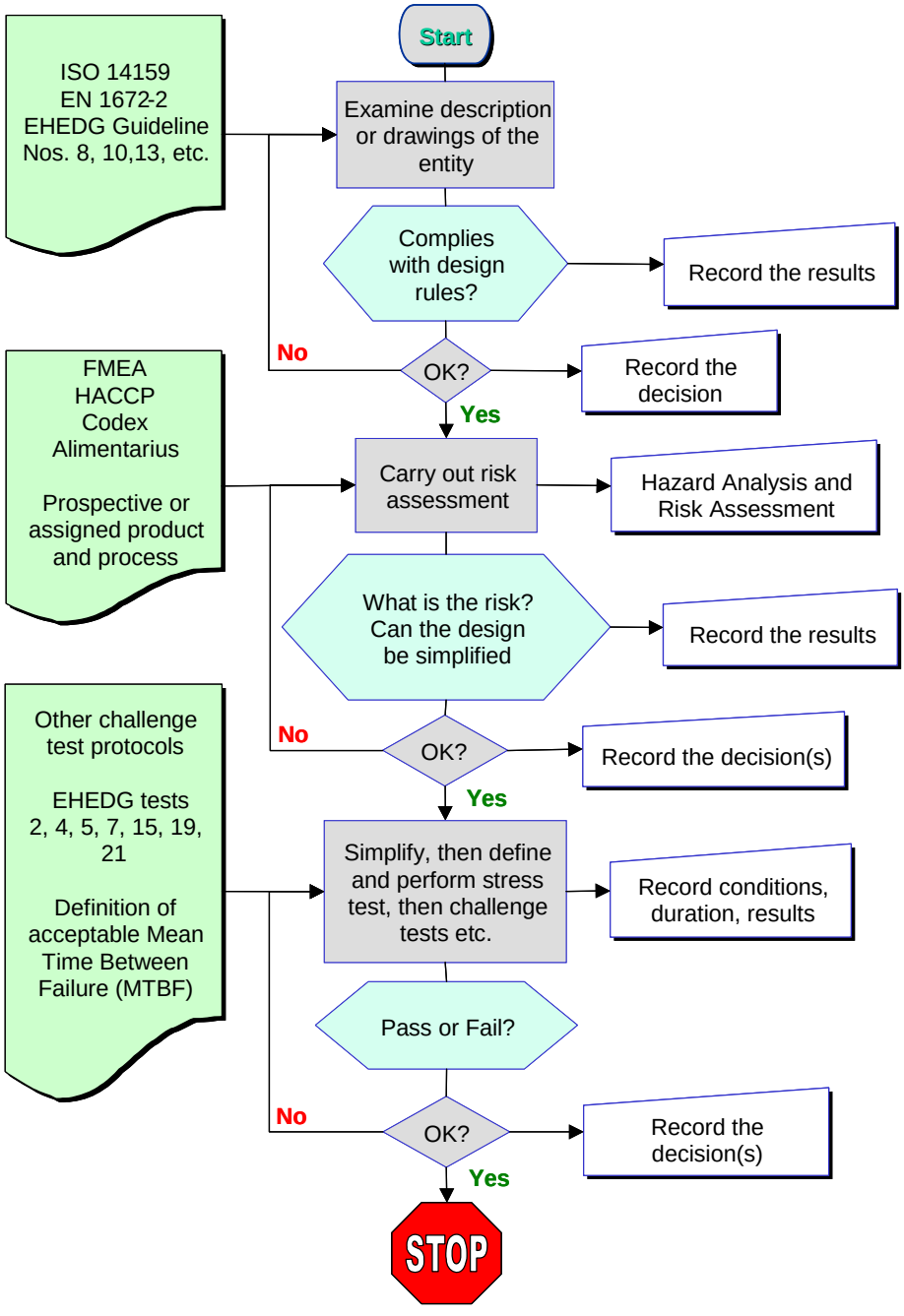


Figure 3. Flow diagram for the integration action 'Validate hygienic performance'.

An example is given here for the ‘Validate hygienic performance’ flowchart (Figure 3). The incoming information for the first step is provided by examining the description or drawing of the entity with respect to the guidelines on the safety of machinery, i.e. NSF 14159 or EN 1672-2, as well as the EHEDG guideline on ‘Hygienic equipment design criteria’. Depending on the entities to be integrated, EHEDG has published guidelines on:

- ‘Hygienic design of closed equipment for the processing of liquid food’,
- ‘Hygienic design of equipment for open processing’,
- ‘General hygienic design criteria for the safe processing of dry particulate materials’,
- ‘Hygienic engineering of plants for the processing of dry particulate materials’ or similar.

The second step is to perform a risk assessment, which in practice means performing a FMEA and HACCP analysis, while the third step is to test the entity. Depending on intended use, one or more of the EHEDG tests for sterilisability, in-place cleanability, or bacteria tightness may be applicable. The acceptable mean time between failures may also be determined at this time.

After completing the validation of the hygienic performance, the entity has been integrated successfully, and can be implemented for the specific process to which it was assigned. If the entity has not been assigned to a particular product or process, it can simply be added to the library of unassigned entities.

Acknowledgement – The author is grateful to European Hygienic Engineering & Design Group, R3-Nordic, Roland Cocker Consulting Ltd as well as colleagues in Denmark and Finland.

HYGIENE CONTROL METHODS IN FOOD PROSESSING – A CASE STORY

Helvi Mustonen
Orion Diagnostica Oy, Espoo, Finland

Migros plays a vital role in Swiss economy. It is one of the largest enterprises in Switzerland, the largest supermarket chain and the largest employer (79.000 employees in 2006).Migros is also involved in over 60 restaurants, furniture shops, and banking. The company was established in 1925 as a cooperative society, the business model that is still in use. Nowadays, a large part of the Swiss population are members of the Migros cooperative – around 2 million of Switzerland's total population of 7,2 million – thus making Migros a supermarket chain that is owned by its customers.

How is process hygiene monitored in the production facilities of Genossenschaft Migros Aare, from where packed raw meat and processed food is delivered to retail sales?

Building and maintaining respectable quality standards is a long process. Migros started this culture over fifteen years ago. In the heart of this process is dedicated personnel, training and motivation, but also appropriate tools for monitoring according to chosen standards. Where this exceptional company really shines, is the quality self-control system they have created. Among the official, government-set regulations Migros has built a systematic tool for evaluating constantly its own and its partners' quality.

Migros way of doing business starts from the point the carcasses are brought inside Migros' premises. The carcass is weighted and each is given a unique ID for tracking the lot from there on, even until the customer pays the groceries at Migros' supermarket. Each carcass is tested with Hygicult TPC before they're processed further. If no or very little contamination is shown, meat can be processed further. Migros' vendors are encouraged to follow the same quality standards. The ideal limit for raw meat is no growth on TPC, however 1–9 colonies are considered acceptable. 10–30 colonies would be traditionally considered as good hygiene level, but in Migros' case 'A minor flaw', which will lead to inquiry.

Each vendor is reviewed constantly and track record is kept for these pre-selected vendors. If minor problems should occur, the vendor is first given a notification with request for explanation and future preventive actions. Second time the vendor is put on quarantine, and no purchases are done until the issue has been confirmed to be cleared. High quality takes time and effort but it pays off as well: during the years, Migros has been able to prolong the sales time for minced meat from one day to five days. This is a value worth of aiming. All this is enabled by superb hygiene standards and continuous improvements.

CONTROLLING HYGIENE IS HUMAN BUSINESS

The basis of good hygiene is in motivated and skilled personnel. Each task is instructed and controlled. Premises of meat production are designed preventing roaming between start and end of production. Needless to say, much attention is paid to appropriate clothes and cleaning stations: one cannot enter to production area without fully automatized disinfectant hand- and shoe washes and protective clothes. Pressurized production area reduces the risk of contamination as well.

Hygicult dipslides are used also to control the critical production spots in manufacturing high quality groceries. Each employee who is involved in handling meat products gives weekly controls – which are published on weekly basis to co-workers. The same method for controlling the raw materials, production equipment and personnel is used throughout the production with same standards. The aim is for excellent result – no contamination in Hygicult TPC.

IMPORTANT POINTS IN HYGIENE

Migros has been using Hygicult TPC to establish a respectable quality system for handling and producing raw meat to safe food. Among with tools to ensure set standards, a comprehensive system and talented people have been required to achieve this. The result is impressive quality standard and a credible self control which has been tested constantly. And has been found convincing by Swiss authorities each time. As always, competition will be present in the future. The benefit of improved product quality and recognized quality level is in the customers' corporate image, making Migros the company they can always trust.

CAMPYLOBACTER SPP. DETECTION IN RISK MANAGEMENT

Mati Roasto
Estonian University of Life Sciences, Tartu, Estonia

Risk management is activity directed towards the assessing, mitigating (to an acceptable level) and monitoring of risks. The main aims of the *Campylobacter* risk management are the reduction and control of the risks related to *Campylobacter* spp. in food production chain and to minimize the *Campylobacter* transmission into food products and thereby to humans. Risk management is often policy base and uses all the available data and scientific information to make accurate and economical decisions at state level. More specific decisions could be made at farm, at slaughterhouse, at industry and at retail/catering level. Scientists should be promoters for state and private sector food safety policy.

Campylobacter detection could be performed at different levels like monitoring of zoonoses at state level, through *Campylobacter* control programs, at EU baseline study level. Additional research should be performed by research institutions. Detection of *Campylobacter* means to follow all the new directions in EU. Disc-diffusion method for susceptibility studies is not advocated for European monitoring because different methodologies are used with different criteria. Only quantitative data on MIC will be accepted. For *Campylobacter* spp. dilution methods shall be performed according to the methods described in Clinical and Laboratory Standards Institute (CLSI) guidelines M31-A3 – Third Edition. Antimicrobials to be included: erythromycin, ciprofloxacin, tetracycline, streptomycin, gentamicin. The quantitative detection of *Campylobacter* spp. shall be done according to EN ISO/TS 10272-2:2006 Part 2: Colony-Count Technique. Detection method can be excluded from the programme. PCR method is the preferred method for *Campylobacter* speciation as phenotypical methods bear a certain risk of giving intermediate or incorrect test results.

Enteric infections caused by *Campylobacter* are the most frequently reported zoonoses in humans in the EU with an incidence rate of approximately 50 confirmed cases per 100,000 population over 17 countries (EFSA, 2009). The majority of cases of

campylobacteriosis are self-limiting with 3–5 days of acute diarrhoea, abdominal pain and fever. However, disease in the very young and elderly can be serious and sequelae of infection, such as polyneuropathies, may result in the need for hospitalisation. Thus the public health and social consequences of campylobacteriosis are significant for the EU. Some cases require antimicrobial treatment and the increasing incidence of antimicrobial resistance is seen as a potential public health issue. Epidemiological studies worldwide indicate that campylobacteriosis is largely food-borne and that poultry meat is a major source. However, the proportion of illness due to poultry meat and the contribution of other potential sources remain unclear. *Campylobacter* can colonise the intestinal tracts of birds at high levels and faecal contamination of poultry carcasses can occur during processing. One of the principle routes of human exposure is considered to be cross-contamination from poultry meat during food preparation in kitchens. Control of *Campylobacter* in poultry meat is a major public health strategy for the prevention of campylobacteriosis.

The purpose of food control is to provide consumers with food that is safe and meets all the appropriate requirements. This is made possible by assuring the conformity of the food-chain as a whole – from producing raw material for food until the moment it become available for the consumer. *Campylobacter jejuni* is a pathogen subject to registration since 2000 in Estonia according to the Infectious Animal Disease Control Act and the Estonian Ministry of Agriculture Regulation No 34 "List of Notifiable Diseases and Diseases subject to Registration". According to the data of the Estonian Program on Monitoring and Surveillance of Animal Infectious Diseases the food samples were taken in the frames of official food surveillance and in the frames of the monitoring program performed at slaughterhouses. In accordance of the national evaluation of the situation of *Campylobacter* spp. the occurrence of *Campylobacter* in fresh broiler meat in Estonia is quite high but shows the decreasing trend: 2004 – 56 samples taken and 26.8% of them were positive, 2005 – 278 samples were taken and 7.5% were positive, and in 2006 80 samples were taken and 6.3% of the broiler chicken meat samples were positive for *Campylobacter* spp. Altogether, 207 food samples were tested in 2006, 5 (2.4%) were positive. All positive samples originate from broiler chicken meat not of Estonian origin. In 2007, a total of 47 caeca and 48 neck skin samples of Estonian broiler chicken origin was examined for *Campylobacter* spp. by the Estonian state monitoring program, and no positive samples were found among Estonian products.

In accordance with the Commission Decision of 19 July 2007 (2007/516/EC: baseline study in 2008) the Member States shall carry out a survey to assess the prevalence of *Campylobacter* spp. in broiler flocks and their antimicrobial resistance as well as *Campylobacter* spp. in broiler carcasses. From January 2008 in Estonia at least 96 slaughter batches shall be sampled. The number of individual broiler chickens per slaughter batch to be sampled is 10 birds for the detection of *Campylobacter* in caeca and one bird for the detection of *Campylobacter* on carcasses. The caeca samples and carcass sample must be from the same slaughter batch. In Estonia one isolate per *Campylobacter* species from each positive slaughter batch shall be included in the antimicrobial resistance monitoring. In cases, in any given year, a lower number of isolates than the target sample size (slaughter batches to be tested) is available, all these isolates shall be included in the antimicrobial resistance monitoring (Official Journal of the European Union, 2007). In the Estonian *Campylobacter* pilot-study in 2002 to 2006 it was found:

1. *Campylobacter* spp. positive samples on fresh chicken products of the small-scale company (35.6%) were significantly more prevalent ($P < 0.001$) than on those originated from the large-scale company (6.3%). The chicken carcasses and wings (28% and 31.3%) had significantly more positive samples ($P < 0.001$) than chicken breasts and thighs (0% and 0%).

2. Proportion of *Campylobacter* positive samples on fresh chicken products of Estonian origin was 9.1% compared to 15.9% obtained from imported frozen raw poultry products at the retail level in Tallinn and Tartu during 2002–2003. Higher proportion of *Campylobacter* positive samples on imported frozen poultry products may indicate the presence of high *Campylobacter* contamination at primary production level.

3. Compared to raw poultry products collected in Tallinn retail outlets, more commonly *Campylobacter* spp. positive samples were obtained from products collected from Tartu markets. One possible reason for differences in positive sample proportions could be differences in transportation time of samples to the laboratories, which for the samples collected from Tallinn was several hours longer (laboratory analyses were made at the University of Helsinki) than in Tartu were analyses were performed almost immediately after sampling. However, more severe contamination of the poultry products at the retail level in Tartu may be associated with the fact that the general hygiene level in Tartu Turg, were most samples were collected, was low during that time and products were sold unpackaged.

4. Analysis of seasonality of *Campylobacter* positive samples indicated that the seasonal peak of *Campylobacter* on chicken meat was from June to October.

5. Our studies showed high serotype and genotype diversity among *Campylobacter* isolates from raw retail poultry meat in Estonia. The serotype distribution did not show association with the origin of the sample. The genotyping of the 70 *Campylobacter* isolates showed *Kpn*I to be more discriminatory, yielding 34 PFGE types compared to 29 obtained by *Sma*I. PFGE with the enzymes *Kpn*I and *Sma*I for digestion proved to be discriminatory, repeatable and reproducible. In practice use of the enzyme *Kpn*I is sufficiently discriminatory. PFGE had good typeability and it was a useful tool in molecular typing of isolates from foods. In our study the majority of the isolates sharing a similar PFGE genotype originated from one country. The association of genotypes with country of origin requires further studies using a larger collection of isolates.

6. Our antimicrobial susceptibility studies of *Campylobacter* strains resulted in high resistance patterns for several antimicrobials. High MICs of both erythromycin and ciprofloxacin pose a problem and because erythromycin is considered as a first-line choice of treatment for human *C. jejuni* infections, the resistance has an important public health impact. Multidrug resistance in Estonian broiler chicken isolates was one of the highest reported in latest studies of broiler chicken *Campylobacter* isolates all over the world. Our findings in 2005 and 2006 suggest that the use of fluoroquinolones may select multiresistant strains since resistance to erythromycin, gentamicin or oxytetracycline was exceptional without simultaneous resistance to fluoroquinolones.

The widespread emergence of multiresistant isolates poses a threat to humans and limits therapeutic medication. In Estonia, more restricted use of antimicrobial agents, especially fluoroquinolones, in food animal production should be implemented. Antimicrobial susceptibility studies need to be continued to find the trends in levels of *Campylobacter* resistance as well as the mechanisms for resistance and potential to decrease the *Campylobacter* resistance in Estonia. Research based risk assessment, risk management and risk communication has to be performed in Estonia in relation with *Campylobacter* spp. in food production chain.

IN-PLACE CLEANING SYSTEMS

Urban Wiik
JohnsonDiversey, Helsinki, Finland

CIP is an automatic and systematic cleaning of the inner surfaces of tanks, heat exchangers, pumps, valves and pipes. The advantages of modern CIP is:

- Strong and hot solutions can be used. The heat, the chemistry and the mechanics can be sustained long.
- The solutions can be reused.
- Can be automated and the reproducibility is good.

Drawbacks are:

- Investment in equipment is high.
- The mechanics are not always sufficient.

A minimum flow velocity 1, 5–2 m/s is a basic requirement. The pipe dimensions and the flow m^3/h of the system should be known and measured.

$$v = \frac{4.Q}{3600.d^2.\Pi}$$

v = flow velocity (m/s)
Q = flow rate (m^3/h)
d = inside pipe diameter (m)

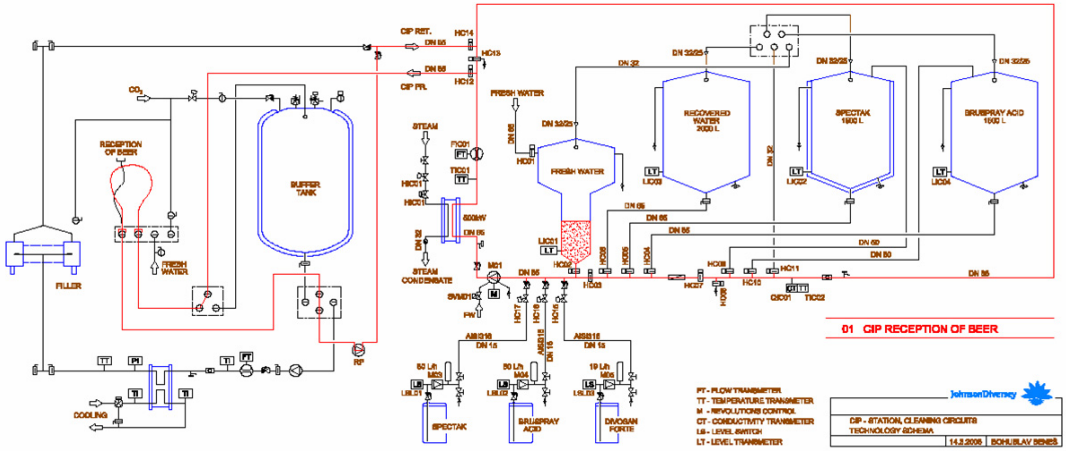
Tank cleaning has different requirements from pipe cleaning. The requirements vary with the soil conditions and the surface properties of the tank. A certain flow per tank circumference is required. This has to be taken in consideration when choosing spray balls. Flow as a function of tank diameter and soil conditions:

$Q_R = D_T * \pi * F_S$

Q_R	=	required flow rate	liters/ minute
D_T	=	vessel diameter	meters
F_S	=	soil factor	liters/(meter*minute)

FS = 27 l/m*min for light soil conditions
FS = 30 l/m*min for medium soil conditions
FS = 32 l/m*min for heavy soil conditions

High pressure rotating sprayheads have different specifications. The flow is lower but the pressure is higher. A minimum time has to be allocated for rotating sprayheads to perform their spraying pattern. Do not install sprayballs and rotating sprayheads on the same circuit. CIP optimizing can save considerable amounts of water, effluent, energy and chemicals. CIP consumption is benchmarked against the circulating volume and not the total volume which includes the CIP hold up tank. The cleaning liquid loss can be measured with simple equipment e.g. with titration and a yardstick. It is important to manage the chemical phase changes in the system. Conductivity based phase management is a popular way of achieving this. The most exact way of managing the phase borders however is volumetric control with flow meters. The fill volume for each object to be cleaned is estimated and the cleaning programs are individually adjusted to this. The CIP flow is best circulated bypassing the CIP tanks with the heating and chemical dosing in the bypass line.



Flow chart for a CIP-system in a brewery.

THE POWER OF IN-PLACE CLEANING TOOLS IN TANK SYSTEMS – TANK CLEANING TECHNOLOGY

Rene Elgaard
Alfa Laval Tank Equipment A/S, Denmark

How the impact from modern Tank Cleaning Machines reduce the consumption of Cleaning Media, Cleaning Chemicals, Cleaning Time and help to reduce the environmental impact of CIP. A tank (or vessel) is part of any processes line manufacturing food. It could be for storage, fermentation, cooking, mixing and many more applications. Depending on the application the complexity of producing the food varies. After processing food in a tank, the internals of the tank must be cleaned to prepare the tank for the next batch. This cleaning is part of the total CIP of the plant and the cleaning is done for various reasons such as:

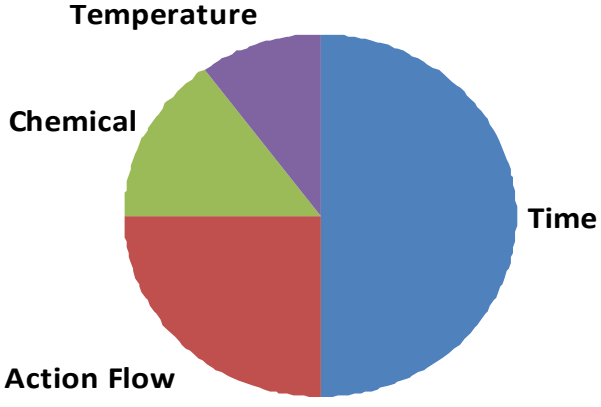
- Guarantee Product Integrity (Re-use Equipment)
- Risk Management, "stay in control"
  - – prevent cross contamination, adulteration and avoidable carryover
  - – removal of bio-film, dried foam, precipitate or sludge/ sediment
- Comply with Legal Requirements
- Prepare Tank for Human Entry i.e. during Maintenance and/or Inspection
- Remove Hazardous or Explosive Atmospheres
- Protect the Equipment against Corrosion.

However, there is another and very up-to-date reason for ensuring that CIP processes are optimised. This is the protection of our environment and safeguarding our resources. There is no doubt that our environment and our water resource in particular is being challenged daily. UN estimates that every sixth person in the world (or some 1.1 billion people) faces inadequate access to safe drinking water. By 2025, UN estimates that water shortages will affect 2.3 billion people in about 50 of the worlds 200 nations.

So there is the operational optimisation of the CIP process and then there are the environmentally issues and our responsibility to safeguard resources. What has all of the above to do with the "Power of in place cleaning tools" = the Tank Cleaning

machines in place. Actual quite a lot. Depending of the Cleaning technology used it has been evidenced that is possible to save up to 90% of the cleaning media used including the cleaning chemicals and reduce the cleaning time with 80%.

The background for all cleaning is described in 1960 by Sinner in Cleaning Philosophy and illustrated in the below Sinners circle. The traditional tank cleaning approach was (and in some applications still is) high-flow and low-pressure cleaning, relying on chemistry and time to remove the sediments / residues. The basis for Sinners cleaning philosophy was based on using Static Spray Balls (SSB), which is being used in many applications, even today. He defined that 4 cleaning parameters needed to be in place for cleaning to take place and he called that TACT = Time – Action – Chemical – Temperature.



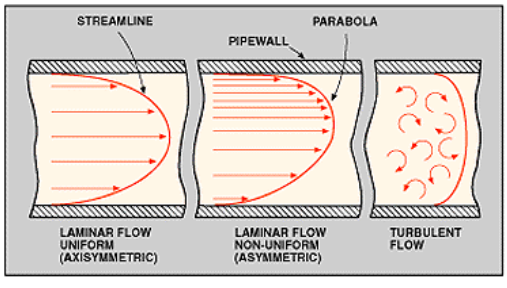
With SSB the cleaning is related to water, chemicals and time. The cleaning water and chemicals are distributed in the top of the tank and the cleaning is done by cascading flow running down the tank wall. The coverage of the cleaning media is partial and large areas inside the tank are not covered at all. The sediments / residues are attacked by the chemistry and the cascading flow. However, there is no or very little impact by the cascading flow, which means that the removals of sediments / residues to a large extend only rely on the chemical reaction and this is only possible when the sediments / residues are being wetted with the chemicals. The action (mechanical force and heat and mass transfer) of the flow is limited to the free falling film and amounts to magnitudes similar to those found for CIP cleaning a straight pipe.

Studies from flow in pipe systems have proven that the cleaning efficiency of the pipe walls is related to the pipe wall shear stress created by the flow. The higher the velocity

of the flow the higher the wall shear stress (Pa). Ensuring turbulent flow ensures higher wall shear stress and better mass and heat transfer to the surface – hence, the Reynolds number has an effect on the cleaning efficiency.

Static Spray ball

Static Spay Ball in action and free falling film



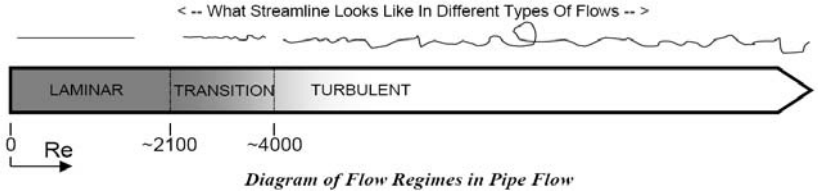


Diagram of Flow Regimes in Pipe Flow

Examples of flow in pipe systems from laminar flow to turbulent flow (above) and a diagram of flow regimes in pipe flow (below)

The wall shear stress from the cascading flow is around 4 Pa at a Reynolds Number of appox. 1000. This means that enough liquid (relative to tank diameter) is required to obtain turbulent flow in the cascading film of water. This sets the lower limit of the volumetric flow rate required using a SSB.

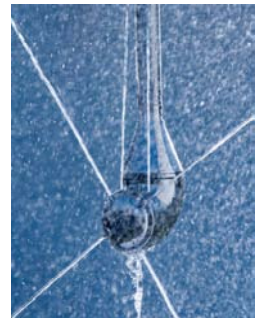
INCREASING THE WALL SHEAR STRESS

We need to increase the wall shear stress in order to remove the sediments / residues more efficient. This can be done by using Rotary Spray Heads (RSH) and Rotary Jet Heads (RJH) where the cleaning action is increased and the coverage of the water on tank wall is better.

Rotary Spray Head

Rotary Spray Head in action

Rotary Jet head

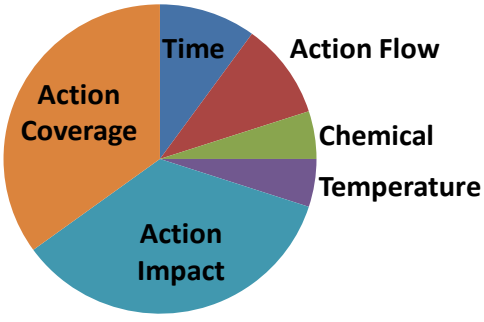


Rotary Jet Head in action

1. First of all the coverage of the cleaning media inside the tank is much better than using a SSB due to the rotation of the spray heads. For the RJH coverage can be made 100%. Secondly the impact from the cleaning media is significant higher than from cascading flow. In fact for RJH the wall shear stress is more than 10 times as high (in the foot-print region) than for a cascading flow. For the direct impact of the jet the force is not comparable with wall shear stress. So basically Sinners TACT still prevails we just improwe coverage of the tank is much better.
2. The impact from the cleaning media is significantly higher creating a higher wall shear stress.

All in all it reduces the Cleaning Time, The Chemical Consumption, The Flow and the total energy consumption from Heating and electricity.

During the last 30 to 40 years the development has taken Tank Cleaning from something that was required to do occasionally to a process where operational optimization, environmental improvement and reducing the bacteria’s in the tanks has proven to be an investment that pays itself back several times during the lifetime of the plant. Below are saving examples from various applications.



Alfa Laval impact cleaning factors and their effects

Examples of savings when using Alfa Laval Toftejorg Tank Cleaning Technology

CIP Savings	Before improvments		After Improvements		Savings			
	Time	**Flow**	**Time**	**Flow**	**Time**	**Time %**	**Flow**	**Flow %**
	min	m^3	min	m^3	min	%	m^3	%
Brewery Fermenter	162	124	84	27	78	48%	97	78%
Yeast Manufacturer	120	72	20	6.33	100	83%	66	91%
Dairy Milk Storage	34	23	24	10	10	29%	13	56%
Dairy Starter Culture	95	40	50	5	45	47%	35	87%

CLEANING AGENTS & DISINFECTANTS IN PRACTICE

Annika Jürgens
Greenclean OÜ, Tallinn, Estonia

Food preparation areas are cleaned according to their areas of expertise or specialization, and the equipment they require for this. The cleaning applications and agents typically range from dry cleaning to wet and/or damp and foam cleaning in exactly defined areas. And in all the public not only expects but demands a safe food supply chain, starting with the producer and on down to retailer of the products. Each surface in kitchen needs a complete different way of handling. We have to consider everything- can we use disinfectants when washing dishes? Can we use neutral detergents when handling floor? We have to consider that all surfaces are together in **one circle**. The aseptic of kitchen comes in. One must not cross-contaminate the other. Not only cross-contamination is the one we have to think of. The following three points must be considered when choosing cleaning agents and disinfectants: 1) ecology and environment, 2) ergonomics and 3) economy. We cannot solely consider the cleaning agents and disinfectants from the chemical point of view and some of the factors are listed below in this paper.

Water can dissolve sugars, salts, many proteins, carry sand particles, fibres and other dust, emulsify many natural fats, like milk, coffee and skin fat, because those come with their own emulsifying agent. Cleaning with plain water, without any additives, is not new in the cleaning industry. Since microfibre materials were introduced, the branch has gotten used to the idea and was convinced by the results, both in general cleaning and in floor mopping- water is a very versatile and effective cleaning agent itself. To enhance the cleaning power of water, one can use mechanical force, like in scrubbing. Another option is to use hot water or we can add cleaning agent and spend more time on the cleaning action. From here we have four ways for cleaning with water:

- *Cold-warm-hot condition*. Normal tap water contains dissolved minerals that bind chemical agents, neutralizing their cleaning power.
- *Demineralisation*. Environmental concerns force machine manufacturers to invest in technological innovations that allow scrubber dryers to run on less or no

chemicals whilst achieving adequate cleaning results. Stationary demineralisation system consists of an ion exchange unit with mixed bed resin cartridge of a scrubber dryer. The tap water is demineralised in the ion exchange unit during the filling process. This water is afterwards being enriched with minerals. The cleaning effect of demineralised water in comparison with normal tap water is based on this effect. Floors that are not extremely dirty can be cleaned with scrubber dryers using clear water.

- *Electrifying*. Two electrodes are placed in the water; at one electrode, acidic water is made with oxygen bubbles (residual gas) and at the other, alkaline water with hydrogen bubbles is formed. This application water electrolysis is used to electrically convert normal tap water to perform as cleaning detergent (based on alkalinity).
- *Steam*. The use of saturated dry and wet steam in cleaning can reduce use of chemicals by more than 90 per cent and water by up to 95 per cent. Energy resources are needed for making the steam.

Detergents are 'helpers', which completes the co-operation with temperature, cleaning method and tool. Cleaning agents and disinfectants in particular need some time to become effective. This is no problem in dishwashing or laundering. In surface cleaning without pre-treatment, cleaning agents have only limited effect. Therefore, it is advised to spray cleaning agent in advance. Chemicals are used that reduce the surface tension of water, the force that holds the water droplets together. Reducing the surface tension allows the water to more easily spread on a surface. In a study on microfibre and traditional cloths initiated by the Dutch Association for Cleaning Research VSR and performed at the Dutch institute TNO, it was shown that chemical agents enhance the cleaning effect of a traditional cloth only by a few per cent of usual stains, like coffee. The main function of chemical agents is to enhance the wetting the surface. Detergent-free cleaning products have also been developed. These products are made from naturally generated ingredients and avoiding the use of surfactants and phosphates.

Cleaning agents in excess increases both costs and environmental burden. Too much cleaning agents are used even in the most professional cleaning companies. This leaves residues on the „cleaned" surface. After repeated cleaning, a visible haze can be formed. Residues can cause dirt to stick to the floor and the surface gets slippery when wet. The website www.isditproductveilig.nl helps domestic users how to interpret information found on the product labels. But for professional users, this information may be insufficient, in particular when larger quantities of cleaning products are being

transported or stored. This type of information can be found on the Material Safety Data Sheet (MSDS). Employers are obliged to instruct their cleaning staff how to safely use cleaning products. The professional users are advised to translate this information to his staff using workplace instruction cards. The website uk.cleanright.eu provides consumers with help to understand and correctly use the cleaning and maintenance products available.

With microfibre cloths we get rid of most dirt, harmful bacteria, oils, acids, carbohydrates (sugar, fruit juices), proteins (blood, milk) and non-soluble dirt (fat, fingerprints, lipstick). In cleaning with microfibre cloths water and few drops of detergent is needed. The microfibre relies upon special characteristics to facilitate optimal soil pickup. The microfibres will struggle to remove dirt e.g. mineral oils and rubber stripes from shoes and tyres are. Traditional cleaning cloths and mops, characterised by their fibreless weave, have less surface area in contact with items cleaned. Tests with traditional wet mops using detergents has shown a reduction of bacteria by approx. 30%, while tests with damp mops have shown a reduction up to 99% of bacteria.

EFFECTIVENESS OF HACCP SYSTEMS IN EGG PRODUCTION AND DISTRIBUTION

Jana Ramus & Peter Raspor*
Chamber of Commerce and Industry of Slovenia, Ljubljana, Slovenia
*University of Ljubljana, Ljubljana, Slovenia

The study presents and discusses the results of quantitative research of factors that have impact on food safety inside the whole egg' food supply chain. Results were collected via anonymous questionnaires in the year 2006. 233 responders on questionnaires were collected by farmers producing hens' eggs, industry producing hens' eggs, food industry producers using eggs as row material or ingredients and by catering units and retailers. Questionnaires were adapted to workers, food safety experts and representatives of board for each mentioned group. Additionally some questions were addressed to a small group of veterinary inspection in primary sector and also some food safety consultants from different institution that help the companies to implement HACCP system or they make a training and education courses in the companies. 12 questionnaires with similar questions were formed to obtain opinions of all stakeholders from different point of view. The first part of questions was related to current situation on efficiency of HACCP system implementation and good hygiene practice within companies, which produce eggs and products including eggs. The second part was oriented to food safety training and education and how this is organized inside the companies. In the third part the cooperation and communication between workers, experts and managers within companies as well as communication forward to authority and consultants institution were discussed. In the fourth in the fifth part some weaknesses and causes regarding food safety issues were handled.

The implementation of preventive HACCP system to food plants is regulation demand in all European countries. By implementation and effective maintaining of HACCP system in practice, the food plants meet many barriers. Through the questionnaires was trying to fond out which barriers are in egg' production and distribution. With the research was confirmed that efficiency of HACCP system in food supply chain is depend on consciousness, understanding and knowledge on food safety area of all

employees in the primary and secondary sector. For successful reestablishment principles of HACCP system and good practices and for successful implementation of those principles the good understanding and active cooperation between food companies and other institutions in process involved are needed. In the frame of the research was found out that in eggs production and distribution there are some deficiencies which influenced on HACCP system respectively food safety. The level of understanding of HACCP principles was different within different sectors of food supply chain and between levels inside food companies. HACCP system was not completely adapted to existent situation inside companies and to their work. There were also not recognised the benefits of the system' prevention approach. There were also found out that employees have lack of knowledge about food safety and there were also some difficulties in organisation and realisation education and training courses inside food business companies. Unenviable was also situation about communication between different working levels within company, between the same branch companies, between different branch companies, professional institution and forward consumers. Gaps of food handlers' knowledge were found especially concerning microbiological hazards on eggshell and egg's content. On the side of authority and consultants higher expectation regarding food safety issues were found compared to companies, especially on those connecting with investments. Upon opinion those who were cooperate in the research there were also some gaps in the consumer knowledge about food safety which could contribute to increase number of food borne diseases. All the weaknesses mentioned above could have bad influence on HACCP system operation in food production and distribution. They could also decrease level of food safety guarantee by the consumers. The results of the research showed that these weaknesses could be result of unsatisfied workers without motivation to work properly and with lack of knowledge about food safety. It was confirmed that lack of knowledge and awareness of own responsibilities food business operators in food production and distribution, bad cooperation and exchange information between food supply chain sectors and other institution involved in the process, present the main barriers for efficiency of the HACCP system. The main conclusion is that effectiveness of HACCP system depends on the human resource management and employees' satisfaction. This indicates that human resource management should become an integral part of HACCP system. All the findings in the frame of the research could help to avoid weaknesses in the food supply chain and they can contribute to better communication and trust within food safety partners.

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FOOD SAFETY RISK MANAGEMENT IN BAKERIES

Helen Ehavald
Fazer Bakeries AS, Tallinn, Estonia

Bakery products are an important part of balanced diet. Wide variety of bakery products such as wheat loafs, rye breads, buns, coffee breads, cakes, cookies, pizza etc are produced and sold in supermarkets. The traditional strategic approach to food safety risk management in bakeries is to implement practices based upon food storage, cleaning and sanitation, pest control, personal hygiene, maintenance program etc. Food hygiene deals with ensuring that food is safe to eat and it should cover all aspects of processing, preparing, transport, handling or serving. It is vital throughout the whole food chain.

Bakery products, like many processed foods, are subject to physical, chemical and biological spoilage. The main routes of contamination are through surfaces, air, water, people and pests. Physical hazard could be pieces of glass, wood, metal, plastic, film, human hair and fingernails, plasters, jewellery, small personal belongings, pests, paper, cardboard etc.

Foreign body detection equipment like sieves, magnets and metal detectors are in use to maximize foreign body detection and to protect customers. Equipment and buildings should be maintained to minimize the risk of product contamination because of floors, walls, ceiling, non-contact surfaces and equipment. It is important to ensure that employees itself does not become a source of contamination. Therefore company should ensure that personnel, who can affect product safety, legality and quality are appropriately trained, supervised and all hygiene rules are followed.

Chemical hazards could be residues of cleaning chemicals, if they are not adequately rinsed after cleaning and disinfection process. Machinery lubricants may contribute to chemical contamination and also synthetic preservatives, for example if the dosing amount exceeds the permitted limits. The use of preservatives should be under control and here again company should provide relevant training as appropriate.

Raw material and product can be contaminated by biological objects like microorganisms and pests. Birds, insects and rodents are potentially a major contamination problem in bakeries. A preventive pest control program shall be maintained covering all areas of plant to minimize pest infestation. Production building should be designed to keep pests out. Ceilings and walls should be designed so that they do not allow insects to live there. During dosing, mixing, molding, cooling and slicing flour dust spreads easily through the air of bakery, therefore the cleaning and disinfecting of floors, walls and equipment is very important. Silos, ingredient dosing systems, mixers, curling chains, conveyor belts, cooling conveyors, packaging machines etc. should be properly cleaned to prevent any infestation outbreaks. In some bakeries the flour handling area is separated from the cooling and packaging area of the finished bread. Good hygienic design, maintenance and an effective cleaning program is very important support to the pest control program and help to prevent the contamination of raw material and product.

Spoilage of most bakery products is caused mainly by moulds, yeast and seldom by bacteria. Reservoir of microbiological contamination can be ingredients, humans, inadequately cleaned equipment and utensils, air, water, packaging material etc. Employees should be trained to understand the risk associated with cross-contamination from raw foods and dirty surfaces coming into contact with equipment, clean surfaces and ready product. Regular and effective hand washing, appropriate and clean clothing is critical to the safety of products, each defined critical control point should be under control.

The most important ingredient of bakery products is flour. It has been estimated that flour contains approxi. 8000 mould spores in 1 g. Flour for instance can be contaminated by moulds and yeasts – species of the genera *Penicillium, Aspergillus, Rhizopus, Eurotium, Fusarium, Cladosporium,* bacteria – mainly of the *Bacillus* species, *Pseudomonas, Streptococcus, Achromobacter, Flavobacterium, Micrococcus,* and *Alcaligenes* and mycotoxins – *Alternaria* toxins, Aflatoxins, Citrinin, Cyclopiazonic acid, Ochratoxin A, Viomelein, and Xanthomegnin. Other bakery ingredients may be also a source of microbial contamination.

The most common bread spoilage moulds are *Penicillium* spp. and *Aspergillus* spp. *Rhizopus (nigricans) stolonifer* is the common black bread mould. *Penicillium* spp. and *Aspergillus* spp. can both produce mycotoxins, which are very resistant and can survive heating process. *Bacillus subtilis* and *Bacillus licheniformis* can cause ropiness in wheat bread; *Bacillus cereus* may survive baking process, because *Bacillus* species can

form endospores. Rope is now rare because of adding preservatives and good bakery hygiene practice. A major reservoir of *Staphylococcus aureus* are humans and some outbreaks have been involved with bakery products, for example filled pies. Raw material, pies, pizzas and cakes should be stored according to the instructions and refrigerated if needed, good hygiene practice also helps to prevent such cases. It is also very important to keep the cold chain of frozen bakery products.

Most bakery products, in general, are not considered as high-risk food products because baking at relatively high temperatures is involved in their preparation. Many bakery products have reduced water activity (a_w) and pH, which also prevent the growth of microbes. An average shelf life of bread is 3–5 days, but if the hygiene and sanitation of a bakery is poor, the shelf life of bread, especially some wheat bread, can be shorter. Preservatives, sourdough, modified atmosphere packaging (MAP), vacuum packaging, microwave and infrared radiation are methods to control the microbial spoilage.

Documented cleaning and sanitation procedures shall be in place for the building, plant and equipment. Good hygienic design of production rooms and equipment prevents the contamination of product and simplifies the cleaning of equipment. Proofing conditions (temperature 30–40°C and humidity 60–80%) are appropriate for the growth of bakery yeast, but also for moulds. Proofing cabinet needs to be cleaned regularly. According to the literature more than 90% of contamination of bread occurs during cooling, slicing or wrapping operations. It is recommended to disinfect slicing and packing machines, to control the hygiene of employees and to take microbial samples from production air, equipment, crates and employees.

For example, according to literature benzalkonium chloride was efficient against most fungi. Some fungi showed to be resistant towards alcohols, but 3% hypochlorite efficiently eliminated them. Quaternary ammonium compounds, 70% ethanol, 70% isopropanol and 30% hypochlorite were effective on yeasts. It is suggested to use at least two different types of disinfectants in rotation during a week to prevent the resistance problems. Problems with mould usually occur more in spring and summer.

To guarantee the safety of a product, good manufacturing practices (GMP) and good hygienic practices (GHP) should be followed. According to HACCP principles all potential hazards should be identified, evaluated, documented and monitored. Some companies have certified their quality management system according to ISO 9001, ISO 22000 or BRC standard and food safety system is an important part of it.

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RISK MANAGEMENT IN A READY-TO-EAT MEAL FACTORY

Asli Kisikkaya
Tübitak Marmara Research Centre, Gebze, Kocaeli, Turkey

Ready-to-eat (RTE) food means food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level microorganism of concern. Foodborne disease outbreaks linked with RTE foods have been associated with various foodborne pathogens. This may in part be attributed to a change in commercial food production such as minimal processing as well as changing consumer demands for ready-to-eat meals. The modern way of life relies heavily on the availability, quality and safety of RTE foods whereas the quality of the starting materials is always of major importance, factors such as handling, processing, transportation and storage can influence the microbial composition of the finished product at the consumer's table.

Food poisoning follows the ingestion of microorganisms that may have been present in already contaminated food, which may have resulted from inadequate food preservation techniques or unsafe handling practices or which may have arisen from cross-contamination from surfaces, equipment, or, less likely, from persons who carry enterotoxigenic staphylococci in their noses or on their skin. In Turkey, there are many issues imposing risk on food safety due to industrialization and mass production, emergence of longer and more complex food chains, fast food consumption, street vendors and growing international trade and tourism. In recent years, public corporations and enterprises in Turkey have attracted attention on the fact that they have oriented themselves towards the policy of handing over food service to private professional institutions with a view to improve the hygienic and taste qualities of foods, and to save labour in Turkey.

The RTE food products provide a source of readily available and nutritious meals for the consumer. However, questions have been raised about the safety and microbial quality of these food products. Since outbreaks of illness in human beings are understood to be caused by consumption of contaminated vegetables, several reports

have been published that describe the bacterial contamination of RTE vegetables. Red meats, poultry, sea foods and vegetables have been documented to serve as vehicles for several bacterial pathogens and foodborne outbreaks have been associated with the consumption of contaminated RTE foods. Other enteric pathogens have been isolated from RTE foods including verocytotoxigenic *Escherichia coli* (VTEC), *Salmonella* spp. and *Campylobacter* spp., emphasizing the risk posed by consumption of these foods.

Risk management in a ready-to-eat meal factory includes consideration of elements that maybe cause risk in the process. As the application of microbial criteria is only one of several management activities to ensure that ready-to-eat foods are of low risk for humans, application of GHP in combination with HACCP should be consistently applied to minimise the initial contamination at manufacturing level, or reducing the potential growth of pathogens and reducing the level of indicator micro-organism. In an HACCP plan for the production of ready-to-eat products, there will never be zero risk. Even though all prerequisite programs are in place such as employee training, certification of ingredients mistakes will happen. The best that management can do is to reduce the chance of a mistake by performing a risk analysis. Risk analysis lays out three steps. First; one performs the HACCP system, procedures, hazards and validated controls for technical management and consumer risk communications. Next; one looks at the risk analysis such as ways that the controls might fail. Management takes action to reduce the risk of failure. The food safety program is then documented in the RTE food factory in HACCP Total Quality Management Manual:

- ***Management – Good Manufacturing Practices*** deals that operational controls validated as capable of very low food safety risk are used by everyone on a daily basis. Management also provides the money necessary to keep the equipment and facilities in adequate operating condition.
- ***Personnel*** work in the facility; this task includes personal hygiene.
- ***Environment*** means control of the environment outside the building, including rodents, insects, contaminated air, and safe water and sewer systems.
- ***Facilities*** include walls, floors, and ceilings, which keep the contaminated environment outside and allow the food to be produced in a semisterile processing plant.
- ***Equipment*** must be designed to be easily cleanable. Floor drains are included in this category, as are equipment corners and niches, which must be scrubbed regularly in order to eliminate accumulation of spoilage microorganisms.

- ***Supplies and foods:*** The supplier certifies the levels of pathogenic substances on the food. If the food is to be processed without any pathogenic substance control, the supplier must certify that the incoming produce is safe to eat without any hazard control procedure because the supplier did the hazard control. During the process; the food is stored, pasteurized, and cooled. Cooking reduces the pathogenes to a safe level, must be based on the sensitivity of customer being served. If the customer removes the food from the facility, labels on the take-out containers warn the customer not to abuse the food; otherwise, spores can grow, thus making the food toxic and harming the customer. Important for the RTE food factory consumption patterns is also age and gender classes.
- ***Modelling the production of RTE food factory – to consumption chain:*** At the earliest stages in the production of a food that can include the effect of the environment. For instances; green vegetables or berry crops might be affected by contamination from soil, silage and the pathogens in them. Pathogens may survive in manure or soil for long periods; inside protozoa; and some may also penetrate the vasculature of leafy plants like lettuce, and alfalfa or mung bean seeds.
- ***Production:*** After harvest, preliminary washing or cleaning of the product may remove some of the initial contamination. Transport may introduce additional or new pathogens. At each of the succeeding stages of production, changes in prevalence and concentration are likely to occur. However, unless actual measurements are taken at each these stages, they must be modelled based on the knowledge that already exists.
- ***Processing and packaging:*** Production steps include holding, mixing, fermentation, heating, pasteurization, brining, smoking and pickling. Some of these steps increase, but most decrease, the prevalence and concentration of pathogens. Much of contamination arises from environmental contamination in the processing plant. For example, aerosols from cleaning water and dirty equipment may be sources. Cooked products, e.g. processed RTE meats, should be free of *L. monocytogenes,* but may become re-contaminated during subsequent handling and contact with equipment before final packaging. Slicing operations appear to be common sources of re-contamination of cooked products.
- ***Transportation:*** Changes in the frequency of contamination can occur after final packaging for products that remained sealed until consumption. The number of microorganism can increase if the food and the storage conditions support the growth of the microorganism.

- ***Retail:*** Changes to populations of the microorganisms can take place during storage and display. The prevalence and levels of a pathogen may change through recontamination from portioning of the opened packaged products through slicing, chopping and then repackaging. Other packages or other RTE foods then may be cross-contaminated by the same process. Ambient temperatures can permit the growth of the pathogen on contaminated slicing equipment, cutting boards, etc., and could increase the level of hazard.
- ***Home and foodservice:*** For foods that support growth of pathogens, time and temperature of storage are the most critical parts of this stage since RTE products may be kept refrigerated for long periods. In addition, cross-contamination to opened RTE food packages may occur in the refrigerator from other foods. For some RTE foods that do not support its growth, such as dry fermented sausages. If there is no final heating step prior to eating, as is the usual case for RTE foods, the concentration of pathogen or the level of indicator microorganism at the end of the storage period in the home or foodservice establishment will be the concentration when the food is eaten.

The magnitude of the contaminating flora in RTE foods at the point of sale depends on the microbial quality of the starting materials, environmental niche, the overall conditions of hygiene during manufacture, packaging and the transportation process. It may be advisable therefore, to take into consideration the contaminating microflora counts and different stages, upon evaluating the quality of RTE foods. The great importance is the use of Good Hygiene Practices and HACCP systems incorporating risk assessment by the food industry to ensure that microorganisms are eliminated or minimized to an extent that they can not cause harm to human health and official controls to audit compliance by food business operations.

RISK MANAGEMENT IN PUBLIC CATERING ESTABLISHMENTS

Aija Melngaile
Latvian University of Agriculture, Jelgava, Latvia

Assurance of food quality and safety is essential prerequisite for consumer protection. In situations, when group food-borne infections arise, catering establishments were more often officially recognized as place of origin of disease. The epidemiological data suggest that food preparation process is associated with microbial contamination risk, and pathogenic microorganisms enter food as a result of contamination. Results of food safety assurance depend on application of adequate methods and resources during implementation process of good manufacturing practice and good hygiene practice. Food safety is based on HACCP principles. Development of preventive food safety assurance systems comprises both the identification of food hazards and the introduction of regular monitoring measures in critical control points of technological processes.

Management of processing in food establishments should be associated with detailed analysis of products, processes and process conditions to evaluate their impact on quality and safety of end products. To take a decision about control measures that are necessary within stages of technological process, application of risk analysis is recommended in food establishments. The results of risk assessment that were obtained with help of methods of mathematical statistics ensures scientific justification of control measures during technological processing of food, improves efficiency of risk management measures and promotes communication between persons engaged in risk management.

On the base of theoretical aspects of food quality and safety management the hypothesis of scientific research has been stated: the methodology of risk analysis can be used in public catering establishments to ensure efficient control procedures of microbial contamination within technological processes of food preparation and to avoid outbreak of group enteric infectious diseases. To verify the hypothesis, the aims of the research were to investigate and to evaluate application of microbial risk analysis in public catering establishments and the following tasks were carried out: 1) to identify microbial hazard that results from technological processing; 2) to assess

exposure to influence of microbial hazard related to food consumption; 3) to characterize microbial hazard in foods and on surface of environmental objects in different public catering establishments; 4) to characterize risk of food-borne group infectious disease outbreaks in public catering establishments & 5) to evaluate efficiency of self-control procedures and to highlight proposals for improvement of microbial risk management and communication processes in public catering establishments.

Trends in spread of microbial contamination of ready-to-eat (RTE) foods were analysed using methods of mathematical statistics. Aerobic Plate Count and data on presence/absence of coliforms, *Staphylococcus aureus* and *Salmonella* spp. contamination were used to describe microbial contamination of foods and environmental surfaces. Results on microbial testing of 17192 food samples and 17604 surface swab samples that were obtained from catering establishments in frame of state's monitoring programs, the data of 90 reports on outbreaks enteric infectious diseases in catering establishments, as well as data on microbial testing of food and surface swab samples in frame of self-control procedures were analysed using mathematical statistics methods. The statistical data were processed by using of SPSS software package SPSS 13.0. Encoding of the data and information was made for statistics purposes: 1) food samples were grouped into 14 identification classes, 31 groups and 142 types, taking into account the food main components and characteristic methods of technological processing; 2) environmental objects were grouped into 16 identification classes, 90 groups and 187 types, taking into account the characteristic application of equipment, utensils, constructions and other objects. Environmental samples that had not precise description within the Database and Work Papers were denoted as "unnamed" objects within certain identification class, group or type of environmental objects; 3) methods for technological processing of foods were grouped into 18 groups taking into account the extent and way of technological processing, including characteristic sequence of technological processes during food preparation processes; 4) public catering establishments were grouped into 23 groups according to the classification system of catering establishments that was used within the Database and Work Papers of the Food and Veterinary Office & 5) analogous grouping system was used for mathematical analysis of state's monitoring data, epidemiological data, and self-control data of public catering establishments. Based on the results obtained the following was concluded:

- Overall microbial contamination risk and probability of coliforms and *S. aureus* contamination of RTE foods was substantially dependent on the processing. Higher contamination risk was demonstrated for foods that were prepared using cold processing – both the mechanical processing of thermally unprocessed foods and

the mechanical processing of thermally processed and chilled foods. Nevertheless, certain microbial contamination was also found after thermally processing of foods, including boiling and frying of foods.

- Overall microbial contamination risk of RTE foods, including coliforms and *S. aureus* contamination risk was substantially dependent on nature of main food ingredients, e.g. different foods with meat components showed higher contamination levels. High contamination risk was demonstrated for salads with components of both animal and plant origin.
- A higher probability of microbial contamination was proven for fried and braised meat and offal foods, especially for fried poultry and fried minced meat foods; pasta foods with meat components; pancakes with meat or curd stuffing; soups using milk basis; rissole soup; raw vegetable salads; cooked vegetable salads; meat and fish entry foods; certain sweets – dessert creams, mousse, sweet porridge; pastry foods with cream stuffing and muffins.
- Comparison of public catering establishments showed that higher microbial contamination risk of RTE foods was characteristic for open-type public caterings e.g.restaurants, cafes, and canteens. Comparison of educational establishments suggests higher contamination risk of foods within all identification classes and the risks in many RTE food groups in general educational establishments were similar to those in open-type public caterings.
- Risks of surface contamination with coliforms and *S. aureus* were substantially dependent on characteristics in both environment and processing. Relatively higher surface contamination risk was proven for storage, display and serving inventory of RTE foods, cutting inventory for preparation of chilled foods, worktables, and personnel hands.
- Presence of coliforms and *S. aureus* contamination on surface of environmental objects was demonstrated in kitchens of vocational schools, general educational establishments and children social care establishments, and in open-type public catering establishments e.g. fast food establishments, cafes and bars. In different public catering establishments substantial differences were revealed for surface contamination risk regarding coliforms, but substantial differences were not detected for surface contamination risk regarding *S. aureus*.
- Different foods belonging to 13 food identification classes, 24 food groups, 40 food types and prepared using 13 different food processing methods, have promoted outbreaks of enteric infectious diseases. This type of diseases was more often promoted by pastry foods with cream stuffing, meat salads, cooked vegetable salads made with mayonnaise sauce and raw vegetable salads made with oil, as well as by

desert creams, including curd cream. If thermally processed foods were compared, enteric infectious diseases was more often promoted by fried poultry and minced meat foods and minced meat sauce.

- Evaluation of the methods of food technological processing demonstrates that outbreaks of enteric infectious diseases were more often promoted by foods that were prepared using cold processing. Nevertheless, food preparation based on boiling, frying, braising, deep-frying in oven and combined methods with final stage of thermal processing promoted enteric infectious diseases.
- During epidemiological investigation the presence of coliforms was demonstrated on surface of 42 different environmental objects. The results suggest about poor hygiene practice in public catering establishments. Coliforms were often found on surface of food processing inventory, worktables, food storage inventory, hands and personnel coveralls.
- Episodically testing of both the RTE food samples and aggregated environmental swab samples in frame of self-control procedures of public catering establishments does not ensure information on trends in distribution of microbial contamination that was necessary for verification of HACCP procedures and implementation of corrective measures. Considerably lower food and surface contamination of coliforms and *S. aureus* on personnel hands was found in self-control procedures than in state's monitoring program results.
- Application of the *Petrifilm* rapid test methods for identification of microbial contamination on environmental object surfaces and for operative assessment of the efficiency of sanitation measures was effectively improving the control of microbial contamination in public catering establishments.
- *Salmonella* spp. contamination on environmental object surfaces was often negative and did not reveal the trends in distribution of microbial contamination within technological processes. Testing of hygiene indicators can be recommended in controlling efficiency of hygiene measures to reveal contamination sources and to ensure preventive risk management arrangements.
- Results on microbial risk assessment ensure science-based guidelines for improvement of both the self-control procedures and the state's monitoring and control procedures in public catering establishments, including setting of priorities for control of microbial hazards. Methodology of risk analysis can be recommended for analysis of trends in transmission of microbial contamination, purposeful implementation of procedures for control of contamination, and for timely prevention of foodborne enteric infectious disease outbreaks in public catering establishments to promote public health protection.

RISK ASSESSMENT OF MICROBIAL CONTAMINATION ON CARCASS SURFACES

František Šišák
Veterinary Research Institute, Brno, Czech Republic

During plant processing, carcass surfaces of broiler chickens and fattening pigs were contaminated with a wide spectrum of microorganisms by both vertical and horizontal transmission. The microorganisms include aerobic, facultative anaerobic and strict anaerobic bacteria which differ not only in species but also in bacterial counts. The amount of saprophytic – spoilage bacteria is of essential importance for microbial quality of fresh meat and its shelf life. Carcass surfaces can also be contaminated with foodborne pathogenic bacteria. With regard to the EU food safety regulations, *Salmonella* spp. and *Campylobacter* spp. appear to be the fundamental causative agents of zoonoses followed by *Yersinia enterocolitica* and *Listeria monocytogenes*. The primary cause of carcass contaminations with pathogens were animals originated from infected herds and flocks. A correlation was found between positive culture from carcasses and samples collected from intestinal tract and lymph nodes. Another very frequent way of pathogen spreading is the secondary contamination of carcasses which occurs when the logistic slaughter is broken, i.e. flocks or herds tested as positive were not slaughtered after flocks or herds which have tested negative. Thus carcasses of broiler chickens from non-infected flocks and swine carcasses from non-infected herds of fattening pigs can be cross-contaminated with pathogens during particular technological processes. Logistic slaughter can be an efficient measure for limiting cross-contamination of carcasses. Pathogenic microorganisms can also survive in biofilms on technological equipment which can be the source of contamination. Carcasses of broiler chickens and fattening pigs present a potential health risk to consumers because of quite frequent contamination with Salmonella and Campylobacter. Health risk to consumers depends on both the prevalence and the level of contamination of carcasses and ultimately on the RTE food. Risk assessment (RA) of carcass contamination with foodborne pathogens is at present a standard tool for veterinary public health inspection in EU member states. This tool has been used for identification and emergence risk analysis at the slaughterhouse level. The objective of

RA analysis is the implementation of effective intervention measures for reduction of prevalence of foodborne pathogens in carcasses, thus minimizing public health risk. In general, risk has been defined as a function of likelihood that hazards in foods will have an adverse impact on human health. Risk assessment can be undertaken qualitatively or quantitatively. Qualitative assessment includes a scale evaluating the risk as negligible, low, medium, and high. Quantitative assessment uses complex mathematic models which have been developed for particular foodborne pathogens in different commodities. Microbial risk assessment is a four step process which includes (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization. The main goal of RA is to provide science-based data for risk management. A current risk communication exists between risk assessment and risk management. RA of microbial contamination in carcass surfaces is an important part of a good manufacturing practice (GMP) and HACCP system in the production chain of poultry meat and pork.

RISK MANAGEMENT OF FOOD PRODUCTION WATER SUPPLIES

Mehlika Borcakli
Tübitak Marmara Research Centre, Gebze, Kocaeli, Turkey

Water is essential in the food industry as an ingredient or as part of a process. Food processors require large quantities of water for different operations, including: food manufacturing, cleaning, ice making, steam production and product transport. Food contact water should have drinking water quality. Food processors must ensure the water and water systems in their facilities are safe and meet national quality standards. However, water in nature is never “pure”. It picks up bits and pieces of everything it comes into contact with, including minerals, silt, vegetation, fertilizers, and agricultural run-off. While most of these substances are harmless, some may pose a health risk. Authorities and governments develop guidelines that set out the maximum acceptable concentrations of these substances in drinking water. These drinking water guidelines are designed to protect the health of the most vulnerable members of society, such as children and the elderly. The guidelines set out the basic parameters that every water system should strive to achieve in order to provide the cleanest, safest and most reliable drinking water possible. Guidelines for Drinking Water Quality deal with microbial, chemical and radiological contaminants. They also address concerns with physical characteristics of water, such as taste and odour.

WATER SOURCES

Most food processors use water from city and municipal suppliers or from wells. Processors are responsible for ensuring the water supply quality using a water testing system. Authorities recommends processors to test their city, municipal or well water supply within required periods in a year to ensure it meets provincial and national quality standards (American Public Health Association, 1999). Processors using river or other natural source waters must ensure that the water supply is properly treated and meets provincial and national quality standards. Water contaminants in processed food, including biological, chemical or physical agents, can affect the health of consumers (Guidelines for Canadian Drinking Water Quality, http://www.gov.mb.ca/watersteward ship/odw/index.html). Enteric pathogens (bacteria, viruses and protozoa) are the main

source of biological contamination. They can exist naturally or can occur as a result of contamination from human or animal waste. Bacteria will not multiply in water if the water does not contain a source of nutrients (U.S. Environmental Protection Agency, 1999). Waterborne pathogens include bacteria (*Aeromonas, Campylobacter,* coliforms*, *E. coli* 0157:H7, *Legionella, Salmonella, Shigella, Yersinia, Vibrio* and *Staphylococcus* (*coliforms are a group of bacteria found in human and animal faecal material, soil and untreated environmental water; it includes strains of *Escherichia* (including *E. coli* O157:H7), *Klebsiella, Enterobacter* and *Citrobacter*. It is easy to destroy coliform bacteria by disinfecting water. Their presence in water indicates a treatment failure or lack of treatment. A total coliform count is used to indicate hygiene levels and the possible presence of enteric pathogens in water)), viruses (enteroviruses, hepatitis A and noroviruses) and protozoa (amoeba, *Cryptosporidium, Cyclospora, Giardia,* roundworms and toxoplasma).

Food processors must have a system that ensures they are using safe, potable water in food production. Steps involved in water safety analysis include water sampling and microbial testing. In the water sampling water in a food facility should be tested from different outlets at regular intervals. A minimum of 200–500 ml water/sample is required. The microbial analysis should include total coliform counts. Escherichia coli is the most specific indicator of faecal contamination and the possible presence of pathogens. The microbial analysis should also include the heterotrophic plate count, which estimates the number of living bacteria equiring carbon for growth. In the interpretation of microbial results water test results need to be interpreted with caution. Pathogens do not distribute uniformly in water, so a negative result does not guarantee the absence of pathogenic bacteria.

Chemical contamination of water can occur from chemical spills, incorrect use of pesticides, improper water treatment or improper disposal of industrial waste into waterways. Harmful chemicals in water can cause adverse health effects and examples of toxic chemicals include: pesticides, mineral salts (nitrates, copper, sulphates etc.), heavy metals (arsenic, lead, mercury, cadmium, silver, iron etc.), volatile organic compounds (phenols etc.), asbestos, organic chemicals and radionuclides (uranium, radium, radon etc.). Maximum acceptable concentrations for chemical contaminants are established by National Guidelines for Drinking Water Quality. Unpleasant taste or odours are most likely caused by organic substances e.g. decaying vegetation, algae or organic chemicals containing carbon.

Physical contaminants of water pose a low health risk to consumers but may affect the quality of water. Water may contain suspended particles of fine sand, clay and

precipitated salts. This cloudiness is called turbidity and can interfere with effective disinfection and purification of water.

HOW CAN THESE WATERBORNE ILLNESSES BE PREVENTED?

Municipal drinking water treatment providing filtration and chlorine disinfection can reduce the risk of contracting giardiasis and cryptosporidiosis. Chlorine by itself is not effective against *Cryptosporidium* but it can inactivate *Giardia*. Research on ultraviolet light has shown inactivation of both organisms. An effective possibility in case of coliform contamination may be to install an ultraviolet disinfection system, which kills both bacteria and other microbes by denaturing the DNA or RNA of their cells. This works well because it does not change the chemistry of the water like some other disinfection methods, such as chlorination, but UV-light is not going to remove the chemical contaminants.

Most food processors use water from city and municipal suppliers or from wells. In case of testing results from a well show an unacceptable level of total coliforms or *E. coli*, it is necessary to shock treat the well and, if possible, find and eliminate the source of contamination. Disinfection can be done using unscented household bleach. Table 1 outlines the quantity of bleach required to properly disinfect new and existing wells, in case the contamination cannot be found and eliminated, the water should subsequently be disinfected continuously (Guidelines for Canadian Drinking Water Quality).

Table 1. Well water disinfection using household bleach (5.2% hypochlorite).

Depth of water in well	Volume of bleach added			
	Casing diameter 15 cm (drilled)		Casing diameter 90 cm (dug)	
	New well*	Existing well*	New well*	Existing well*
1.0 m	100 ml	20 ml	3.2 l	0.6 l
3.0 m	300 ml	60 ml	9.8 l	2.0 l
5.0 m	500 ml	100 ml	16.5 l	3.0 l
10.0 m	1000 ml	200 ml	32.0 l	6.5 l

* In new wells 250 ppm chlorine for effective disinfection whereas existing wells require 50 ppm

Disinfecting our drinking water ensures it is free of the microorganisms that can cause serious and life-threatening diseases, such as cholera and typhoid fever. However, chlorine also reacts with the organic matter, naturally present in water. This chemical reaction forms a group of chemicals known as disinfection by-products which the most

common of these are trihalomethanes (THMs), which include chloroform. Although other disinfectants are available, chlorine remains the choice of water treatment experts. When used with modern water filtration methods, chlorine is effective against virtually all microorganisms. Chlorine is easy to apply and small amounts of the chemical remain in the water as it travels in the distribution system from the treatment plant to the production line.

A number of cities use ozone to disinfect their water, because ozonation does not produce THMs. Although ozone is a very effective disinfectant, it breaks down quickly and cannot be used to maintain disinfection in the distribution system. Small amounts of chlorine or other disinfectants still must be added. Examples of other disinfectants include chloramines and chlorine dioxide (ClO_2). Chloramine is a weaker disinfectant than chlorine, but is very effective in the distribution system. ClO_2 can be used in the treatment plant, but it is not very effective in the distribution system. The environment-friendly agent ClO_2 can be applied in various fields e.g. foods, drinking water and environmental elements. It has effect on a wide range of microbes at various conditions. It shows rapid killing, low corrosive activity, low toxicity, microbial biodegradability, action without hazardous by-products e.g. THM. As a pretreatment ClO2 aids in prevention of carcinogens in drinking water by selectively oxidizing containments that may otherwise be transformed by chlorine into THMs. ClO_2 also acts as a biocide inactivating harmful microbe e.g. *E. coli* and *Cryptosporidium* with minimum contact time.

Electrolyzed water is considered as another means of drinking water treatment. Use of electrolyzed water is the product of a new concept developed in Japan and getting popularity in other countries as well. It has double effect; the acid electrolyzed water that is produced by the same electrolysis process (at the same time) is used as a disinfectant and pesticide and the alkaline electrolyzed water assists in the alleviation of gastrointestinal disorders, acidosis, chronic diarrhoea, and poor digestion. It possesses high bactericidal, virucidal, and moderate fungicidal properties. Studies on this emerging technology have been carried out in Japan, China, and USA in the food processing field. It has a lot of potential possessing environment friendly properties and is cost effective. To produce it, an apparatus is required that utilizes common salt and an electric source. It can be produced at site, as the size of the machine is quite small. Studies have been done on its use as a sanitizer for fruits, utensils, and cutting boards. It can also be used as a fungicide at postharvest processing of fruits and vegetables, and as a sanitizer for washing carcasses of meat and poultry.

PILOT CASE AND WORKSHOP PRESENTATIONS

PILOT CASE I – FOOD SAFETY AND HYGIENE ENQUIRY IN SOME CYPRIOT FOOD INDUSTRIES COMBINED WITH PILOT STUDIES

Savvas Gennaris
Veterinary Services, Nicosia, Cyprus

The hygiene in food industries is an issue of utmost importance since high hygienic levels assure the safety and quality of end products and therefore consumer's health. A survey combined with pilot studies aiming in identifying possible sources of microbial hazards and evaluating the hygienic conditions using different sampling and detection methods was performed in six food producing companies in Cyprus. The outcome was a general overview of the hygienic status concerning also some of the main food pathogens of interest. Results have shown that some hygienic aspects need to be improved inside the Cypriot food industries. The results will also be used to further evaluate the hygienic status of food enterprises and in planning future research needs in the area of food safety and hygiene.

BACKGROUND TO THE FOOD SAFETY AND HYGIENE ENQUIRY

The survey was conducted in April 2007 in six Cypriot small and medium-sized food companies (SMEs) in order to investigate their needs, to focus future European research activities and to incorporate SMEs in the research area of food safety and hygiene. The Cypriot companies involved in this survey were asked to fill in the questionnaire. The questionnaires and hygiene surveys were part of SAFOODNET-project (Food Safety and Hygiene Networking within New EU Member States and Associated Candidate Countries; FP6-022808; http://safoodnet.vtt.fi). The companies that participated in the hygiene survey belonged in primary production, secondary processing or both. All companies were to some extent involved in the distribution of their final products.

Information on the Participating Food SMEs

All the companies involved in the survey had a HACCP system in place. Four of them were certified, whereas the other two were planning to proceed with certification soon. Two companies were in the process of implementation of ISO 22000. All companies except one mentioned that they had had fewer customer complaints after they had implemented these safety and quality systems. Nevertheless all companies believe that the implementation of such systems has helped in improving their products' quality and safety.

The daily time needed to complete the Hazard Analysis Critical Control Points (HACCP) documents varied between companies (1–3 h). All companies mentioned ways to reduce the paper workload within their HACCP system i.e. electronic keeping of documents, proper team work and distribution of tasks, simplifying of HACCP documents, automated/constant recording of parameters and reduction of Critical Control Points (CCPs). All interviewees replied positively regarding their ability of carrying out the different tasks required in their HACCP system. Two mentioned that more resources both in equipment and personnel were needed. All interviewees mentioned that their management had pointed out the importance of having a HACCP system in place. The person responsible for the implementation of the HACCP system was reported to be either the HACCP coordinator, the quality manager, or the production manager.

Raw Materials

The raw materials were either processed or not prior to their arrival depending on the type of activities in each company. The quality of the raw materials was checked in all the companies. The raw materials were inspected visually and tested using analytical methods in all industries. Only one company mentioned that it did not carry out any analytical testing regarding raw materials. It relied only on the certificates provided by the suppliers. Apart from the controls perform by the companies themselves, Competent Authorities may perform sampling and analysis of raw materials based on a risk assessment approach. None of the companies reported significant fluctuations regarding the quality of raw materials.

All companies had specifications for their raw materials based both on legal requirements i.e. *Salmonella* free, antibiotic free, clean hide and heavy metals and quality requirements i.e. production method, size, weight, fat and protein content,

intact packaging and satisfactory labelling. They considered those requirements as adequate. All companies mentioned that they had certain procedures for approving and monitoring their suppliers.

Raw materials that were examined for the presence of any microbial or chemical hazard were quarantined until the final analytical results were available. In the case that raw materials did not meet the quality and safety standards, they were either sent back to the supplier or destroyed. The Cypriot companies considered the efficiency of their safety and quality control systems for raw materials adequate.

Food Production Facilities

The daily work in the food companies was organised in one-shift work programme. The production schedule for each day was made either by the production manger or the company manager. Two companies reported that they sometimes work according to a two-shift work programme. In some companies the second or third shift was responsible for the cleaning and disinfection. In all the companies the main criteria used to decide the order of production were: 1) market/customer needs, 2) availability of raw material 3) production procedures e.g. foodstuffs needing more time for preparation were usually the first ones to be produced and 4) logistics.

The production rooms' temperature was monitored with either constant recording systems or manually recorded using calibrated room temperature thermometers. All companies reported that cleaning was carried out by their own personnel. The decision regarding the time that should be spent on cleaning was made either by the production manager or the personnel responsible for cleaning. Cleaning instructions were available in all companies. Visual checking was generally carried out afterwards in order to verify that the cleaning had been carried out properly. All interviewees reported that there was enough time set aside for cleaning. Two companies though reported that too much time was spent on cleaning. One company stated that the cleaning efforts were not satisfactory enough and that improvement in cleaning procedures was necessary. Most companies reported that they considered having adequate knowledge about efficient cleaning, cleaning mechanisms and agents. One company mentioned that more training was needed in order for the personnel to be in position of performing cleaning and disinfection properly and more information about special types of cleaning methods and mechanisms was necessary e.g. cleaning of plastic transportation crates.

Most of the information regarding the cleaning procedures, mechanisms and methods had been provided by the companies selling the detergents and disinfectants. Generally the decision on which cleaning agents were going to be used was made by the production manager or the HACCP coordinator although there were cleaning protocols available that were set up by company in collaboration with the cleaning agency and sometimes an external consultant. In only one company it was reported that they had difficulties with the cleaning procedures due to lack of knowledge regarding the proper use of cleaning agents and limited time spent on cleaning.

The efficiency of cleaning was checked both visually and analytically. All companies reported that they cooperated with an accredited laboratory that conducted microbial screening for them. Two companies reported that they also had their own microbial laboratory. Three companies reported that they were using the ATP-test method for checking their cleaning regularly. All interviewees reported that they had sufficient knowledge concerning foodborne pathogens and spoilage microbes although they consider that a more focused approach on some microbes of interest and on contamination routes would have been useful. They considered their cleaning results satisfactory, although they reported that there could be some improvements in the cleaning efforts.

The companies participating in the survey reported that training of staff on basic hygiene issues was conducted with regular intervals e.g. every 1, 2, 3, 6 months or 1 year. All training courses were documented. A follow up on issues concerning Good Hygiene Practice (GHP) was made by the production manager, the HACCP coordinator and the manager. Their intervention was made when necessary, either by guiding individuals or by organising common training courses. The language was mentioned as an obstacle in training foreign personnel. The fact that workers in those industries were not permanent made it very difficult to follow up their training progress. Another problem mentioned was the difficultly of repeating detailed training courses just for a few beginners. All companies reported that re-training courses were arranged regularly according to identified needs.

In case of spillage during production all companies reported that cleaning took place immediately. Care was taken to avoid any cross-contamination between spillage material and products. It was reported that the personnel generally respected the hygiene rules although some problems were occasionally reported. Zoning i.e. clean and dirty area, high and low risk area, production and packaging of ready-to-eat (RTE) products,

final products and wastes, applied in all companies. Other measures used to secure safe production were: different storage rooms for RTE and other products, air filtration and strict rules concerning the flow of materials and the personnel inside the factory.

All companies reported that hand washing facilities were appropriate and sufficient. There were also changing rooms available for personnel working in different zones. All reported that the entrance in their premises was controlled and that specific rules also applied for the external waste areas e.g. cleaning and waste collection, regular checking of drainages and septic ditches. These rules were not as strictly followed as the hygiene rules inside the premises. The pest control in all the companies was carried out by external, approved pest contractors once a month and additionally all companies had internal pest control programme e.g. visual inspection for the detection of insects or rodent droppings, inspection of packaging material for any damages due to insects or rodents and traps both for rodents and insects. The packaging area was reported as the high risk area in all companies. In companies producing ready-to-eat products this area was kept under close supervision. The temperature control and monitoring of the chilling and freezing/deep freezing storage rooms were also considered crucial. Some of the companies reported that they considered the chlorination tank and equipment as high risk area along with the water source/storage and ice production machines.

Food Packaging Facilities

All companies reported that there was a separated area for packaging within which strict hygiene rules applied i.e. more frequent hand washing, limited entrance, protective clothing including gloves and face masks, inspection of packaging material to be used during production and tidiness of the facilities. All packaging lines reported in this survey were open except one. The packaging was performed either manually or semiautomatic. Packaging materials used were polystyrene boxes – trays, plastic food grade films or plastic containers. All companies reported that the cleaning of the packaging line took place at defined periods of time and also when the product's type changed. The decision when to clean was usually made by the production manager, the quality manager or the person responsible for the packaging line. All interviewees reported that the packaging equipment and facilities were manually cleaned except in one case where there was an automatic cleaning system in place (CIP). No problems concerning the cleaning efficiency of packaging equipment were reported. The cleaning was checked both visually and analytically and the analytical methods used included both microbial and chemical tests. The maintenance of the packaging line e.g.

repair and greasing took place during shut down and specific rules applied for cleaning after maintenance had been preformed.

All companies reported that they had a certain supplier for food-grade packaging materials and special quality criteria applied for different packaging materials. None of the companies was cleaning or sterilising the packaging materials prior use. The procedure mainly followed was that the packing materials were inspected at arrival visually. One company occasionally performed microbiological testing of the packaging materials. All final products were packed in disposable containers or wrappings. A stock rotation system was in place in all companies both for raw and packaging materials. Packaging materials were stored in common storage rooms that were used for that purpose. All companies reported that non conforming products were quarantined and destroyed. The companies stated that this procedure was documented and the reasons of deviation from the standard quality criteria were noted.

Food Storage and Transportation Facilities

All companies had their own cleaning programme for vehicles and storage areas. Vehicles were cleaned mainly with the same cleaning agents as those ones used in the factory, and the cleaning was taking place after every use according to the instructions of each company. Storage areas including chilling and freezing storage rooms were also cleaned regularly.

Digital thermometers were installed in all vehicles used for transportation of final products. Some companies reported that the temperature was automatically and constantly recorded, whereas others reported that recording was done manually. All companies reported that vehicles never carried various types of products e.g. packaging/raw materials and end products at the same time. If it was necessary that the same vehicle carried more than one type of products the same day; a cleaning procedure took place between the different transportations. The vehicles in all companies were loaded both manually and by using simple manual or automatic lifts. The temperature during loading in the loading corridors was kept at low level.

The storage areas were generally designed to be both convenient and to guarantee the good quality and safety of the stored material/product. They were located away from possible sources of contaminants or pollutants and the doors were always kept closed. There were special storage rooms for cleaning agents and cleaning equipment.

Regarding the storage rooms for end products those were either cooling/chilling storage rooms for fresh products at temperatures of 0–4°C or freezing storage rooms for temperatures of -18°C. These storage rooms had usually one or two entrances and were hygienically designed.

The products inside were either kept on shelf stands, in plastic boxes and on pallets. There were different storage rooms for raw materials, products undergoing maturation or other processing, final products and packaging materials. The final products after wrapping were packed inside carton boxes or put in plastic transportation crates during storage and delivery.

General Issues in Food Safety and Hygiene

All companies reported that they were informed about both legal and scientific issues concerning hygiene and safety by their external consultants, equipment manufactures, and Competent Authorities. Some interviewees mentioned that members of their staff had attended seminars organised either by Competent Authorities in Cyprus or by European organizations and institutions abroad. The use of internet was also mentioned as one way of obtaining information on food hygiene and food production in general but also relevant information on European legislation (EURLEX). The interviewees stated that the research and researches could be more useful for SMEs by disseminating their knowledge and experience through on spot visits within the companies and by organising training seminars/workshops. The following hygiene relating topics were considered to be useful for Cypriot companies:

- contamination sources regarding *Listeria* spp. in cold smoked fish products,
- growth rate and optimal growth conditions for pathogens,
- cool chain from production to consumer's table,
- easy to follow, simple and sufficient training of personnel (multi-language, interesting educational methods avoiding too much scientific terminology),
- identification of spoilage microbes sources and means to combat them,
- new cleaning and sterilization methods e.g. transportation crates,
- new, cheap and easy to clean materials used for the storage of cheese during maturation period,
- air cleaning methods e.g. air filtering and disinfectant fogging,
- avoiding mould growth on the fermented cheeses during maturation and

- microbial quality of brine used for halloumi (traditional Cypriot cheese) preservation.

The following improvements were needed according to the companies:

- cleaning methods and equipment,
- packaging and food handling,
- metal detection and shelf-life,
- ventilation and air quality as well as personnel training,
- food handling and hygiene,
- improvements in the interior structure of buildings.

The following special topics of interest were mentioned:

- ventilation, shelf-life of raw products, right packaging material for expanding shelf-life and use of indicators both for temperature abuse during storage and transport but also for bacterial growth in products,
- intelligent indicators (temperature and safety/quality), shelf-life, and novel cleaning mechanisms,
- tracing of contamination routes with rapid and reliable methods,
- cleaning mechanisms, agents, equipment and protocols – procedures,
- novel decontamination technologies,
- novel packaging, food storage and processing surface materials,
- interactive and easy ways of getting information about development in the field/communication with researchers and research institutes.

PILOT STUDIES IN FOOD ENTERPRISES

The pilot studies in Cyprus took place the period from 6th to 12th of June 2007. The six food processing companies that had participated in the hygiene survey were visited in order to perform the pilot studies. The companies were visited on different days, prior the beginning of the production. The samples were taken both from surfaces coming in direct contact with food and also non-food contact surfaces. Environmental samples were also collected. The sampling places were chosen by the visiting staff in cooperation with the food company representatives, giving more importance to those surfaces in contact with food especially in the packaging areas. Other surfaces were chosen based on difficulties in cleaning and disinfecting e.g. electric control panels and drainage systems and other problematic areas e.g. porous material surfaces that were found inside each establishment.

Surfaces sampled were production line surfaces, packaging machine surfaces, conveyors, slicing machines, balances, knives, floors and walls in the production areas, drains, plastic boxes, containers, wooden pallets, door handles, buttons in equipment, hoses, taps, protective clothing and storage room surfaces e.g. doors, walls, floors and shelve surfaces. The sampling was made taking all the necessary precautions to avoid any cross-contamination both of sampling materials and surfaces. All personnel participating in the sampling was properly dressed with disposable overalls, caps, gloves and shoe protection boots and ethanol 70% was use for the disinfection of both operator's hands and gloves when needed.

Twenty surface samples were taken from each establishment using two different sampling techniques, contact agar dishes made from selective chromogenic agars (OXOID®) and commercial dry agars (RIDA®COUNT) which were moistened before sampling. Apart from that, ten of these places were also sampled using microbial transport swabs and non-woven clothes. Temperature, humidity and pH were recorded during sampling at each sampling place. The sampling surfaces were differentiated depending on the material they were made off, their contact or not with food and if the sampling took place during production or not.

Furthermore five water samples from different locations inside each establishment were taken. Ice, brine and drainage water were also collected for analysis where applicable. Packaging material and final product samples were taken from each company in order to investigate the microbial status of the packaging material and examine the final product at the best-before-end day in order to find out whether their quality complied with the legal microbial criteria and company's quality requirements.

Sampling Methods in the Pilot Studies

Four different types of contact agars were used in this study: CM 1036 chromogenic *Bacillus cereus* agar, CM 1046 chromogenic *E. coli* / Coliform selective medium, CM 1084chromogenic *Listeria* agar and CM 1007chromogenic *Salmonella* medium (Oxoid Ltd, Hampshire, UK). The chromogenic agars were prepared according to the manufacture's instructions and the necessary volume was placed in contact petri dishes (Nunc™, Roskilde, Denmark). Each dish was filled with approximately 13–15 ml of the agar so that the agar surface after solidifying would project slightly above the edge of the dish. One contact plate of each of the 4 different agars was pressed 10 times for 3 s each time on the sampling surface in a way that if applicable the whole agar surface

comes in contact with the sampling surface. The plates were incubated at 36°±1°C for 48 h. The colonies were counted following the instructions provided by the manufacture. If the enumeration was not possible due to the presence of too many colonies then the colonies of one cm^2 were counted and an estimation of the total number of colony forming units (cfu) per plate was reported as a final result. The results were expressed as cfu per cm^2.

The RIDA®COUNT are medium sheets, coated with dry culture medium, for the quantitative detection of microorganisms from food and environment. The culture medium is covered by a fabric, which allows absorbance of the applied sample solution or moistening liquid. The transparent cover film on the top of the fabric prevents undesired contaminations of the medium sheet in the three different RIDA®COUNTs used: RIDA®COUNT Total, RIDA®COUNT *Enteribacteriaceae* and RIDA®COUNT Yeast and Mould (R-biopharm AG, Darmstadt, Germany). The sheets were moistened with 1 ml peptone saline 1 d before the sampling. Each RIDA®COUNT was pressed once for 3 s at each sampling place. Effort was made that the whole surface of the sheet would come in contact with the sampling area. The sheets were incubated at 36°±1°C for 48 h and then the colonies were counted following the manufacture's instructions. Yeast and mould test was evaluated after four days of incubation in room temperature at 25°±2°C. If the enumeration was not possible due to too many colonies then the colony counting guidance sheets provided by the manufacturer were used. The results were expressed as cfu/cm^2.

PROBACT transport swabs (Labema Oy, Kerava, Finland) were used for collecting samples for *Listeria* and *Salmonella* analysis. These swabs are comprised by a synthetic sterile swab tip on a plastic shaft and a container with cap. The tubes contain amies transport medium with charcoal which is recommend for the safe collection, transportation and preservation of microbial specimens. The swabs were sealed in sterile plastic film rapping so no further preparations were necessary prior their use. Ten swabs samples were taken per establishment. Each transport swab was moistened with sterile saline if needed and after swabbing the defined area the sample was put into the swab tube containing the transport medium and the tube was properly closed.

The swabs were kept in refrigerator and were all analysed at the end of the whole sampling. Each tip was removed aseptically from the shaft and was diluted in 6 ml of peptone saline. The sample was vortexed for 30 s and 1 ml of each sample was used for cultivation on plate count agar (PCA) (dilution series were used from 10^{-1} to 10^{-4}). Half

of the remaining sample was put into *Salmonella* pre-enrichment broth (buffered peptone water) and the other half into *Listeria* pre-enrichment broth (1/2 Fraser). The volume of enrichment broth used for both cases was 10 ml. All pre-enriched samples were incubated for 24h at 37°C and 30°C respectively. Then the samples were treated according to the *Salmonella* detection method in foods (NMKL method No 71 5.ed., 1999) and the *L. monocytogenes* detection method (ISO method 11290-1). The detection and enumeration of *L. monocytogenes* according to ISO 11290-1 was modified so that LMBA agar was used instead of PALCAM agar. Also the secondary enrichment step as mentioned in the ISO method was not followed since the aim was a qualitative and not a quantitative result. Furthermore real-time PCR technique with iQ-Check™ *Salnomella* Kit and iQ-Check™ *Listeria monocytogenes* II Kit (BIORAD, Marnes-la-conquette, France) were used. Any positive results were documented.

Non-woven cloth samples were taken by using Mesoft® gauzes previously cut to half of the original size (14,5 x 8 cm^2) and sterilised in autoclave. Ten gauze samples were taken per establishment. Equal number test tubes were previously filled with 5 ml peptone saline (Maximum Recovery Dilent LAB M™, Lancashire, UK). The sampling was always carried out by using gloves. The non-woven cloths were moisten with sterile peptone saline to wipe the area sampled and then were put in the test tubes containing 5 ml sterile peptone saline. The sampled non-woven cloths were stored in refrigerator and were further analysed after the completion of all samplings. From each tube half of the sample (half peptone saline and half non-woven cloth) was placed into the *Salmonella* pre-enrichment broth and the other half sample into *Listeria* pre-enrichment broth. Ten ml of each enrichment broth were used. The pre-enrichment, enrichment broths and culturing procedures followed for the isolation and identification, were the same as the ones described for the transport swabs.

Water Samples

Five water samples of 500 ml were collected from each establishment. Each sample was collect into disposable plastic bottle treated with sodium thiosulfate (Sterilin®, Mid-Glamorgan, UK). Regarding water analysis beside the traditional cultivation on agars, also the Compact Dry plates (HyServe, Uffing, Germany) were used. Compact Dry is an easy-to-use method for counting microorganisms. A liquid sample placed onto the plate diffuses homogenous over the whole plate. Then the plates were incubated at the recommended temperatures. The grown colonies are pigmented within different colour, developed by chromogenic substrates and redox indicators. The type

of bacterial growth can then be identified by the produced colour. The Compact Dry plates that were used were: Compact Dry TC for total count, Compact Dry ETB for *Enterobacteriaceae* and Compact Dry YM for yeast and mould.

The water samples were taken similarly as to the way the water is used for its normal purposes i.e. directly without sterilising the tab or generally the water outcome, or allowing running of some water first to drain. This technique was applied to evaluate and investigate the real microbial burden of water which remains inside pipes and hoses overnight and which represents the worst case scenario.

The samples were kept in refrigerator after collection and were analysed later on the same day. Before filtering, samples were shaken to homogenise their content. Four volumes of 100 ml of each sample were filtered using sterilised cellulose nitrate filter (Sartorius AG, Goettingen, Germany) 50 mM in diameter with pore size 0.45 or 0.2 μm depending on the turbidity of the examined liquid. Generally the filters intended to be used for the cultivation concerning different pathogens and later used for the PCR detection method were selected to be the ones with 0.2 μm pore size.

For filtering the water samples the Millipore Filter and Water Containers (Millipore Filtering System) were used. Three filters from the same water sample were placed on PCA (Plate Count Agar), VRBGA (Violet Red Bile Glucose Agar) and PDA (Potato Dextrose Agar) Petri dishes respectively. The fourth filter was put in 10 ml sterilised peptone saline and the test tube containing the filter was vortexed for 30 s. The volumes of 0.1 ml and 1 ml from each tube were transferred on each chromogenic agar (chromogenic *Bacillus cereus* agar, chromogenic *E. coli*/coliform selective medium, chromogenic *Listeria* agar-ISO, and chromogenic *Salmonella* medium). One ml of each sample was pipetted onto each of the three different Compact Dry media used. Furthermore another 1 ml of the initial sample was also pipetted on another series of Compact Dry media.

The remaining volumes of the filter samples contained in tubes were put into refrigerator and after the completion of the samplings half of the sample was put in 10 ml of *Listeria* pre-enrichment broth and the other half in 10 ml of *Salmonella* pre-enrichment broth. The cultivation and confirmation procedures for these samples were the same like the ones described for both transport swabs and non-woven cloths.

The plates were incubated in different temperatures according to the manufactures instruction or ISO methods. PCA solid agars were incubated in 30°C for 48 h whereas the Compact Dry TC, Compact Dry ETB and chromogenic media at 36°±1°C for 48 hours. Both PDA agars and Compact Dry YM were incubated at 25°±2°C for 4 d. After the end of the incubation period colonies were counted. If too numerous colonies were present on the filters, or on the chromogenic and Compact Dry media an estimation of the total number of cfu was made.

Final Products

The microbial analysis of the final products sample took place on the best before day as it was indicated from the manufacturer. The temperature was recorded using data loggers during transportation of the samples. Ten grams from each sample were weighted and put in a filter stomacher bag, 90 ml of peptone saline (Maximum Recovery Diluent LAB M™, Lancashire, UK) were added and then samples were homogenised using Stomacher 400-Laboratory blender for 60 s at normal speed. Dilutions 10^{-2}–10^{-5} were made and 0.1 ml of each dilution was pipetted on the agar surface and spread with a Z-rod. Only for the first dilution 10^{-2} the pour plate technique was used and only for total bacterial count and *Enterobactreriaceae*. For yeasts and moulds only the spread technique with the Z-rod was used. The solid media used were the PCA for total bacterial count, VRBGA for *Enterobacteriaceae* and PDA for yeasts and moulds. The plates were incubated at 30°C for 48h, 36°±1°C for 48h and 25°±2°C for 4 days respectively. After incubation plates with more than 10 and less than 300 colonies were selected and the colonies counted. All the results were documented. All products were examined for the presence of *Salmonella* spp. and *Listeria monocytogenes* following the NMKL No 71 5.ed, 1999 method for the detection of *Salmonella* in foods and ISO method 11290-1 for the detection and enumeration of *Listeria monocytogenes.*

RESULTS AND DISCUSSION

Process surfaces

Generally the majority of surfaces that were in contact with food were found to be satisfactory clean although in some cases high numbers of microorganisms were counted (Figures 1 and 3). Unfortunately even less attention is given to surfaces that do not come in direct contact with food and other surfaces that are difficult to clean and

disinfect. Samples from all establishments were positive for *Listeria* spp. The presence of *Listeria* spp. though is indicative of the possibility that pathogenic *Listeria monocytogenes* can be present and grow on the same places since the growth requirements of all microbes belonging to this family are the same. It is necessary to prevent spoilage and contamination of final products and therefore more attention to the sanitation of non-food contact surfaces should be paid. Moreover the cleaning procedures should be better planned and intensified in order to eliminate the *Listeria* spp. niches in all establishments.

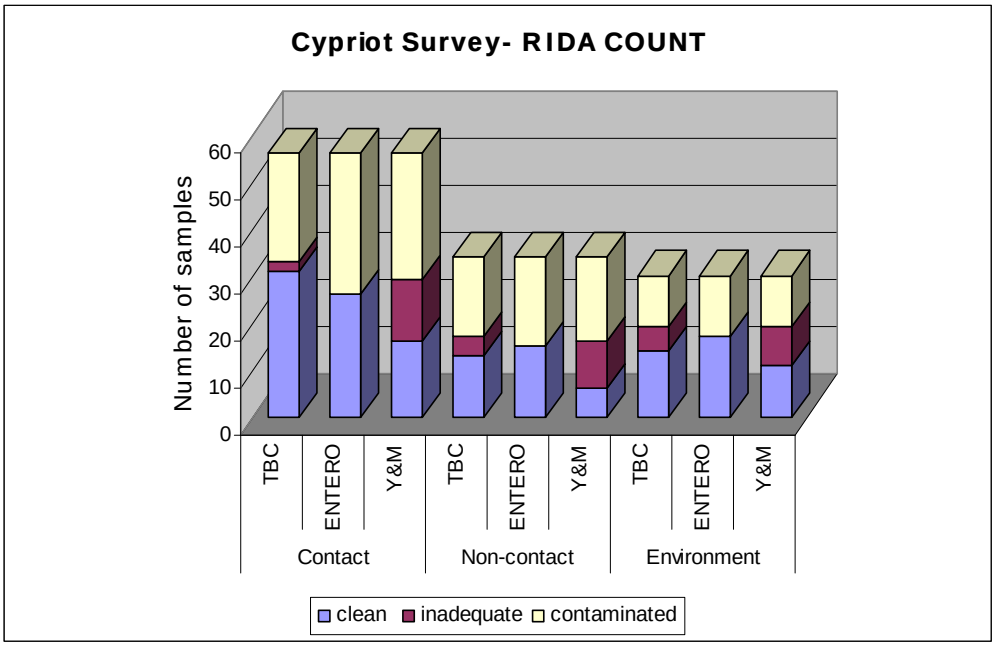

Figure 1. Results based on RIDA®COUNT method of Total bacteria, Enterobacteriaceae and Yeast and Mould in the hygiene surveys: the results are categorized as clean (< 2 cfu/cm^2 for Total bacteria and < 0.1 cfu/cm^2 for Enterobacteriaceae and Yeast and Mould), inadequate (2–5 cfu/cm^2 for Total bacteria and 0.1–1 cfu/cm^2 for Yeast and Mould) and contaminated (> 5 cfu/cm^2 for Total bacteria, > 0.1 cfu/cm^2 for Enterobacteriaceae and > 1 cfu/cm^2 for Yeast and Mould). In the figure, the samples are divided in contact, non contact and environment surface samples.

Process Environment

Attention on the process environments should also be paid since it was indicated through this survey that certain parts in the process environment can pose significant threat for the final product's safety. Therefore more intensive cleaning protocols should

also be applied for doors, walls, ceilings, floors and drainage systems. Although the air quality was not examined during this survey, the presence of moulds on walls in one of the companies indirectly indicates the need for improved cleaning protocols, air purification and the necessity of keeping the facilities and equipment dry at all times.

Water

The majority of the water samples examined complied with the microbial criteria that are mentioned in the legislation (Figure 2). There were some exceptions though in which the waters were heavily contaminated with different bacteria. Great attention should be paid on this issue as well, since water is used for cleaning and rinsing purposes of the processing equipment. If the quality of the water used for this purpose is inadequate then there is no benefit from applying even the more sophisticated cleaning protocols. Rinsing with bad quality water will re-contaminate the cleaned surfaces. It is worth mentioning that the high temperatures that predominate inside the establishments in Cyprus during the shut-down periods and especially during the summer season may affect the microbical load of the water since microbes present in the piping and hoses can multiply. This should be further investigated in order to find preventative measures.

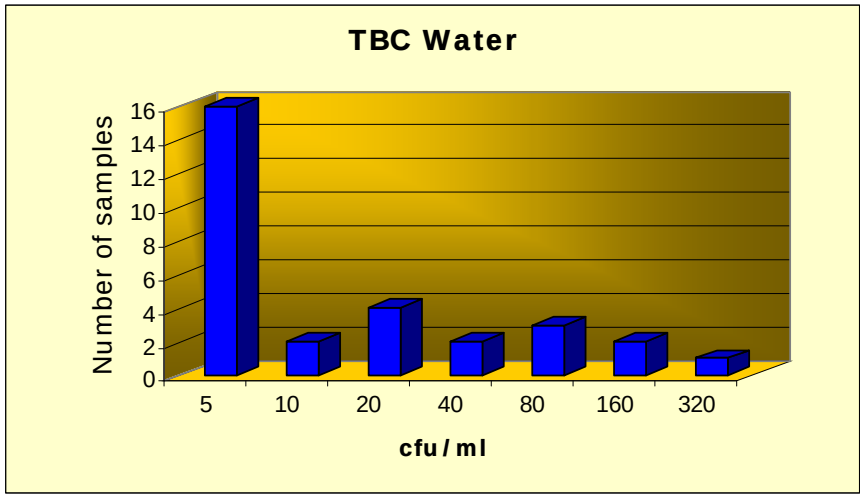

Figure 2. Microbial quality of water samples collected (Total Bacterial Count). The majority of the samples complied with the limits set in the legislation (20 cfu/ml, 37°C).

Products

Some product samples analysed on the best-before date had exceeded the minimum microbial criteria stated in the legislation. This can partially be due to the fact that the temperature during the transport fluctuated and for some time it had exceeded 8°C for more that 12 h. *Salmonella* spp. was not isolated from any of the final products whereas *L. monocytogenes* was isolated from one of the final products. No problem was identified concerning the packaging materials.

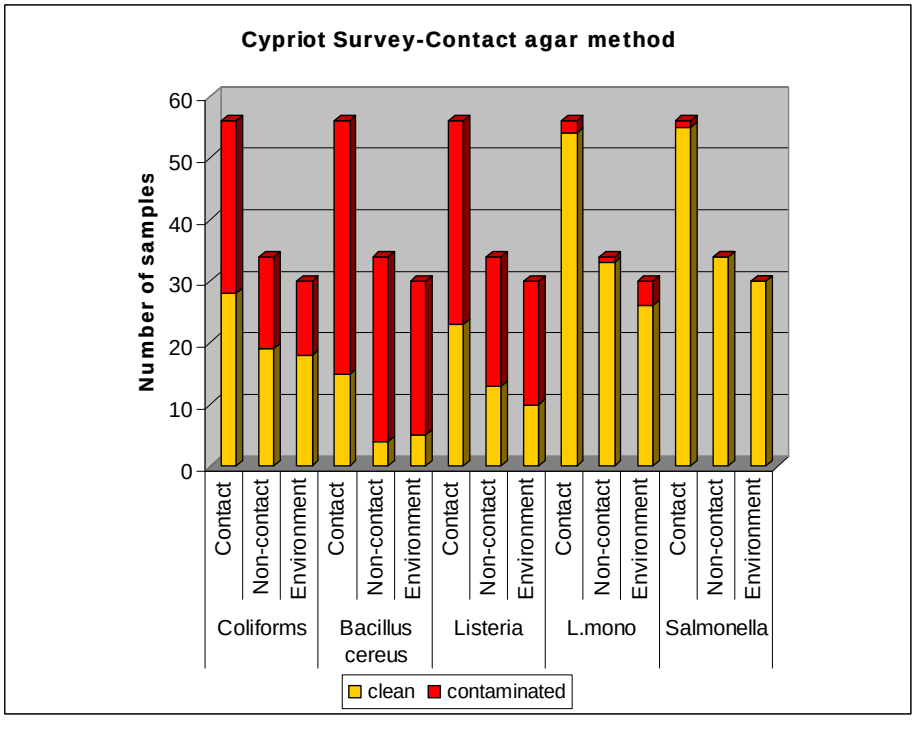

Figure 3. Results based on the contact agar method for Bacillus cereus, E. coli / Coliform, Listeria spp and Salmonella spp. The presence of the characteristic colonies on the agar was considered to be a positive (contaminated) sample.

CONCLUSIONS AND RECOMMENDATIONS

All companies participating in the surveys and pilot studies were informed with a written report for the outcome of the sampling and recommendation were made in cases of identified problems. In general, there is need for a more effective implementation of the cleaning and disinfection program and of a better training for the

personnel on these issues in the majority of the visited food industries. The main goal should be to improve the hygiene status and eliminate any pathogens that might be present within the industries; towards that direction the following general recommendations were made:

- Cleaning and Sanitation: Improvement of cleaning and disinfection programs is necessary. When cleaning procedure is done manually i.e. scrubbing or by using special mobs, wipers and sponges these should always kept clean and dry and regularly replaced. Movable equipment and tools, even if not coming in direct contact with food should be cleaned and sanitised. Extra attention should be paid to difficult to clean and to reach areas e.g. ceilings and plastic grades. The cleaning procedure should be closely supervised and regularly checked and verified either visually or by using other analytical methods. Only good quality water should be used for cleaning and water that might have stayed still for prolong periods inside hoses or other water compartments should be discarded. Extra attention should be paid to the fact that during the shutdown of the factory and after cleaning and disinfection has taken place the surfaces and equipment must be kept dry and away of any source of pollution. In high risk areas another disinfection should take place prior of the initiation of production each day.
- Personnel should follow the rules of personal hygiene such as frequent hand washing, using a head cover that should cover all the hair, gloves (disposable or reusable) and face masks if necessary. The personnel should always use clean uniforms and garments (any personal clothing like scars and overcoats should not be worn inside the production areas). The personnel responsible for performing the cleaning should be adequately trained.
- Hygienic design, equipment and structures: The hygienic design of the equipment should always be taken into consideration. Difficult to reach and to clean surfaces should be avoided. Good ventilation is necessary in order to avoid formation of moisture and condensation of vapours. Wood should be avoided and any worn equipment should be replaced. Wires and cables should be covered and control panels should not be placed in the production areas since their cleaning can be both problematic and dangerous. Badly welded surfaces should be cleaned thoroughly and floors should be maintained regularly in order to facilitate sufficient cleaning and drainage. The whole production process should be frequently re-evaluated taking into account any changes in the equipment and in the production in order to be able to better control, reduce, eliminate and avoid the hazards concerning food.

- Sampling methods: Compact Dry plates are an easy-to-use method for counting microorganisms when analysing liquid samples. Non-woven clothes were found to be a better sampling material regarding foodborne pathogens compared to transport swabs probably because of the larger contact surface with the sampled area compare to swabbing. RIDA®COUNT and chromogenic contact agars were found to be easy and useful ways for the evaluation of the cleanliness and identification of the microorganisms present on surfaces respectively. On the other hand in both cases further differentiation is necessary using other techniques and evaluation of the result can be problematic when too many colonies are present and enumeration is impossible. In some case the interpretation of some types of chromogenic contact agar plates is difficult due to the variation of colours. Real-time PCR kits of iQ-Check™ *Salmonella* Kit and iQ-Check™ *Listeria monocytogenes* II Kit were found to be quite useful in doubtful cases.

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PILOT CASE II – PRACTICAL HYGIENE SURVEY IN FOUR ESTONIAN DAIRIES

R. Vokk, T. Veskus, L. Kutsar, M. Mets, S. Salo* & G. Wirtanen*
Tallinn University of Technology, Tallinn, Estonia
*VTT Technical Research Centre of Finland, Espoo, Finland

Description of the Work

The current work was performed during summer 2007 as a part of a specific support action project SAFOODNET – Food Safety and Hygiene Networking within New Member States and Associated Candidate Countries (FP6-022808) funded by EU. VTT Technical Research Centre of Finland in association with Tallinn University of Technology was involved into the pilot case. In order to obtain relevant information on current status of safety and hygiene issues in SMEs, a questionnaire was used for interviewing industrial partners before the pilot actions. Based on the questionnaires short-term objectives have been choosen. Most of the dairies were very concerned about the product quality. During the pilot case microbial survey throughout the whole production line was performed. In order to fulfil the industrial partners´ expectations, the hygiene study team has performed following activities: preliminary and second samplings, Hygiene training as an additional tool for hygiene improvement and summary reports to the enterprises. During the preliminary visits the detailed plan of the study for individual enterprises has been prepared. Before the second sampling the following improvements were implemented in 3 dairies: ultrasonication cleaning procedures and personnel hand hygiene training as well as general hygiene practices (ozonation of cloths) performed in a pilot scale with *Listeria monocytogenes* contaminated handkerchiefs.Second sampling was performed to evaluate the impact of the additional hygiene improvement methods used at the places.

The main objective of the study was to evaluate the hygiene conditions in Estonian dairies and to monitor the adherence of micro-organisms on direct food contact and indirect contact surfaces. In order to define the current level of hygiene in Estonian dairies, hygiene survey including detection of total viable bacteria count, *Enterobacteriaceae*, moulds & yeasts, *Listeria* spp*, Listeria monocytogenes, coli*-forms

and *Bacillus cereus* was carried out. Additional hygiene improvement methods were used to guarantee better hygiene conditions in evaluated dairies.

In the present survey sampling places were divided into three different groups – direct product contact surfaces, indirect product contact surfaces and environmental surfaces. The basic sampling places were different tanks, production lines, packaging machines but also control switches, transportation boxes, hand washing sinks and even drains. The location of problem areas was similar between dairies. Typically, microbes like to adhere on pipe elbows, rubber seals, gaskets, conveyor belts, caps in dead-end areas, floors, drains, plate-heat exchangers, pasteurizers and other surfaces. Bacteria can form microcolonies on the equipment surfaces or other areas and form biofilms.

Microbial samples were taken aseptically from surfaces with 3 various contact plates and 3 various Petrifilms, non-woven cloths and *Listeria* Isolation Transport swabs as follows: 1) Surface analyses with contact agar (Selective *E. coli* / *Coli*form Chromogenic Agar, Chromogenic *B. cereus* Agar & Chromogenic *Listeria* spp. Agar) plates, Petrifilms (Aerobic Count, *Enterobacteriaceae* andYeast and Mould) and other materials (non-woven cloth & Transport swabs – "*Listeria* Isolation Transport" for *L. monocytogenes*).

Experimental Results and Discussion

Generally, the direct product contact surfaces were clean and did not show any critical points were special attention is needed, except the packaging machine and tank for milk before pasteurization. According to the hygiene survey results obtained from Estonian dairies the amount of total viable bacteria counts and *Enterobacteriaceae* in direct and indirect product contact surfaces varied between dairies. *Listeria monocytogenes* was detected in all four dairies: 8 surfaces, 4 raw milk samples, 1 product sample, 1 personnel sample, 3 water samples and the drain samples (altogether 247 samples were studied; Table 1). In cases where the amounts of aerobic bacteria and *Enterobacteriaceae* were high, *Listeria monocytogenes* was not found and when the amount of aerobic bacteria and *Enterobacteriaceae* was lower, *Listeria monocytogenes* was found in the same sampling places more frequently. The design of equipment plays important role in combating the biofilm formation and if the machine has hard to clean areas then the contamination risk is bigger. In processing plants, common sites of postprocessing *L. monocytogenes* contamination are specifically filling and packaging machines. This survey also showed that the packaging machines in dairies were the places where microbes tend to adhere. About 36.3% of the sampling

places had aerobic bacteria counts in range of > 300 cfu/20 cm^2 and only 9.1% of the sampling places had counts in the range of < 1 cfu/20 cm^2. The amount of *Enterobacteriaceae* was also high on packaging equipment surfaces. In one dairy enterprise there were 3 samples of *Enterobacteriaceae* in a maximum range. This is the point where dairies should make some improvements because occurrence of *Enterobacteriaceae* in direct contact surfaces increase the potential risk of contamination with pathogens like *E. coli*. *Listeria* spp. were detected from 8 packaging machine samples, and even 2 positive *L. monocytogenes* samples were found in the packaging machines in two dairies. More attention should be paid on the design of equipment, on the surface materials and on the effeciency of cleaning. Different surface materials were investigated (Table 2). No significant differences were obtained although application of the specific plastic materials for the direct food contact surfaces seems to be promising. The survey indicated that packaging machine is one of the most critical points in the dairy industry and cause serious problems of cross-contamination. The study verified the microbial adherence in cracks and cornes of packaging machines. In addition, the indirect product contact surfaces and environmental surfaces had high bacterial counts. The hygiene of indirect product contact surfaces and environmental surfaces influence the general hygiene level in the dairy factory but often dairies are focused more on direct contact surfaces. Unclean areas even not directly in production facilities can increase the risk of contamination. Postprocessing contamination in the dairy production environment is the main source of spoilage microorganisms found in the milk. For example, microbes can enter into the pasteurized product through the contact with pre-contaminated processing equipment. The microbial quality of air was not appropriate in all dairies. The microbial air quality was similar in all sampling places due to the unrestricted airflow. Special attention should be paid to closing doors and controlling filters in the ventilation system. According to the findings of the current study the personnel was a potential contamination source, especially in the production area where a lot of manual work phases were performed. Therefore a training course was organized after the first sampling.

CONCLUSIONS

The described Pilot case II was a practical approach in a pilot scale i.e. not monitoring the hygiene level in the whole dairies. The hand washing training and ultrasonication cleaning procedure of utensils did not show the clear improvement of the results. However, regarding the ultrasonic cleaning, the used equipment was small i.e. only small parts fitted into ultrasonic bath. Furthermore, the ozone disinfection reduced

Listeria in samples taken from cloths in pilot tests. These results proved that main concern is not only product quality. The contamination routes were shown. These hygiene surveys changed the way of thinking and the main attention will in the future be paid to all elements in the production.

Table 1. Aerobic bacteria in direct product contact surfaces.

Microbial load (cfu/20 cm^2)	Amount of samples (%)					
	Tank[1]	Tank[2]	Tank[3]	Production line	Packaging machine	Packaging material
< 1	0	0	66.6	12.5	0	0
1–10	12.5	0	0	12.5	0	66.7
11–100	50.0	22.2	16.7	62.5	66.7	33.3
101–300	25.0	33.3	0	12.5	0	0
> 300	12.5	44.5	16.7	0	33.3	0

[1] raw material; [2] milk before pasteurization; [3] milk after pasteurization

Table 2. Comparison of aerobic counts on stainless steel and plastic surfaces.

Colony forming units cfu/20 cm^{22}	Amount of samples (%)			
	Stainless steel (direct)	Plastic (direct)	Stainless steel (indirect)	Stainless steel (indirect)
< 1	12.9	16.7	20.0	0
1–10	12.9	16.7	20.0	25.0
11–100	27.5	50.2	40.0	33.3
101–300	16.1	16.7	0	8.4
> 300	30.6	0	20.0	33.3

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PILOT CASE III – MODEL FOR READY-MADE MEALS

Hanne Løje, Peter Reimer Stubbe & Alan Friis
National Food Institute, DTU, Denmark

A model for prediction of the shelf life for a ready made chilled dish has been developed. The model gives a good estimation of what the shelf-life for a given ready made chilled dish is likely to be. The model can be a useful tool to give information about shelf life and it can be used as a teaching tool. The model may also be used as tool in production planning to get an idea about important steps in the food production regarding shelf life.

DESCRIPTION OF THE WORK

The aim of the pilot case III was to identify safety criteria for chilled food products consisting of a combination of different types of raw materials. In addition the focus was to be able to predict safety of new products. First the work was conducted as a bachelor project for a student at DTU, Anna Kousholt. The student made a report consisting of literature studies and developed an excel tool for simulation of the prediction of safety. The excel tool was a basic tool for decision making and needed more work to be optimal to use for teaching of students. The next step was to further develope the tool and to repair the bugs which were found in the first tool. This work was done by MSc Peter Reimer Stubbe, National Food Institute, DTU. A new tool has been made based on the excel tool using Javascript which is more optimal for modelling.

INTRODUCTION

All foods will change over time no matter type and composition of the food and no matter the conditions of the surroundings. The three most important factors contributing to the changes in quality are water activity, pH and temperature. These factors determine the rate of activity for chemical and enzymatic processes in the product and the growth rate for micro-organisms. In this project only temperature has been taken into consideration, because the water activity in practice can be considered to be one or very close to 1 and pH approximately neutral ~ 7.

TEMPERATURE

The temperature history is one of the most important parameters to be aware of if one wants to ensure a good quality. The fact is that both the reaction and growth rates increase with temperature until a point where the enzymes and micro-organisms become inactivated or die. In addition some micro-organisms are not capable of multiplying at low temperatures. Therefore it is crucial to be able to control the product temperature in all steps during the shelf-life of the product. The deterioration rate for a given product changes with temperature is shown in Figure 1.

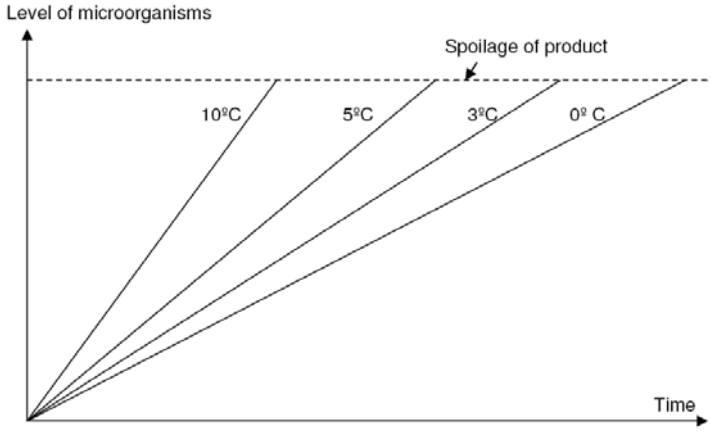


Figure 1. Sketch illustrating of how temperature affects the rate of deterioration and spoilage of a product caused by micro-organisms.

The Arrhenius equation describes the influence of temperature on the influence of temperature on the reaction rate constants:

$$k_T = A_o \cdot e^{-E_a/(R \cdot T)}$$

Where k_T is the reaction rate, T is the absolute temperature [K], E_a is the activation energy [J/mole], R is the gas constant [8.3 J/(mole·K)] and A_o is the rate constant as T approaches infinity (the frequency factor).

MICROBIAL GROWTH

During manufacturing of chilled ready made dish, the food undergoes a heat treatment. This process reduces the level of micro-organisms, which is desired as the lower the

initial population of micro-organisms the longer the time before the critical level is reached. The degree of heat applied to the product will affect some types of micro-organisms ability to survive. Some bacteria produce spores which are heat resistant and they will survive. Most other bacteria in foods are not able to survive; other species may grow relatively unhindered because of the lack of competitive flora. However post-heating contamination may occur, bringing the heat-labile micro-organisms back into the product.

Microbial growth can be divided into three major phases as illustrated in Figure 2. The lag phase is the time the micro-organism needs to adapt to new conditions in this phase there is no apparent growth; it is followed by the exponential phase where the number of microorganisms increases following the equation:

$$N_t = N_0 * 2^{t/\tau}$$

Where N_t and N_0 is the number of microorganisms at times t and 0 and τ is the specific growth rate.

The next phase is the stationary phase, where the concentration of microorganisms is steady, this can be due to inhibitory metabolites or that the nutrients are depleted. It is the exponential rate that is of most concern when estimating the shelf-life of a product. To ease the prediction of the shelf-life the lag phase has not been taken into consideration in this model and it is assumed that the product spoilage will reach a level where it is unsuitable for consumption before the microorganisms reach the stationary phase.

SHELF LIFE MODELLING

The ability to predict shelf life of a product is of great value to the food industry. It is important to be able to identify which factors determine the shelf life of the product, which may be microbial, chemical or physical depending on the product, the process, packaging and the storage conditions. The work has contained a development of safety criteria for combined products produced for cooling. The process in developing the model has consisted of several steps, 1) To define quality in relation to ready made chilled meals, 2) To generate data and knowledge through literature study, including the study of other shelf life predictive models for inspiration, 3) To make decisions on the delimiting process or processes, types of microorganism, indicator enzymes etc and

4) To build the model; choosing the microbial and enzymatic hazards relevant for the model and modelling the thermal reduction and subsequently growth of microorganisms and enzymatic reduction during storage. The student project has been limited to building the model rather than finding all the variables for the microorganism and enzymes included in the model. The aim of the tool had been to determine limiting factors in the process chain and relate it to the shelf life. Two theoretical food technology approaches were made: 1) Inactivation kinetics of common microorganisms and enzymes are affecting consumer safety and shelf life and 2) A theoretical shelf life prediction is based on the inactivation kinetics and time/temperature profile in processing, storage, transport etc.

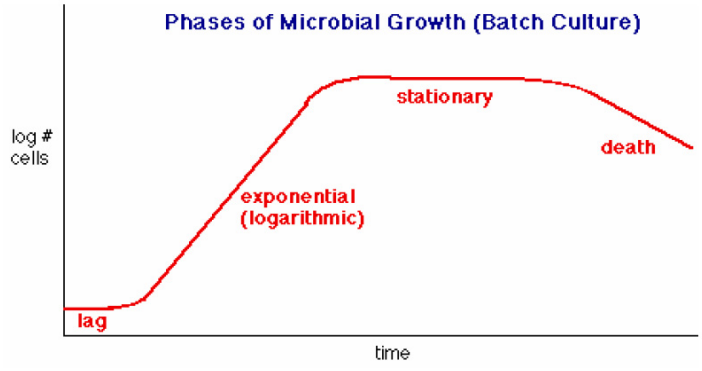


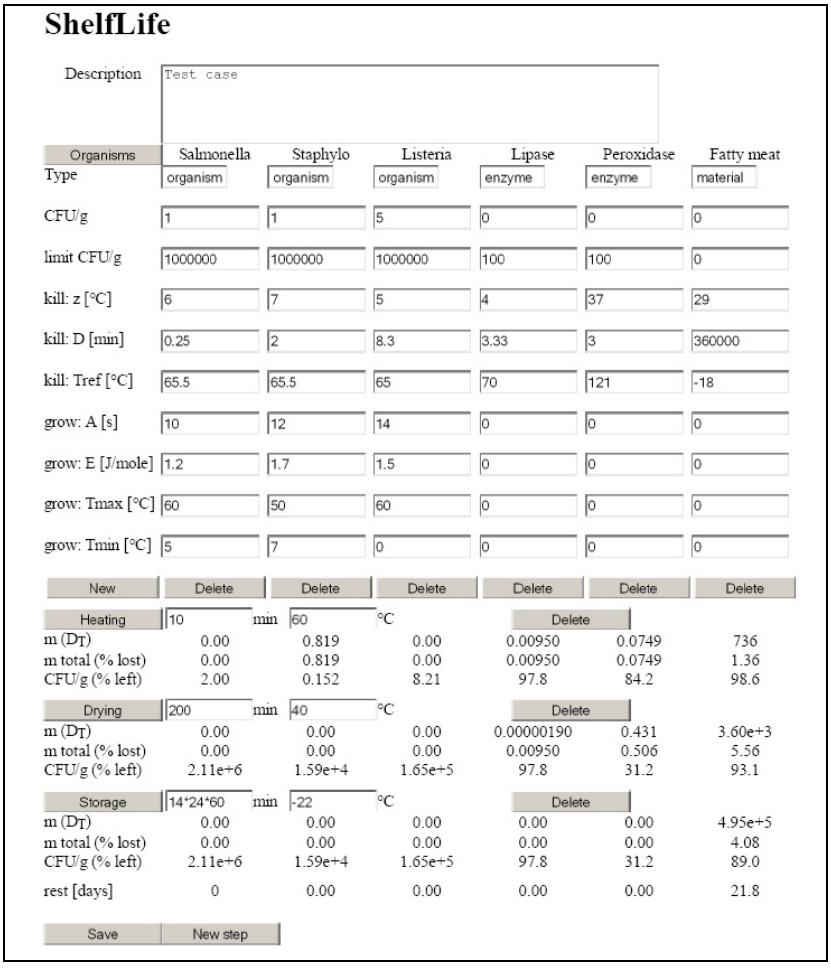
Figure 2. Microbial growth depicted with log cells against time. The three growth phases can be seen; and the forth phase is the death phase, caused by lack of nutritions among other things.

In Figure 3 is shown an example of the model. First data for the relevant micro-organisms and enzymes for the particularly product are added in the scheme. The next is to entry data for heating, drying, storage etc. More processes and/or storage steps can be added. The model will calculate the remaining days of storage for the given product under the giving conditions.

LIMITATIONS OF THE MODEL

The model for calculation of the shelf life can be improved by altering water activity and pH. This has not been implemented in the model. In addition the model does not include preservation additives or modified atmosphere packaging. The complexity of spoilage is a major limitation in order to be able to precisely predict the shelf-life of food products. It is not just the growth of one single micro-organisms that will determine the shelf-life of a product, but many different factors may influence the

growth of micro-organisms and the activity of enzymes. Model assumptions have been made in order to get a simpler model, however there are still issues which are not take into consideration. The main factor is the interactions between micro-organisms. To be able to account for these interactions thorough experiments must be carried out on how the micro-organisms interacts and in what way it affects the shelf life of foods. Another issue that needs attention is the residual enzyme concentration after processing. This concentration is seldom known in practice and it has not been possible to find information on how this affects the shelf life of the product. Thus it would be advisable to make further investigations on how the enzymes influence the shelf life of a chilled-ready to eat dish.



ShelfLife

Description	Test case					
Organisms	Salmonella	Staphylo	Listeria	Lipase	Peroxidase	Fatty meat
Type	organism	organism	organism	enzyme	enzyme	material
CFU/g	1	1	5	0	0	0
limit CFU/g	1000000	1000000	1000000	100	100	0
kill: z [°C]	6	7	5	4	37	29
kill: D [min]	0.25	2	8.3	3.33	3	360000
kill: Tref [°C]	65.5	65.5	65	70	121	-18
grow: A [s]	10	12	14	0	0	0
grow: E [J/mole]	1.2	1.7	1.5	0	0	0
grow: Tmax [°C]	60	50	60	0	0	0
grow: Tmin [°C]	5	7	0	0	0	0
New	Delete	Delete	Delete	Delete	Delete	Delete
Heating	10 min	60 °C		Delete		
m (DT)	0.00	0.819	0.00	0.00950	0.0749	736
m total (% lost)	0.00	0.819	0.00	0.00950	0.0749	1.36
CFU/g (% left)	2.00	0.152	8.21	97.8	84.2	98.6
Drying	200 min	40 °C		Delete		
m (DT)	0.00	0.00	0.00	0.00000190	0.431	3.60e+3
m total (% lost)	0.00	0.00	0.00	0.00950	0.506	5.56
CFU/g (% left)	2.11e+6	1.59e+4	1.65e+5	97.8	31.2	93.1
Storage	14*24*60 min	-22 °C		Delete		
m (DT)	0.00	0.00	0.00	0.00	0.00	4.95e+5
m total (% lost)	0.00	0.00	0.00	0.00	0.00	4.08
CFU/g (% left)	2.11e+6	1.59e+4	1.65e+5	97.8	31.2	89.0
rest [days]	0	0.00	0.00	0.00	0.00	21.8

Save | New step

Figure 3. An example of how the tool works. Here is shown what to entry at the top and underneath is the result given for this particularly test product.

PILOT CASE V – HYGIENE SURVEY IN ROMANIAN BAKERIES

Alina Dobre, Satu Salo* & Gun Wirtanen
Institutul de Bioresurse Alimentare, Bucharest, Romania
* VTT Technical Research Centre of Finland, Espoo, Finland

A series of hygiene surveys was performed in six Romanian bakeries. The work was carried out at VTT Technical Research Centre of Finland (Espoo, Finland) and IBA, Institutul de Bioresurse Alimentare (Bucharest, Romania) from October 2008 to February 2009. The main aim of the work was to detect microbial contaminants from bakery environment including process lines and evaluate the hygiene status in Romanian bakeries.

Surface samples were taken from process environment, equipment and packaging material. Altogether 164 surfaces were studied in bakeries, including environmental, non- and food contact equipment samples. In addition contact samples were taken from protective clothing of workers. All these contact samples were taken with 4 different types of commercial Petrifilm agars to determine the amount of aerobic bacteria, yeasts and moulds, *Enterobacteriaceae*, coliforms and *Escherichia coli*. Swab samples were taken from process environment to define the presence of *Listeria* spp., *L. monocytogenes* and *Salmonella* spp. Water samples were analysed to determine the amount of aerobic bacteria, yeasts and moulds, *Enterobacteriaceae*, coliforms, *E. coli*, *Bacillus cereus* and bacterial spores. Air samples were collected using MAS-100 air sampler and the amount of aerobic bacteria, yeasts and moulds was determined. From raw material and product samples the amount of aerobic bacteria, yeasts and moulds, *Enterobacteriaceae*, coliforms, *E. coli*, *B. cereus* and bacterial spores and presence of *Listeria* spp., *Listeria monocytogenes* and *Salmonella* spp. were analysed.

The interpretation of results was based on experience obtained in hygiene surveys performed at VTT and recommendations found in literature. Properly cleaned surfaces should not contain coliforms and the amount of total aerobic bacteria should also be below 100 colony forming units (cfu) / 20 cm². The presence of coliforms indicates

poor hygiene. The surfaces that are not in direct contact with food must also be cleaned in order to avoid cross contamination.

In general the bakeries were visually in good condition. In all bakeries no unnecessary utensils were in the process area and the personnel were wearing protective clothing according to instructions. Compared to Finnish bakeries there was a lot of personnel in the factories. Personnel are one of the main contamination sources. More attention should thus be paid to the working routines and protective clothing hygiene. The amount of microbes in water varied in the different sampling places. Damaged, dirty hoses and taps can contaminate the water taken from the main water pipeline. The water intakes available in the factory must all be in regular use to avoid accumulation of biofilms in the pipelines and hoses.

In many bakeries the total bacterial count of air inside the factory was at the same level than in the outdoor air indicating that the doors and windows were not kept closed as they should. To improve the air quality the bakeries were informed that the airborne contamination risks can be reduced by keeping windows closed, by installing air filters, by separating areas and by arranging the air to flow from the high hygiene areas to the lower hygiene areas. In all of these six hygiene surveys neither *Salmonella* spp. nor *Listeria monocytogenes* were found.

PILOT CASE VII – SURFACE, WATER AND AIR HYGIENE IN TURKISH FOOD PREMISES

Mehlika Borcakli, Neşe Aslı Kısıkkaya Öncü & Gun Wirtanen*
Tübitak Marmara Research Centre, Turkey
*VTT Technical Research Centre of Finland, Espoo, Finland

Hygiene surveys will perform in three Companies in Turkey. Two of them Meat & Meat products companies and one of them is a Dairy company. The work will be carried out at TÜBİTAK Marmara Research Center Food Institute and VTT Technical Research Centre of Finland in May-June 2009. The main aims of the work detecting microbial contaminants from process lines and evaluate the hygiene status in these three Turkish food factories. Surface samples will be taken from process environment, equipment, contact and non-contact surfaces. All these samples will be taken with for different types of commercial petrifilms such as Total Viable Count, *Enterobacteriacea, Escherichia-coli* and Yeast & mould. Two different contact agars will be used in this study; *Hygislide PCA/ CHR ECC* for personel hygiene; and *Hygislide Baird Parker A./CHR ECC* for surfaces. In addition to this swab samples will be taken from process environment to define the presence of *L.monocytogenes* and *Salmonella spp*. The samples will be taken before and after cleaning process of the company. Water samples will be analyzed for total viable count, coliforms & *Enterococcus* spp. Ten water samples will be analyzed for each company. Air Samples will be taken from each company using active air sampler and the amount of aerobic bacteria, yeast and mould will be determined from different places especially during the production. Furthermore investigation the microbial status of the final product will be performed in order to find out whether their quality complies with the legal microbial criteria and company's quality requirements.

REPORT ON THE 1ST WORKSHOP – DETECTION AND IDENTIFICATION OF HARMFUL MICROBES

Ivan Rychlik
Veterinary Research Institute, Czech Republic

The main objective of Workpackage 3 was to organise two workshops focusing on the detection and identification of harmful microbes and microbiological risk management in food processes. The workshops were aimed for young scientists and junior employees within science, state administration and industry and provided these target groups with current scientific and practical knowledge on microbial risk management and novel detection and identification methods. Activities performed under Good Management Practice (GMP) such as pest control, personal hygiene, waste disposal and continuous measures at critical control points in the process were also covered. The both workshops consisted of theoretical presentations followed by practical training as well as an exam at the end. Each workshop consisted also form group work during which the participants were asked to prepare their own opinion on the specific topic which was related to their specialisation and present their opinion in front of the others. The first workshop on detection and identification of harmful microbes is being presented below and second workshop will be presented in the next chapter.

The workshop on 'Detection and identification of harmful microbes' was held in Brno in Czech Republic in December 10–12, 2007. The duration of the workshop was 3 working days. It was led by Ivan Rychlik from VRI (P3). The 1st workshop arrangements were started at the 2nd MT-meeting at Tübitak in Gebze (Turkey) in December 2006 and the flyer for the 1st SAFOODNET workshop was published on the web-site in August 2007. The workshop was attended by 24 young scientists and junior employees from food industry. Participants from VRI were 11 speakers and organisers as well as 9 attendees visiting only the lectures. The participants came from Bosnia-Herzegovina, Bulgaria, Czech Republic, Cyprus, Estonia, Slovakia, Slovenia and Turkey. The following project partners attended the workshop: Gun Wirtanen (P1), Satu Salo (P1), Mehlika Borcakli (P6) and Jana Ramus (P5). The practical part of the event went on as planned. However the time schedule were a little pressed on the first day, but this seemed fine. After the workshop, in February 2008, the proceedings containing abstracts written by speakers

and participants as well as an exam on 24 questions (multiple choices) with solutions were published. Some questions had just one right answer and some had as many as four correct answers. In the exam 19 out of 22 participants passed. Five students passed it with excellence. There was also an evaluation of the workshop. The participants were very enthusiastic and eager to learn. They worked intensively with the practical tasks.Programme of the workshop was as follows:

Monday, December 10, 2007 – Detection and identification of pathogens

9.00 – 9.30	Registration at the VRI
9.30 – 9.40	Welcome address, director of the Veterinary Research Institute Brno (VRI Brno), Prof. Toman
9.40 – 10.00	Welcome address, SAFOODNET project coordinator, Dr. Gun Wirtanen, VTT Espoo, Finland
10.00 – 10.20	Sampling for microbiological analysis in food industry, Gun Wirtanen, VTT
10.20 – 10.40	Sampling for laboratory detection of pathogenic microorganisms, Satu Salo, VTT
10.40 – 11.00	*Coffee/tea break*
11.00 – 11.20	Sampling for *Salmonella* detection in poultry and pig farms and slaughter houses, Frantisek Sisak, VRI
11.20 – 11.40	Immunomagnetic separation of selected bacterial species, Renata Karpiskova, SZU Brno, Czech Republic
11.40 – 12.00	PCR detection of pathogenic microorganisms, Milan Bartos, GeneProof, Czech Republic
12.00 – 13.00	*Lunch*
13.00 – 17.30	Practical sessions: 3 groups focusing on detection of: 1) *Salmonella* spp., 2) *Mycobacterium* spp. and 3) *Listeria* spp. and *Staphylococcus* spp. The practical sessions on detection of pathogenic microorganism will include: • sample processing for culture detection of specified bacterial species • plating pure cultures on specific selective agars • bacterial species identification and confirmation, serology, PCR etc. • ELISA detection of particular microorganisms (if applicable) • sample processing for PCR detection, PCR design • quantification of bacterial load by quantitative real time PCR (if applicable)
19.00 – 22.00	*Brno city mini-tour and workshop dinner*

Tuesday, December 11, 2007 – Differentiation of pathogens

9.00 – 9.20	Characterisation of bacterial species by biochemical properties, Alois Cizek, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
9.20 – 9.40	Antibiotic resistance in pathogenic microorganism, Helena Hradecka, VRI Brno, Czech Republic
9.40 – 10.00	Phage typing of bacterial species, Renata Karpiskova, SZU Brno
10.00 – 10.30	*Coffee/tea break*
10.30 – 11.30	Molecular epidemiology of mycobacterial infections, Ivo Pavlik, VRI Brno, Czech Republic
11.30 – 12.30	*Lunch*
12.30 – 17.00	Practical sessions: 3 groups focusing on differentiation of: 1) *Salmonella* spp., 2) *Mycobacterium* spp. and 3) *Listeria* spp. and *Staphylococcus* spp. The practical sessions on detection of pathogenic microorganism will include: • Serological characterization • Phage typing • Plasmid profile analysis • DNA fingerprinting • Pulsed field gel electrophoresis • PCR detection of mobile DNA • DNA sequencing of resulting PCR products • Microarray genomotyping
18.30 →	*Mendel museum followed by workshop dinner*

Wednesday, December 12, 2007 – Continuation of the session on differentiation of pathogens

8.00 – 11.45	Continuation of practical session on differentiation of pathogenic microorganism
11.45 – 12.05	*Coffee/tea break*
12.05 – 12.35	Overview of DNA typing methods (plasmid profile analysis, different PCRs, DNA fingerprinting, Ivan Rychlik VRI Brno, Czech Republic
12.40 – 13.50	Exam based on multiple choice questions and questions with answers to be listed
13.50 – 14.00	Closing the workshop
14.00 →	*Lunch and departure of participants*

SUMMARY REPORT ON THE 2ND WORKSHOP – MICROBIAL RISK MANAGEMENT IN FOOD PROCESSES

Hanne Løje & Alan Friis
National Food Institute, DTU, Lyngby, Denmark

The 2nd SAFOODNET workshop took place at Technical University of Denmark (DTU) from the 13 to the 15 October 2008. The title of the workshop was "Microbial risk management in food processes". The aim of the workshop was to combine theoretical presentations with practical exercises in microbial risk assessment and management. The workshop was organised by Gun Wirtanen and Satu Salo, VTT, Finland and Hanne Løje and Alan Friis, DTU National Food Institute, Denmark.

There was a great interest of young scientists and junior employees in the workshop. There were 29 participants in the workshop and the participants came from 'new' EU-countries and associated candidate countries and they represented countries such as: Croatia, Cyprus, Czech Republic, Estonia, Latvia, Romania, Slovakia, Slovenia and Turkey. The participants arrived the day before the workshop started and check in to the hotel, which was situated in Copenhagen city centre. In the evening "welcome to Denmark event" was arranged at the hotel.

The first day of the workshop consisted of presentations given by different speakers. There were invited two speakers from U.K, Tony Hasting from Birmingham University and Kathryn Whitehead from Manchester Metropolitan University. In additions there were two speakers from Turkey, Aysegül Eyigör and Seran Temelli, Uludag University, Turkey. From Cyprus, Savvas Gennaris, gave a presentation. More over there were five speakers from DTU and industries in Denmark; Birthe Fonnesbech Vogel and Paw Dalgaard, DTU Aqua, Tina Beck Hansen, DTU National Food Institute and Lars Mikkelsen, Ecolab and Eigil Pedersen, Bactoforce. There were also five presentations by SAFOODNET partners; Gun Wirtanen and Satu Salo, VTT, Finland, Hanne Løje and Alan Friis, DTU National Food Institute, Denmark, and Raivo Vokk, Technical University of Tallinn (TUT), Estonia.

The practical work consisted of three tasks, and the task work took place on day 2 and 3. The participants were divided into three teams. Each team had three hour for each task. The groups were organised in the way that some from each of the groups performing the practical group work participated in the preparation of each of the three tasks.

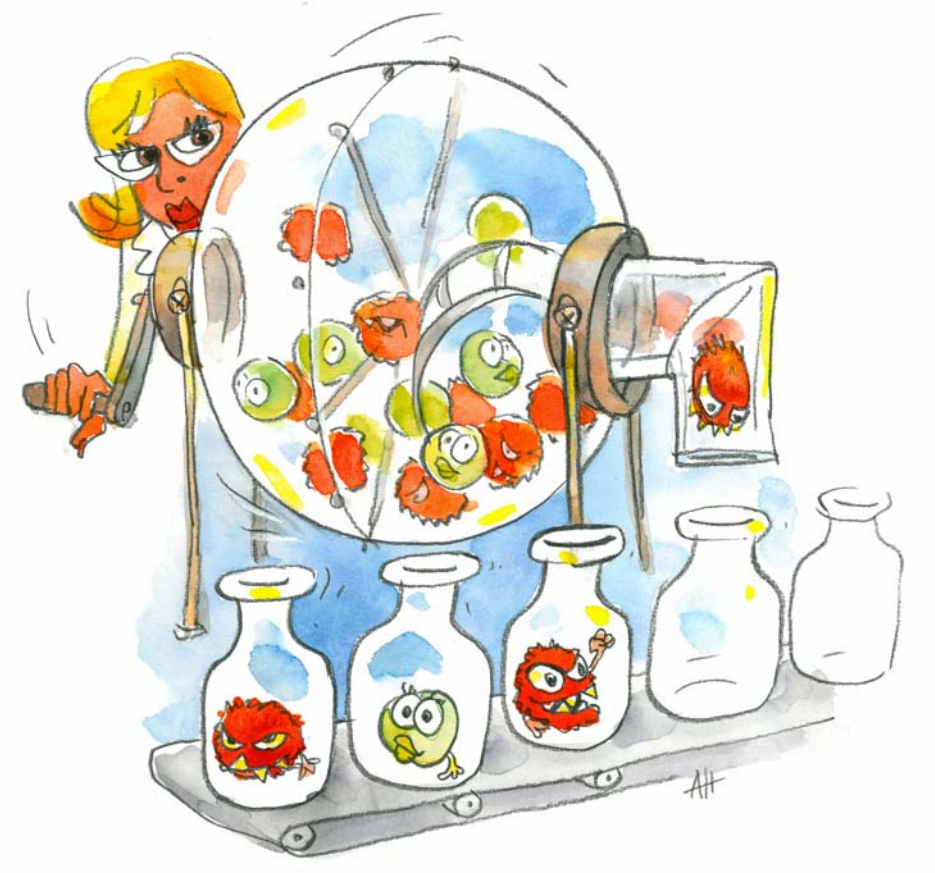
The first task focused on 1) Hygienic design based on cleaning flows in pipelines and dismantling of small equipment (supervised by the DTU team) with subtasks on a) Removing soil from process surfaces. Cleaning of a transparent T-piece soiled in mustard by fluid flow alone by applying different set up; b) Evaluation of the hygienic design of an ultra filtration unit. Use of European standard and theoretical knowledge to decide interested design features, examine the unit, disassemble and evaluate the hygienic design & 3) Evaluation of the hygienic design of a large scale fermentor. Use of European standard and theoretical knowledge to decide interested design features, examine the unit, disassemble and evaluate the hygienic design.

These two other tasks were supervised by Satu Salo and Gun Wirtanen from VTT. Task 2 focused on disinfection – efficacy, residues and detection with subtasks on efficacy test (Do normal recommended concentrates kill microbes?), chemical residue tests (Clean surface should neither contain microbes nor toxic chemicals) and detection of cleanliness (How to observe the cleaning unit of a fermentation tank?). Task 3 dealt with HACCP-based risk assessment using HYGRAM®. The purpose was to estimate the microbial risk of smoked poultry ham through all process steps using selected parts of hygiene and hazard modules.

On the last day there was an ending session before the exam which included presentation of work done with Estonian companies by Raivo Vokk, TUT, Estonia and a wrap up lecture by Alan Friis, DTU National Food Institute, DK.

The exam consisted of a questionnaire based on 34 questions (multiple choices). Some questions with just one answer and some with as much as four correct answers. All students participated in the exam and the result was that five students passed with excellence, 26 students passed and three students failed to pass. The evaluation of the workshop was the practical side all went on as planned. However the time schedule were a little packed on the first day, but this seemed fine. The participants were very enthusiastic and eager to learn. They worked intensively with the practical tasks.

PARTICIPANTS' ABSTRACTS



EFFECTS OF DRY ICE CLEANING IN ORGANIC STUFFED VINE LEAVE PRODUCTION

Taner Baysal
Ege University Engineering Faculty Food Engineering Dept.,
Bornova, Izmir, Turkey

Environmental sensitivity and demand for healthy foods have been showing an escalating trend foremost in developing countries in last decades. In this respect, productions of agricultural products have become more important in many countries around the world. Production of organic products began to increase in Turkey after 1980.

Organic foods need to be produced according to certain standards; meaning agricultural products need to be grown and process without the use of conventional pesticides and artificial fertilizers, free from contamination by human or industrial waste, ionizing radiation or food additives. In many countries require producers to obtain special certification in order to market foods as organic within their borders. On the other hand most of the food companies are not processing only organic products but also conventional products at same processing line.

Thus cleaning of the processing line prior to an organic production run is very important. It is not allowed to use non-organic chemicals as disinfection and cleaning agent at processing line before organic production run.

One of the product which is produced as organic in Turkey is stuffed vine leaves. Stuffing step is most important step where the contamination can occur from tables. In this research, dry ice (solid state CO_2) used for cleaning and disinfection purposes of processing tables (stuffing tables) where the labours are stuffing the vine leaves. Total viable count, coliform and spore forming bacteria were analyzed before and after cleaning of stuffing tables.

CO_2 has no liquid state at pressures below 5.1 atm. At 1 atm. it is a solid at temperatures below -78°C. In its solid state CO_2 is commonly called dry ice. It is

colourless and odourless and at atmospheric conditions -78°C CO_2 changes directly from a solid phase to a gaseous phase through sublimation or gaseous to solid deposition. In general, it has been observed that applying dry ice decreased both total microbial and coliform load effectively. However, the same effect was not found for neither spore forming bacteria (~2 log) nor faecal streptococci.

QUANTITATIVE RISK ASSESSMENT MODELS FOR FOOD PATHOGENS

A. Handan Baysal & Sevcan Ünlütürk
Izmir Institute of Technology, Gülbahçe, Urla, Izmir, Turkey

Risk assessment (RA) which is a holistic approach involves hazard identification, exposure assessment, dose-response assessment, and risk characterization (Oscar, 2004). Microbiological risk assessments (MRAs) have been subdivided into four steps, which comprise, hazard identification, hazard characterization, exposure assessment and risk characterization. RAs are well developed for chemical hazards, and much effort has been put into the application of this type analysis to microbial food safety risks, especially for human foodborne diseases. The methods used for MRA, as well as the underlying concepts for evaluating risk derived from foodborne organisms and the effects of control measures on reducing risk are still new to many countries including developing countries. It is important to demonstrate examples of practical data generation and modelling even if they are limited in data size.

The typical approach taken is to construct a quantitative risk assessment model (QRAM) in a computer spreadsheet using probability distributions to model the variability and uncertainty of important risk factors, such as time, temperature and pathogen density. The QRAM is then simulated using a spreadsheet add-in program that randomly samples the probability distributions and uses the random numbers generated to perform calculations and penetrate output distributions.

Many researchers have discussed the importance of modelling growth, survival, and inactivation of pathogens, thereby developing the concept of “predictive microbiology” in regard to food safety. Others have utilized more comprehensive techniques, such as Monte Carlo simulation, to develop QRAMs to describe large processes with multiple interaction steps, including growth and inactivation models.

QRAM for food pathogens using this approach and which are published in the scientific literature includes *S. enteritidis* (pasteurized liquid eggs, shell eggs), *E. coli*

O157:H7 (ground beef hamburgers, raw fermented sausages, apples, ground beef, beef burgers, beef trimmings, unpasteurized milk), *Salmonella* spp. (cooked poultry patty, whole chicken, turkey cordon bleu, almond), *Bacillus cereus* (Chinese-style rice, pasteurized & chilled courgette purees), *L. monocytogenes* (smoked salmon & trout, cold smoked salmon), *S. aureus* (Unripened cheese) and pathogens (sprout).

Monte Carlo simulation utilizes probability distribution functions such as the normal distribution as input variables in a set of governing equations in order to calculate likely distributions of defined outputs. A random number generator is used to populate the input function in a series of iterations or trials that represent different values of the input (incoming concentration of bacteria of an infected beef trimming) relative to the desired output (prevalence and concentration of the bacteria in the finished product).

QRA Modelling is a holistic approach that has a great potential as a decision analysis tool for the food industry. The advantage of QRAM over other approaches such as in-plant HACCP, is that post processing risk factors, such as food-handling practices and consumer demographics are considered in the evaluation of the microbial safety of food. More basic research is needed in assessment to support development of models that address the complex effects for the interacting microbial populations in foods. Extension of the body of scientific knowledge of predictive microbiology in foods will support improvements in risk assessment modelling and management of foodborne risks.

LOW TEMPERATURE PLASMA: A NEW DISINFECTION METHOD FOR FOOD AND FOOD CONTACT SURFACES

A.Handan Baysal[1] & Taner Baysal[2]
[1]Izmir Institute of Technology, Gülbahçe, Urla, Izmir, Turkey
[2]Ege University, Bornova, Izmir,Turkey

The need for ensuring microbial food safety and quality, without adversely affecting the nutritional, functional and sensory characteristics of foods, has led to an increase in low-temperature or non-thermal processing technologies for food preservation. These emerging technologies including high hydrostatic pressures, pulsed electric field and low-temperature plasmas inactivate microorganisms at ambient or moderately elevated temperatures and short treatment times.

Plasma is a matter containing partially or wholly ionized gas with a net neutral charge and is often referred to as the fourth state of matter as it shares properties similar to both those of gases and liquids. Low temperature plasmas (LTP) at both atmospheric and low pressures were applied for the sterilization and functional modification of the surfaces of biomedical materials and devices manufactured from heat sensitive plastics. LTP created by excitation of gaseous atoms a derivative species have been also used as a means of inactivating microorganisms on the surface of heat sensitive materials that need to be sterilized for food contact use.

A neutral gas can be converted to plasma by the application of energy in form of thermal, electric or magnetic fields and radio or microwave frequencies, resulting in an increase in the kinetic energy of electrons of constituent gas atoms. This causes a cascade of collisions in the gas resulting in the formation of plasma products of electrons, ions, radicals and radiation of varying wavelengths including that in the UV ranges. The effectiveness of plasma to inactivate microorganisms on inert surfaces will depend greatly on the equipment design and operating conditions like gas type, flow rate and pressure.

The chemical composition of LTP of nitrogen, oxygen and carbon dioxide gas mixtures are dominated by ions free radicals and highly reactive intermediate species. If water vapour is present highly reactive species (H_2O^+, H, OH, HO_2) and cluster ions containing H_2O are formed. The generation of UV radiation occurs between 0–290 nm and the wavelengths above 200 nm have cidal effects on microorganisms.

Plasma inactivation of vegetative cells and bacterial endospores attributed to destruction of DNA by UV irradiation, volatilization of compounds from the spore surface by UV photons and erosion (etching) of the spore surface by adsorption of reactive species such as free radicals. It has been shown that LTP inactivated microbial cells and spores on surfaces and *E. coli* adsorbed to the surfaces of almonds with > 4 and up to 5 log reductions, respectively.

LTP could be used as an effective way of disinfecting solid surfaces for microbial inactivation and enhancement of food safety. Thus, in order to use LTP commercially the selected equipment and conditions should be validated with the food product itself and target microorganisms.

SANITATION OF LIQUID EGG PRODUCTS BY NONTHERMAL PROCESSES: HACCP AND INACTIVATION STUDIES

A. Handan Baysal
Izmir Institute of Technology, Gülbahçe, Urla, Izmir, Turkey

Foodborne disease outbreaks involving *Escherichia coli* O157:H7 and *Salmonella enteriditis* in liquid egg products (LEPs) are the major public health concern. Egg is a principal source of the *S. enteritidis* able to colonize the ovarian tissue and is present within the contents of intact shell eggs. Most of the *S. enteritidis* outbreaks have been associated with shell eggs or egg containing products. Although statutory action has been taken at breeding flock level, contaminated eggs and LEPs remain the main source of infection. The special facility of *S. enteritidis* to cause prolonged infection of the avian reproductive tract has been a major factor in vertical transmission of the organism from breeding flocks and internal contamination of eggs is thought to have been the major factor in its spread *S. enteritidis* localizes in glandular parts of the reproductive tract. The level of shell contamination usually correlates with visible faecal contamination of shells and with the degree of excretion of *Salmonella* in faeces but *S. enteritidis* originating from the oviduct can be found on shells even when no *Salmonella* is present in faeces. *Salmonella* on egg shell shows a rapid natural reduction in ambient conditions but survival may be prolonged in more humid or cold conditions and some strains of *S. enteritidis* show more prolonged survival than others. Contaminated shell may also cause cross contamination in the kitchen or fragments may become included in bulked LEPs. Vaccination has a beneficial effect on egg contamination but there is still some contamination risk associated with the presence of *S. enteritidis* in infected vaccinated flocks. Rapid cooling by forced air, especially after washing, which increases the heat retention of stored eggs, can be used to reduce the opportunity for bacterial multiplication but lower temperatures can enhance survival of *Salmonella* on shells. Food safety programmes focus increasingly on the farm-to-table approach as an effective means of reducing hazards. This holistic approach to the control of food-related risks involves the consideration of every step in the chain, from raw material to food consumption. Hazards can enter the food chain on the farm and

can continue to be introduced at any point in the chain until the food reaches the consumer. In order to eliminate *Salmonella*, the primary control must be at farm level.

As a result, these products must be processed in sanitary facilities under continuous inspection and pasteurized before distributed for consumption. In the production of ready to use and shelf stable liquid egg products (LEP); pasteurization is the fundamental process to eliminate pathogenic microbes from the product. The most common pasteurization method for LEP is the thermal treatment (for the egg yolk 60°C/6.2 min, egg white 55.6°C/6.2 min, whole egg 60°C/3.5 min). Thermal pasteurization is the most available and best understood technique, however, it may affect the physical (coagulation, foaming and emulsifying) and functional properties (technological and nutritional) and decrease the quality of LEPs.

Alternative pasteurization methods including UV-C radiation, ultrasonic wave treatment, high electric field pulses, high hydrostatic pressure or ultrapasteurization combined with aseptic packaging have been explored to extend the shelf life and minimize disadvantages of thermal processing of LEPs. In spite of the fact that some of these methods have limitations, nonthermal food processes can be a food safety tool that serves as a complement to other food safety technologies such as HACCP. The dose required for each individual application should be established by risk analysis, taking into consideration the contamination level, the hazard involved, nonthermal process parameters, and environmental factors such as oxygen presence, the efficiency of the nonthermal treatment as well as the fate of critical organisms during manufacturing and storage.

COMPARATIVE ANALYSIS OF THE HYGIENE PROGRAMS EFFICIENCY IN DAIRY PLANTS

Alina Dobre[1], Gabriela Nedita[1], Elena Marcu[2]
[1]Institute of Food Bioresources, Bucharest, Romania
[2] SC Asi Nature SRL, Tichindeal, com. Marpod, Sibiu County, Romania

This paper aims to present the results of the hygiene monitoring in a dairy products plant started from the specific aspects, related to implementation and conformity with new food legislation, as are mentioned in "Hygiene package". The dairy products plant under survey is located in central region of Romania, in a rural area from Sibiu County. It is a small food plant, which is highly concerned to keep the specific quality of their products, in the same time with the innovation improvement. In order to assess if the hygienic conditions comply with the sanitation programs included in food safety management system, two different methods were used.

This comparative survey has been done both with rapid tests (System SURE II device and Ultra Snap tests based on ATP method) and classical microbial tests, for 4 months (between 30th of June 2008 – 20th of October 2008), in 10 trials, at one to two weeks each. The hygienic tests had been performed on direct and indirect contact surface and also on the hands of the workers. The main indicators that had been tested with these two methods are: total aerobic bacteria, yeast and moulds and *E. coli*. The mediums used in classical microbiology tests are: PCA medium for aerobic bacteria, AGC for yeast and moulds and EC broth for *E. coli.*

For the determination of yeast and moulds had been performed 12 tests 1 week apart and for the amount of total bacteria and *E. coli* 16 tests at one – two weeks apart. The results from hygiene survey had shown a correlation between ATP values and classical microbiology. The amount of yeast and moulds detected on the contact surfaces by classical microbiology is in legal limit and has an equal value with the ATP value detected with Ultra Snap tests. Total aerobic bacteria amount detected on PCA agar is similar with ATP value and *E. coli* had been found absent, on the working surface and on the hands of the workers. 75% of the results for yeast and moulds of the ATP

method were less than 10 RLU indicating a clean area and 25% were in the precaution zone (< 30 RLU relative light units), and for total aerobic bacteria 56% of the result were < 30 and the rest (44%) were over 30, but less than 40 RLU. During these 4 months had been possible to evaluate the cleanness of the working areas and of the personal and the correlation shows a similarity of 98% between the results of these methods, which could be used as a valuable argument for a food company to use rapid assessment.

Thus as a conclusion, it is safe to say that a monitoring hygiene system, based on ATP, is efficient, by rapid identification of possible sources of contamination in dairy plants which allows to take corrective actions in time. These results are important for the sanitation program included in the management system of the company and had provided many answers related to the use of rapid methods for efficient monitoring of hygienic conditions from the unit.

HACCP AND QUALITY MANAGEMENT SYSTEMS

Helen Ehavald
Fazer Bakeries Estonia, Tallinn, Estonia

Hazard Analysis and Critical Control Point (HACCP) is a food safety management system which uses the approach of controlling critical control points (CCP) in food and drink production. Codex Alimentarius Commission had produced principles and guidelines for the uniform application and implementation of HACCP. HACCP concentrates prevention strategies on known hazards and the risks of them occurring at specific points in the food chain. According to the regulation (EC) no. 852/2004 the implementation of HACCP is obligatory for all food productions.

Many companies have formal or informal quality system and this can be defined as the organisational structure, responsibilities, procedures and recourses for implementing quality management. Food safety, prerequisite programmes (Good Manufacturing Practices – GMP; Good Hygiene Practices – GHP; Food Hygiene Practices – FHP) and quality control are integrated to a company's quality management system. Different types of quality management standards are in use. HACCP can be incorporated into ISO 9001, BRC Food standard, ISO 22000 or Total Quality Management (TQM) principles.

International Organization for Standardization had published ISO 9001 and ISO 22000 management standards. ISO 22000, which was published in 2005, specifically aims at managing safety in the food chain. The standard integrates the principles of HACCP and application steps developed by the Codex Alimentarius Commission. The standard applies to organizations ranking from feed producers, primary producers through food manufacturers, transport and storage operators and subcontractors to retail and food service outlets.

A new version of the ISO 9001 quality management system standard was published in 2008 and it specifies requirements for a company's quality management system. It focuses on the effectiveness of the quality management system in meeting customer requirements. It is widely implemented in all sectors, but does not specifically address food safety, ISO 22000 standard extends to ISO 9001.

Some companies follow Total Quality Management (TQM) which is a tool and focuses on processes and process improvement. TQM provides a business system by which the whole organisation can be harnessed to meet the needs of customer requirements.

Since the 1990s many private food quality and safety standards have been published, like BRC Global Standard – Food, IFS, SQF and other. BRC standard is created and published by British Retail Consortium. The BRC standard sets out the requirements for the manufacture of processed foods and the preparation of primary products supplied as retailer branded products, branded food products and food or ingredients for use by food service companies, catering companies and food manufacturers. The standard has been developed to specify the safety, quality and operational criteria for food manufacturing companies to protect and fulfil customer expectation. BRC standard requires the development of a food safety plan based on HACCP.

Auditing is a fundamental part of a food safety or quality system, whether it be auditing to certify a supplier or a quality system, or internal auditing to assess compliance to Good Manufacturing Practice (GMP), to verify a HACCP plan or to monitor internal compliance to quality systems and procedures.

Food quality and safety is continuously evolving and the foods industry needs to keep abreast of these changes to remain competitive and meet customer requirements. Adopting of a HACCP based quality management system can help to facilitate trade nationally and internationally by demonstrating to customers the company's commitment to food safety. Functional and systematic quality management system can help to assure product quality and safety.

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PRESENCE OF *SALMONELLA* IN RETAIL TURKEY MEAT AND RED-MEAT: COMPARATIVE EVALUATION OF REAL-TIME PCR AND BACTERIOLOGY

Aysegul Eyigor, Seran Temelli & K. Tayfun Carli
Uludag University, Faculty of Veterinary Medicine, Gorukle Campus, Bursa, Turkey

This study aims to detect *Salmonella* from turkey meat and red meat by real-time PCR and two internationally-approved bacteriological detection methods, FDA-BAM and ISO6579-2002, and to obtain data on serogroup information of the isolates.

A total of 45 samples, comprised of 15 turkey meat (5 neck, 4 thigh-skin on, 6 cubed meat with skin), and 30 red meat samples (4 various deboned veal cuts, 7 veal ground-meat, 14 lamb cuts, 1 lamb ground meat) were collected from retail stores in Bursa, Turkey from June to November 2008. All samples were examined for the presence of *Salmonella* spp. by two bacteriological methods and by real-time PCR.

Fourteen (93%) out of 15 turkey meat samples yielded *Salmonella*-suspect colonies in selective agar plates in FDA-BAM and ISO6579-2002 methods, respectively. After biochemical tests, 5 (33.3%) of these samples were identified positive for *Salmonella* by ISO6579-2002 method, whereas only one (6.66%) sample was positive by PCR.

Three (10%) out of 30 red meat samples yielded *Salmonella*-suspect colonies in selective agar plates of only ISO6579-2002 method. After biochemical tests, 1 (3%) sample was positive for *Salmonella* by bacteriology, whereas 18 (60%) samples were positive by PCR.

1–3 isolated colonies from XLD agar plates of 5 *Salmonella* positive turkey meat samples and 1 positive red meat sample were selected, subcultured on to Mac Conkey agar plates, and serogrouped by available antisera. Results of the 12 isolated turkey meat *Salmonella* colonies were: 5 [41.66%] Poly A and Poly B negative, 1 [8.33%] Group C2, 1 [8.33%] Group B, 3 [25%] Group B/D, 2 [16.66%] Group E2/E3/E4. Of these 5 *Salmonella* positive samples; 2 had 3 and 3 had 2 different *Salmonella*

serogroups. The only Salmonella positive red meat isolate was Poly A and Poly B negative.

In conclusion the results showed: 1) *Salmonella* still is a problem in retail turkey meats and red meats of well-known brands in Turkey.

2) ISO6579-2002 was superior to FDA-BAM method in detecting *Salmonella* from both turkey meats and red meats. ISO6579-2002 results were more reliable for precise percentage of *Salmonella* in these meat types.

3) Bacteriology results showed a lower *Salmonella* load in red meat samples; however PCR revealed that more than half of the samples harboured *Salmonella*. This indicates that the selective enrichment, particularly the selected agars (XLD and XLT4 agars) in FDA-BAM and ISO6579-2002 methods were somehow not suitable for/insufficient in detecting *Salmonella* in red meat. Further studies should be performed to determine the most suitable selective agar(s) or their combination(s) to be used.

4) Significantly lower *Salmonella* detection rate by PCR compared to bacteriology occurred as an unforeseen instance. In this study, all DNA templates were prepared from pre-enrichments (as indicated by DNA template preparation kit) of all meat samples, and this worked well with all samples except for turkey meat samples. PCR efficiency should be re-evaluated with templates prepared from both pre-enrichment and primary enrichments of the same samples.

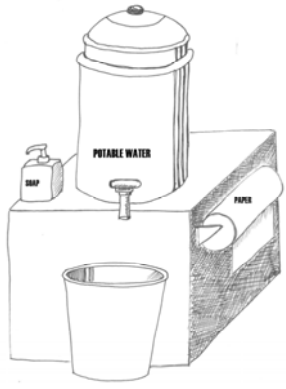
5) Detection of more than one isolate from a turkey meat sample is not uncommon if serogrouping is applied by selecting multiple colonies of a sample plate. These isolates should to be serotyped in full, and genotyped, as well, for them to be related to salmonellosis cases and outbreaks in Turkey and worldwide.

BASIC HYGIENE PRINCIPLES ON-BOARD SMALL FISHING VESSELS

Savvas Gennaris
Veterinary Services, Cyprus

Small fishing vessels represent the great majority of fishing vessels in Cyprus (around 500 of them were licensed in 2008) whereas there are only few big fishing vessels (41 in 2008). Generally when referring to small fishing vessels we mean vessels less than 12 meters long. It is quite clear that professional fishing is mainly small-scale coastal fishing. The catch of small fishing vessels is normally some tens of kilos each but when adding up the whole quantity it represents a quite considerable amount of the annual fish harvest. The fishing activity undertaken by these small and very small vessels/crafts usually only lasts a few hours and fish is consumed or sold on a daily basis. Nevertheless the beneficial effects of applying the basic principles of good hygiene practice, hygienic design and sanitation can be apparent for a wide range of fishery activities, both small and large scale, and for virtually all fish species. All species of fish, when properly handled, chilled and stored will stay fresh for longer periods than those that are not handle correctly.

In small fishing vessels that usually fish near the coast the rules and principles of good hygiene practice are difficult to apply due to shortage of clean or potable water and no necessary hygiene facilities. Furthermore proper cleaning is difficult to take place upon the small surface of such vessels during fishing. Fish on the other hand is one of the most perishable foodstuffs and requires special attention during handling, storage and transportation. Good handling of the fish caught will assure that it is safe to consume and of high quality. Although the application of proper hygienic design and satisfactory sanitation program along with the personal hygiene might seem problematic in the beginning, the adoption of simple hygienic rules and guidelines can be well worthy. The personnel involved in the fishing activities should respect and follow the rules of basic personal hygiene. This includes wearing clean clothes; washing hands frequently and avoiding involvement in fishing activities when not healthy. It is true that in the absence of water posts on-board, washing hands can be problematic. Nevertheless, a simple water container with soap and paper can be used as illustrated in the picture.



The equipment and surfaces that come in direct contact with the fish should be kept clean and if necessary disinfected. Surfaces should be smooth, water resistant and made from non-corrosive materials easy to clean and disinfect. Any parts of the deck and compartments that come in direct contact with fish should be made of non-toxic, waterproof material, or alternatively covered with proper material that is suitable for food (e.g. plastic). If paints or resins are used to cover rusted or wooden surfaces those should be certified food-grade. Any equipment in direct contact with fish (e.g. fishing tools, plastic grades and containers) should be adequately cleaned and disinfected when necessary, especially prior use. This can take place during the time that the boat is at shore. If the cleaning procedure is done at shore it is easier to perform and any toxic effluents originating the detergents and disinfectants used can be collected safely without polluting the sea and marine environment. During fishing apart from potable water, clean sea water can be used for a simple cleaning/flushing of the equipment. This requires that water should only be collected from the open sea. Closed and polluted harbours or places where accumulation of dirt is visible with naked eye must be avoided.

Boat construction should also be taken into consideration when addressing food safety issues. Curves on vessels should be smooth if possible and any store compartments should be cleaned and drainaged adequately. Cleaning can be done by using the proper detergents and a brush to remove away any dirt. The accumulation of dirt on fishing vessels along with corrosive effect of sea salt leads inevitably to a faster wear of the equipment. Therefore “the less you clean the more you pay” in renewing you equipment. Pest control should also be applied when necessary by using simple mouse and fly traps. The collection of fish, depending on the method of fishing, should be carried out as soon as possible and the fish should be washed with potable or clean sea water to remove any dirt. The storage of the fish after it has been collected should be done in way that it is protected from the sun or any other source of heat or pollution.

Fish should be stored away from bilge water, lubricants, fuels, smoke and other toxic substances. The fish can be stored in a clean container with an adequate quantity of small or fragmentary ready for use ice. Ice should be of good quality (potable or clean sea water) and stored properly. In the absence of a chilling room or refrigerated hold on-board, the storage of fish can be done in the shade inside adequately insulated ice boxes, containers and fish holds either from polyester or other material suitable for food. The purpose of chilling is to prolong the shelf-life of fish by slowing the action of enzymes and bacteria and the chemical and physical processes that can affect quality. Fresh fish is an extremely perishable food and deteriorates very rapidly at normal temperatures. Reducing the temperature at which the fish is kept lowers the rate of deterioration. During chilling the temperature is reduced to that of melting ice (0°C/32°F). Containers made out of wood should be avoided otherwise they can be used for storing fish only after the inner surface has been covered with a plastic food grade film.

Adding salt (2–3%) to freezing water/ice lowers the temperature at which the water/salt solution freezes. In other words, adding salt to ice causes melting of ice, not because the salt raises the temperature but rather because the new solution formed has a lower freezing point. The new solution aids in keeping the fish cold by allowing better contact with the fish. Water from the melting of ice should not stay in contact with the fish products as it can be a good substrate for the bacteria to grow. Any damage due to the handling of fish especially on the flesh should be avoided as it allows spoilage bacteria to enter into the flesh. The right ratio of ice should be used (2 parts fish and 1 part of ice) and the ice should be well dispersed amongst the fish and should also surround it in sufficient amounts to keep it out of contact with the container surfaces. During the uploading and transport extra care should be taken to keep fish in good condition and proper temperature i.e. follow the quote: “Be clean, gentle and cool and if you can not be cool be fast”.

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THE USE OF ENTEROCIN AS-48 TO INHIBIT *LISTERIA MONOCYTOGENES* IN SELECTED FRUIT AND FRUIT JUICES

M. Guldas[1]*, N. Georgiadis[2], O. Gurbuz[3], S. Gonenc[4]
[1]Uludag University, Karacabey Vocational School, Bursa Turkey
[2]Alexander Technological Educational Institute of Thessaloniki, Greece
[3/4]Uludag University, Faculty of Agriculture, Bursa, Turkey

In this study, it was aimed to investigate the effects of enterocin AS-48 and its combinations with different antimicrobial agents on *Listeria monocytogenes* growth in five fruits (peach, apple, orange, mandarin and quince) and three fruit juices (orange, mandarin and apple). At first, the fruit samples were contaminated with *L. monocytogenes* suspension by immersion method with enterocin AS-48 (30 mu g/ml) and then stored at different temperatures (2, 5, 10, 15 and 20°C). As control group, the samples were dipped into the sterile distilled water.

Enterocin treatments significantly inactivated or destroyed *L. monocytogenes* in peach, apple and quince stored at 20°C for up to 2 days and in peaches and apples at 15°C for up to 8 days. Immersion treatment with enterocin AS-48 also reduced the total viable counts in sliced fruits (peach, apple and quince), but did not completely inhibit the growth of these microorganisms during storage at 15 and 20°C. Enterocin (30 mu g/ml) addition into the selected citrus fruits juices as orange, mandarin and apple completely inactivated *L. monocytogenes* after 12 hours.

On the other hand, enterocin (AS-48) was also investigated in combination with selected antimicrobials (p-hydroxybenzoic acid, n-propyl p-hydroxybenzoate trisodium trimetaphosphate, sodium hypochlorite, hydrogen peroxide and polyphosphoric acid) on sliced fruits (peach, apple and quince) at 20°C. The combinations of enterocin and the antimicrobials were more effective on *L. monocytogenes* then the each antimicrobial tested ($P < 0.01$). The combination with sodium hypochlorite (20 mM) and p-hydroxybenzoic acid (120 mM) caused to decrease in the *L. monocytogenes* growth during storage at 20°C ($P < 0.01$). We concluded that enterocin AS-48 alone or in combination with other antimicrobials can be used successfully to inhibit *L. monocytogenes* in fruits and fruit juices.

RISK MANAGEMENT AND DETERMINING CONTAMINATION SOURCES TO "MANYAS CHEESE": A TRADITIONAL RAW MILK CHEESE

Reyhan Irkin
Balikesir University, Susurluk College, Susurluk, Balikesir, Turkey

Traditional raw milk cheeses are recognised as outstandingly tasty products with complex characteristics. Raw milk cheeses have pointed out as risk products and the level of pathogens in these cheeses must comply with legislations and regulations. This study was carried out to investigate contamination sources of pathogen microorganisms to Manyas cheese which is a Turkish traditional raw milk cheese and to determine survival period of these pathogens during storage time. Manyas cheese is a hard texture and high salty type unpasteurized cow's milk cheese it has typically large gas holes (3–4 mM) resulted from propionic acid fermentation at the center of it. In this research, Manyas cheese was manufactured from unpasteurized milk in a commercial dairy plant in traditional ways in Manyas region of Balikesir, Turkey. In this respect, contamination sources of *Staphylococcus aureus*, *Escherichia coli*, *Enterobacteriaceae*, Coliforms, *Pseudomonas*, *Listeria monocytogenes*, *Salmonella* spp., yeast and moulds, total mesophilic aerobic counts have been examined in the Manyas cheese samples and the contact surfaces of the dairy plant. The samples from raw milk, milk in cheese vat, heated milk (58°C), curd, heated curd, moulded cheese before salting, moulded cheese after salting, one, two and three stored cheeses were collected and immediately analysed. Also, the samples and/or swabs from the rennet, brine, cheese vats, milk stirrer, curd cutting knife, from the walls, the hands of the workers, packaging material and the storage room air were taken aseptically and analysed. Traditional cultivation techniques were used in the microbial analysis. All the analyses were replicated three times on different occasions with different samples in the year of 2008. The means of the *E. coli* counts were 1.67 log cfu/g, *S.aureus* 1.9 log cfu/g, *Salmonella* spp. 1.18 log cfu/g, *L. monocytogenes* < 0.27 log cfu/g, total mesophilic aerobic counts 6.14 log cfu/g, *Enterobacteriaceae* 2.6 log cfu/g, *Pseudomonas* spp. 1.47 log cfu/g, coliforms 1.77 log cfu/g were determined in the three months stored cheese samples and all the results did not complied with the Turkish legislations. These data showed that quality of Manyas

cheese was very poor. The raw milk, was significantly ($p < 0.05$) important contamination sources of *S. aureus*, *Salmonella* spp., *L.monocytogenes*, *E. coli* and other microorganisms. Workers's hands and rennet were primary sources of *S. aureus* also. Brine solution, cheese vats, the walls and the packaging material were not contaminated with the pathogens significantly level ($p > 0.05$). Yeast and mould counts were big problems in the production area and the storage room air during the storage period. Nevertheless, in the process heating of the curd (40–42°C) could be encourage of the pathogen microorganism counts and it was determined that all the pathogens in the samples increased after the heating process proportionally.

It is important to initiate good hygiene practise applications in the farm level since it is very important to prevent contamination of raw milk which is the essential contamination source. Also using an active starter culture in the production and implementation of a HACCP system will help to produce cheese free from pathogens. Beside of them training of personnel and determining risk management applications for these type of cheeses would be very important for to prevent public food-borne illnesses.

FOOD SAFETY IN BAKERY PRODUCT DEVELOPMENT

Kärt Kontram
Fazer Eesti AS, Tallinn, Estonia

Consumers and buyers are becoming increasingly aware of the importance of safe and high quality food products. When making a new product quality and food safety should be developed hand in hand with the product and one has to go through all the hazards that may occur until the final product is made. Hazard Analysis Critical Control Points (HACCP) system can be used to control any point in the food production process that could contribute to a hazardous situation whether it be contaminants, pathogenic microorganisms, foreign objects and involves a systematic study of the raw material/ingredients, the special conditions of manufacturing process, handling, storage, packaging and distribution of food products and consumer use. Food quality is based on a sensorial properties, chemical composition, physical properties and microbial flora. Fungi are the most common spoilers in bakery products. Commonly, a shelf-life around 3–4 days may be expected of unpreserved products. Apart from the repelling sight of visible growth, fungi are responsible for off-flavour formation and the production of mycotoxins and allergenic compounds. Research on weak organic acids (propionic, benzoic, and sorbic), packaging material and/or modified atmosphere packaging is needed. Processing, packaging and transportation are also to be kept in mind. The passage of food material over a surface leaves residual food debris which encourages the growth of microorganisms. Over time these can multiply to sufficient numbers that they affect the safety or quality of the food. Non-product contact surfaces, such as floors, walls, ceilings, are also important. As well as being reservoirs of microbial contamination, they can also be a source of physical and chemical contamination. They need to be designed so that they are durable and can be effectively cleaned. Fazer Eesti AS is a company that makes not only fresh products (such as different kind of bread, buns, cakes), but also frozen products (coffee breads, pies with different filling, baguettes, buns). Lastly named products are baked in different in-store baking outlets all over Estonia. In those outlets there are made also different products with meat, vegetable and/or dairy filling. Since Fazer bakery makes so many different products the range of clients is very high. Every company wants to give to its client the best product. To provide high quality also in baking outlets we use own-checking plan (OCP).

RISK MANAGEMENT OF READY-TO-EAT MEAT PRODUCTS CONTAMINATED WITH *LISTERIA MONOCYTOGENES*

Figen Korel
Izmir Institute of Technology, Urla, Izmir, Turkey

Nowadays, the modern life style relies heavily on the availability, quality, and safety of ready-to-eat (RTE) food products. The quality of the raw material, handling, processing, transportation and storage are the important factors influencing the microbial quality of the finished product. The mostly consumed RTE meat products are hamburgers, frankfurters, hot dogs, dry/semi-dry fermented sausages, salami, and deli meats. Several pathogens could be found on RTE meat products, including *Escherichia coli* O157:H7, *Salmonella*, *Listeria monocytogenes,* and *Staphylococcus aureus*. Vegetative pathogens are destroyed during thorough cooking and processing of these products. Contamination during post-processing in the plant, and further contamination and improper handling during storage, at retail or in home cause microbial growth. Pathogens can multiply under inappropriate conditions causing foodborne diseases and outbreaks.

One of the important pathogens found on RTE meat products posing a public health risk is *Listeria monocytogenes*. This pathogen results in about 2500 cases of listeriosis annually in U.S. and of these cases, 500 people die. Those at great risk of listeriosis are the elderly, those with suppressed or compromised immune systems, pregnant women and infants. This pathogen grows at low oxygen conditions and refrigeration temperatures. It also survives long period of time in processing plant environments, on foods, and in household refrigerators. To protect public against *L. monocytogenes,* food safety management strategies must be established especially in RTE meats. The author identified and characterized the hazard and developed the dose-response curves. Exposure assessment was made and the risk was characterized based on the individual RTE meat product. It was found that not reheated deli-meats and frankfurters were relatively often contaminated with *L. monocytogenes*, and the potential for rapid microbial growth to high levels. These products were stored for longer period of time

and had relatively high consumption rates. Dry/semi-dry fermented sausages and frankfurters (reheated) had moderate risk since these products included a bactericidal step or inhibitors. Immediate risk management action is needed to be taken for products having very high and moderate risks. New control strategies and consumer education programs should be developed. Food control measures including reformulating foods to have antimicrobials to prevent/retard growth of *L. monocytogenes* to high numbers, post packaging listericidal treatments, reduction of shelf life, or use of competitive flora to reduce the growth of *L. monocytogenes* should be taken into account. Good hygiene practices, good manufacturing practices and hazard analysis critical control point systems should be implemented to reduce the risks associated with *L. monocytogenes* in RTE meat products.

EVALUATION OF CLEANLINESS OF DAIRY PLANTS AND INNOVATIONS FOR IMPROVING HYGIENE

L. Kutsar[a], M. Mets[a], M. Rätto[b], S. Salo[b], T. Veskus[a] & G. Wirtanen[b]
[a]Tallinn University of Technology, Tallinn, Estonia
[b]VTT Technical Research Centre of Finland, Espoo, Finland

Hygiene has an important role in food industry to produce healthy and high quality products. The aim of the study was to evaluate cleanliness of dairy plants. Hygiene level was investigated before and after improvement in the hygiene practices in four Estonian dairies. To make improvement dairies had used ultrasound equipment for cleaning of small utensils and personnel had practical hand hygiene course. The microbial surveys were performed in dairies during summer 2007 and each dairy was visited two times. Survey sampling plans included sampling points in different type of surfaces like indirect/direct product contact and environmental surfaces. In addition some raw material and product, water and air samples were added in sampling plan. Microbial samples were taken aseptically from 171 different surfaces with 3 various contact plates (contac dishes were filled with Oxoid chromogenic agars for detection of *E. coli*/coliform, *Listeria* spp. and *B. cereus*), 3 commercial contact agars (Aerobic Count, *Enterobacteriaceae* and Yeast&Mould Petrifilms), non-woven cloths and *Listeria* Isolation Transport swabs. Also 34 water samples, 42 raw material and product samples were taken. In addition 29 air samples were taken with 3 different methods using Microbial Air Sampler MAS and MD8 air sampler. Temperature, humidity and pH were also measured at each sampling point. Microbial analyses were performed at VTT laboratory in Finland using traditional methods and PCR detection (iQ-Check™ *Listeria monocytogenes* II Kit). According to the results cleaning methods used in dairies were effective to provide the cleanliness of direct product contact surfaces, but indirect product contact surfaces and environmental samples were containing higher microbial counts. Protective clothing of personnel, hand washing sink and drains were potential sources of contamination. Results of detecting pathogens from dairies demonstrated that cleaning method used in dairies should be more effective to minimize the potential risk of pathogens. Amounts of detected *E. coli* and *B. cereus* were not disturbing, but *L. monocytogenes* was found from some surfaces (packaging

and filling machine). Air samples demonstrated quite high aerobic bacteria and yeasts and mould counts. Besides survey in dairies, ozone as one of the advanced disinfection technique was tested in laboratory study. Elozo ozone cabin was used and 3 disinfection times (10, 30 and 60 min) were tested. Results of ozonation test indicated that ozonated air has effective impact to destroy *L. monocytogenes* from cloths in laboratory test (> 5 log reduction).

The hygiene survey results from Estonian dairies demonstrated that hygiene level between two samplings did not improve notably and the awareness of microbe amounts in process area did not show better cleaning results. According to ozone laboratory test, the use of ozone cabin can be recommended to dairies to improve for example the hygiene of protective clothing.

PROCESSING AND WASTEWATER MANAGEMENT OF FRESH-CUT VEGETABLES

Marja Lehto[1], Risto Kuisma[2], Ilkka Sipilä[1], Maarit Mäki[3], Jenni Määttä[2], Hanna-Riitta Kymäläinen[2] & Anna-Maija Sjöberg[2]
[1]MTT Agrifood Research Finland, Vihti, Finland
[2]University of Helsinki, Helsinki, Finland
[3]MTT Agrifood Research Finland, Jokioinen, Finland

One background motivator of this study in Finland was that poor-quality carrots grown during the previous year had been delivered to school kitchens in the two municipalities, causing severe regional epidemics. During investigations of these cases, identical serotypes and genotypes of *Y. pseudotuberculosis* were found in the patient samples and in the environmental samples collected from the carrot distributor's storage facility, but the original source and the mechanism of contamination of the carrots remained unclear. On the basis of these epidemics arising from stored carrots, farmers, vegetable-processing plants and institutional kitchens have been informed by the authorities of the risk of *Y. pseudotuberculosis* in order to prevent outbreaks in the future.

Vegetable processing generates considerable amounts of wastes and wastewater, which should be treated so that there will be no risk to the environment or to human health. Wastewater should be treated before reuse, release to the recipient or use for irrigation. The wastewaters generated in various processes differ with regard to volume, organic and inorganic nutrient concentrations and microbial quality. Approximately 75% of the total organic matter of vegetable processing waters is soluble, and biological methods should be used for its treatment. Simple, efficient and cost-effective farm scale applications of wastewater treatment processes are needed.

The aim of this study was to develop Finnish small-scale production of fresh-cut vegetables, mainly carrots and lettuce. The main focuses were on the quality of raw material, processing, disinfection techniques and process water management. Microbial safety during processing must be ensured.

PRESENTATION OF THE HACCP TEAM AT MLINOTEST D.D.

Primož Likar
Mlinotest d.d., Ajdovščina, Slovenia

Company Mlinotest d.d. produce many products that are made of cereals. Main groups of products are: milling products (flour, meal, mixes for bakery), pasta (dried, fresh, tortellini), gnocchi, sandwiches and pastry products. The products manufactured must be safe and thus R&D department have to plan safe food from the beginning of developing new products. New products have to be safe for consumers. Knowledge on risk management is needed for performing the work as efficiently as possible. Primož Likar, a manager of the R&D department at the company Mlinotest d.d. and also a member of HACCP team participates risk management seminar in order to obtain information which helps in mastering the critical points in the food production.

PRESENTATION OF TASKS IN THE HACCP TEAM AT MLEKARNA CELEIA D.O.O.

Aleksander Maher
Mlekarna Celeia d.o.o., Velenje, Slovenia

Aleksander Maher is a member of the HACCP team, the R&D team and the investments team at Mlekarna Celeia d.o.o. and he is also giving lectures to students in Faculty of Agriculture and Life Sciences (Maribor) now over 3 years about technology in dairying. Here follows a list of tasks he is involved in at work:

- Reception of raw milk:
  Receiving raw milk into dairy tanks: controlling,
  Take care for quality CIP agents CENTRAL CIP (working solutions; conductivity, efficacy for each cleaning: trucks, changing programs for CIP cleaning),
  To assure the almost same total bacterial count in raw milk in tank as we get from farmers. That means quick pasteurization after receiving raw milk.
- Pasteurization:
  • Take care for undisturbed process of pasteurization. That all machines (pasteur, separator, alfast, bactofuges, etc.) works properly,
  • To assure all described parameters for pasteurized milk,
  • To assure proper work of computer (automatization, searching optimal set points)
  • To have 24 h control of whole system by mobile internet,
  • Take care for quality CIP agents SATELITE CIP (working solutions; conductivity, efficacy of each cleaning: tanks, lines, and if we need: changing programs for CIP cleaning, etc.). Many things about parameters of base, acid, disinfectants,
  • Take care for quality COP agents (working solutions; conductivity, efficacy for each cleaning,
  • Many things about bacteriophages, CIP, technology, GMP, disinfection of atmosphere in production, machines, tanks, lines and milk of course. Main issue in production is how to get rid of bacteriophages with minimum costs as quick as possible.
  • Processing of raw whey (skimming) in to a product – whey concentrate.
- Fermentation:
  • Microorganisms and their behaviour, fermentation (acidification, product density, proper conditions for undisturbed process of fermentation).

CHARACTERISATION OF THREE LACTATE DEHYDROGENASE KNOCKOUT STRAINS IN *ENTEROCOCCUS FAECALIS* V583

Ibrahim Mehmeti, Maria Jonsson & Ingolf F. Nes
Norwegian University of Life Science, Ås, Norway

In order get a better understanding of regulation and control of central metabolic processes in *Enetrococcus faecalis* V583, we constructed three mutants deficient in lactate dehydrogenase. The first part of the study was a detailed metabolic characterization of the strains. To follow up these experiments, we wanted to assess differences in proteome/transcriptome between the ldh-negative mutants and the wild type V583. The wild type and the ldh-deficient strains were studied at the proteomic level using 2-D gel poly acrylamide gel electrophoresis. Preliminary experiments show clear differences in the spot pattern between the gels from the four strains .Based on the metabolic analyses of the strains, we expect the main differences to be in proteins related to glucose metabolism, like acetolactctate sythase, pyruvate formate lyase and pyruvate dehidrogenase. Results from 2D-gels will be correlated with transcriptome analyses using microarrays. By microarray we saw the differences are been into the glucose metabolism. The expression profile of metabolically important genes, initially ldh, also is analyzed in detail by reverse transcription-PCR. The results shows the pattern seen in LDH activity assays and lactate determinations, namely that the expression of the individual ldh genes. There is not a great deal change between the wild type and the mutants, since there is very efficient machinery to compensate for the loss of lactate dehydrogenase.

THE PREVALENCE OF *CAMPYLOBACTER JEJUNI* IN ESTONIAN RAW POULTRY MEAT IN 2006–2007

Kadrin Meremäe & Mati Roasto
Estonian University of Life Sciences, Tartu, Estonia

The aim of the present study was to investigate the prevalence of *Campylobacter jejuni* in Estonian raw poultry meat in 2006–2007. A total of 193 and 46 raw poultry meat were collected in 2006 and in 2007, respectively. *C. jejuni* was detected using NMKL Method no 119, 2nd ed. 1990. A 25 g sample of chicken meat with skin was transferred to the sterile plastic bag and 250 ml Preston enrichment broth was added. After 24 h of incubation in Preston enrichment broth at 42 ± 0.5°C in microaerobic conditions, 10 µl of the enrichment broth was plated on modified charcoal cefoperazone deoxycholate agar (mCCDA) (Oxoid; Basingstoke, Hampshire, UK) and incubated for 48 h at 42 ± 0.5°C microaerobic conditions. Typical *Campylobacter* colonies on mCCDA plates were streaked on Brucella blood agar (Oxoid) plates, which were incubated for 24 h at 42 ± 0.5°C in the microaerobic conditions. Gram staining, motility analysis, oxidase and catalase tests, hippurate and indoxyl acetate hydrolysis tests were performed as the identification tests of *C. jejuni*. *C. jejuni* were isolated in 14 (7.3%) of 193 and in 1 (2.2%) of 46 poultry meat samples. A total of 1, 5 and 8 positive raw poultry meat samples were obtained in March, range from April to June and from July to September in 2006, respectively. All investigated samples from raw poultry meat were negative for *Campylobacter* from January to February and from October to December in 2006. All investigated raw poultry meat samples were also negative for *Campylobacter* from January to May and from July to December in 2007. Therefore, the higher proportions of *Campylobacter* positive raw poultry meat samples were from July to September in 2006 and in June in 2007. In conclusion, results showed that the number of *Campylobacter* positive samples of raw poultry meat in Estonia were low.

WATER COOLERS: A MICROBIAL RISK?

Maarten Overes, Wilma Hazeleger & Rijkelt Beumer
Wageningen University, Wageningen, The Netherlands

The consumption of water from water coolers gained popularity during the last decade. Drinking water from the tap in the Netherlands is of decent quality: its microbial load should be < 100 colony forming units (cfu) per ml. Despite the fact that water in so called bottle water coolers is stored longer compared to tap water, it is perceived to have a higher quality. From a microbial point of view, this is doubtful. Organic compounds from the container will leak in the water and may be used as a substrate by the microbes present in the water. Moreover, on sites of the container that are in contact with the water, often biofilms are formed. This could be a risk, if pathogens are present. The aim of this research was to determine if it is safe to drink water from water coolers. Therefore, we sampled 25 water coolers for the presence of two waterborne pathogens: *Pseudomonas aeruginosa* and *Aeromonas hydrophila*. In all samples the total colony count at 22°C using R2A was also determined. Furthermore, challenge tests were performed to investigate if *P. aeruginosa* and *A. hydrophila* were able to grow in water from water coolers. We investigated two types of water coolers: point-of-use water coolers directly connected to the tap and bottle water coolers with a large bottle on top of the apparatus. Point-of-use water coolers had an average colony count of 3.2 log cfu/ml ($\sigma = 0.6$), whereas bottle water coolers had an average count of 4.0 cfu/ml ($\sigma = 0.4$). Both counts are considerable higher than the counts in tap water: < 2.0 cfu/ml. The high number of colonies in bottle water coolers may be due to the presence of plasticizers and low amounts of cleaning agents in the water, long keeping time, aeration of the water and still-standing water. *P. aeruginosa* was found in 24% of the water coolers (n = 25). Furthermore, this pathogen was able to grow in water from water coolers. The presence of *P. aeruginosa* will probably not cause health risks, because it is not able to cause foodborne infections in healthy people. *A. hydrophila* was not detected in these water coolers. In the challenge tests this pathogen showed a slight growth (n = 3). Although *A. hydrophila* is able to cause a foodborne infection, it is known from literature that high numbers must be ingested to show symptoms of waterborne infections. In conclusion it can be stated that there is only little evidence that drinking water from water coolers is a risky action despite the observed high counts of total bacteria in the water coolers.

PRESENTATION OF TASKS IN THE HACCP TEAM AT PEKARNA PECJAK D.O.O.

Darja Peterka
Pekarna Pecjak d.o.o., Škofljica, Slovenia

Darja Peterka finished the study of Food Science and Technology in 1998. The title of her thesis was Fatty acid composition of meat lipids and adipoze fat laying hens. In 2000 she started to work in a small dairy as a food technologist – production manager where she worked 3 years. The main task was implementation of HACCP system in a production site. After that she has worked as a sales representative in a company Johnson Diversey d.o.o. in section Food and Bevarage. The main task was selling chemical agents and offering solutions for food industry (especially for tanks for collecting milk and for small plants for production meat products). In 2005 she started to work in the company Pekarna Pečjak, d.o.o. as a HACCP team leader. The company is one of the biggest one in Slovenia for production quick frozen products made from dough and for production pasta. Her main task is food safety of the products (in general). During this time the company has been successful in to gain the certificate ISO 9001 and IFS version 5. In her job Darja must follow all the EU legislation and they must implement it in their HACCP. Furthermore beside HACCP they discover new problems in production site which must be solved rapidly. Actually this is never-ending story for which they need more experience how to solve the problems fast and efficiently.

CONCLUSIONS OF PHD WORK "*CAMPYLOBACTER* SPP. IN POULTRY AND RAW POULTRY MEAT PRODUCTS IN ESTONIA WITH SPECIAL REFERENCE TO SUBTYPING AND ANTIMICROBIAL SUSCEPTIBILITY"

Mati Roasto
Estonian University of Life Sciences, Tartu, Estonia

Nowadays, *Campylobacter jejuni* and *C. coli* are the most common registered bacterial causes of human intestinal infections in many developed countries. Several epidemiological studies have shown that handling or eating poultry is an important risk factor for acquisition of campylobacteriosis. Commission Regulation (EC) No 2073/2005 on microbial criteria for foodstuffs, contains microbial criteria for specific food/microbe combinations and the implementing rules to be complied with by food business operators at all stages of the food chain. To date no criteria have been established for *Campylobacter* spp. in foodstuffs.

The objectives of the present study were: 1) To determine *Campylobacter* spp. in raw retail poultry meat in Estonia in order to provide data for understanding the significance of poultry as a potential source of human *Campylobacter* infection in Estonia, 2) To serotype and PFGE genotype *Campylobacter* isolates originating from raw retail poultry meat to understand the distribution and diversity of serotypes and PFGE genotypes in Estonia, 3) To determine the antimicrobial susceptibility of the isolated *Campylobacter* strains in order to compare it to respective levels in other EU countries and to understand the problem severity in Estonia.

Campylobacter spp. positive samples on fresh chicken products of the small-scale company (35.6%) were significantly more prevalent ($P < 0.001$) than on those originated from the large-scale company (6.3%). The chicken carcasses and wings (28% and 31.3%) had significantly more positive samples ($P < 0.001$) than chicken breasts and thighs (0% and 0%).

Proportion of *Campylobacter* positive samples on fresh chicken products of Estonian origin was 9.1% compared to 15.9% obtained from imported frozen raw poultry products at the retail level in Tallinn and Tartu during 2002–2003. Higher proportion of *Campylobacter* positive samples on imported frozen poultry products may indicate the presence of high *Campylobacter* contamination at primary production level.

Compared to raw poultry products collected in Tallinn retail outlets, more commonly *Campylobacter* spp. positive samples were obtained from products collected from Tartu markets. One possible reason for differences in positive sample proportions could be differences in transportation time of samples to the laboratories, which for the samples collected from Tallinn was several hours longer (laboratory analyses were made at the University of Helsinki) than in Tartu were analyses were performed almost immediately after sampling. However, more severe contamination of the poultry products at the retail level in Tartu may be associated with the fact that the general hygiene level in Tartu Turg, were most samples were collected, was low during that time and products were sold unpackaged.

Analysis of seasonality of *Campylobacter* positive samples indicated that the seasonal peak of *Campylobacter* on chicken meat was from June to October. Our studies showed high serotype and genotype diversity among *Campylobacter* isolates from raw retail poultry meat in Estonia. The serotype distribution did not show association with the origin of the sample. The genotyping of the 70 *Campylobacter* isolates showed *Kpn*I to be more discriminatory, yielding 34 PFGE types compared to 29 obtained by *Sma*I. PFGE with the enzymes *Kpn*I and *Sma*I for digestion proved to be discriminatory, repeatable and reproducible. In practice use of the enzyme *Kpn*I is sufficiently discriminatory. PFGE had good typeability and it was a useful tool in molecular typing of isolates from foods. In our study the majority of the isolates sharing a similar PFGE genotype originated from one country. The association of genotypes with country of origin requires further studies using a larger collection of isolates.

Our antimicrobial susceptibility studies of *Campylobacter* strains resulted in high resistance patterns for several antimicrobials. High MICs of both erythromycin and ciprofloxacin pose a problem and because erythromycin is considered as a first-line choice of treatment for human *C. jejuni* infections, the resistance has an important public health impact. Multidrug resistance in Estonian broiler chicken isolates was one of the highest reported in latest studies of broiler chicken *Campylobacter* isolates all over the world. Our findings in 2005 and 2006 suggest that the use of fluoroquinolones

may select multiresistant strains since resistance to erythromycin, gentamicin or oxytetracycline was exceptional without simultaneous resistance to fluoroquinolones.

In summary, this study which was the first of its kind performed in Estonia, revealed that there are several areas where further studies are required. More studies to monitor the potential *Campylobacter* levels and the reasons for changes in contamination levels with time are needed in Estonia. Furthermore, similar *Campylobacter* spp. control programs used in the Nordic countries could be applied in Estonia. The general focus of those programs is to high level of biosecurity at the farm level to prevent flocks from being infected and to logistic slaughter i.e. slaughtering positive flocks at the end of the day to prevent cross-contamination at the slaughterhouse. Furthermore, carcasses from positive flocks may be frozen or subjected to heat treatment. Additionally, more effective cooperation between human medicine and veterinary medicine in *Campylobacter* research is needed in Estonia in order to have the best knowledge of *Campylobacter* infection trends and finally to prevent or decrease human *Campylobacter* infections. Multiresistant strains may reflect the past history of antimicrobial usage during a longer period. This phenomenon may partly explain a rather high number of multiresistant strains in our study as well. The widespread emergence of multiresistant isolates poses a threat to humans and limits therapeutic medication. In Estonia, more restricted use of antimicrobial agents, especially fluoroquinolones, in food animal production should be implemented. Antimicrobial susceptibility studies need to be continued to find the trends in levels of *Campylobacter* resistance as well as the mechanisms for resistance and potential to decrease the *Campylobacter* resistance in Estonia. Research based risk assessment, risk management and risk communication has to be performed in Estonia in relation with *Campylobacter* spp. in food production chain.

ARTIFICIAL SWEETENERS IN FOOD PRODUCTS

Relika Ivask & Terje Tamme.
Estonian University of Life Sciences, Tartu, Estonia

The aim of the present work was to give a review of the qualities of artificial sweeteners that are used in food technology and also of their potential impact on consumer's health, and to perform the statistical processing of imported and Estonian artificial sweeteners in the years 2001–2006.

The most used artificial sweeteners, acesulfame K, aspartame, saccharine and cyclamate, were studied in the thesis. Altogether 255 products were analyzed, from them 74% were imported products from 20 countries. On the total 830 analyses were performed. The results of the analyses were compared to the maximum level of food additives established in Estonia.

Mostly beverages (37%), milk desserts (14%), confectionery products and chewing-gums (9%), beverage concentrates (8%), fish products (7%) and pastilles (5%) were analyzed. Less investigated areas were (less than 3%) refreshing confectionery, sauces, tablets, assorted bakery products, jams and snacks.

The maximum level of artificial sweetening content established in Estonia was exceeded by 7.8% of the studied products. Domestic products exceeded the maximum level by 6%, imported products by 8.5%. Artificial sweetener aspartame exceeded the maximum level most in sugar-free pastilles by 58%.

The domestic products contained the sweetener acesulfame K 2.4 on the maximum level and the imported products contained aspartame 2.6, saccharine 2.4 and cyclamate 2.1 times more than the maximum level.

In total the maximum level of sweeteners was exceeded in three product groups: fish products, confectionery and assorted bakery products. From the imported products six product groups exceeded the maximum level: pastille, refreshing confectionery, beverages, fish products, milk desserts and confectionery products.

HEAT TREATED TURKISH STYLE SUCUK: EVALUATION OF MICROBIAL CONTAMINATIONS IN THE VARIOUS PROCESSING STEPS

Seran Temelli*, Sahsene Anar, Cem Sen & Devrim Beyaz
Uludag University, Faculty of Veterinary Medicine, Gorukle Campus, Bursa, Turkey

In this work, we evaluated the microbial status of heat treated Turkish style sucuk by determining the contamination sources and assigning the relationship of the routes of contamination by specific microbial groups.

Turkish heat treated sucuks were prepared in a local meat processing plant in Bursa, Turkey for 7 months, and was replicated 10 times. Samples were taken from deboned and cubed meat, post-blending and kneading, post-grinding, post-filling and from the final product after heat treatment. We also analysed samples of spices, casing, knife, meat cutting surface, batter vat, grinding machine, filling machine, workers' aprons, workers' hands, potable water used in the plant and production and cold room area air as possible origins for contamination and/or recontamination.

Results. Statistical data revealed the following enlisted sources as primary agent(s) of contamination at indicated steps: knife (enterococci, $p < 0.001$), meat cutting surface and cold room area (total aerobic mesophilic bacteria, $p < 0.05$) in deboning; spices (enterococci, yeast and mold, $p < 0.01$) in post-blending; batter vat (yeast and mold, $p < 0.05$) and grinding machine (enterococci, $p < 0.001$) in post-grinding; filling machine (total aerobic mesophilic bacteria, $p < 0.05$; coliform, $p < 0.01$) and casing (coliform, $p < 0.01$; yeast and mold, $p < 0.001$) in post-filling. Workers' hands were very important contamination/recontamination sources in blending (enterococci, staphylococci, yeast and mold, $p < 0.05$), in grinding (enterococci, $p < 0.001$; staphylococci, $p < 0.05$) and in filling (coliform, $p < 0.001$) steps.

Conclusions. This study showed that heat treatment resulted in reduction of microbial counts in the final product, which should not be the sole step to be depended on for production of safe sucuk. Data analysed in this work revealed that there were various

sources, which contribute to contamination and recontamination to the process or to the final product. These can be summarized as follows: total aerobic mesophilic bacteria increase due to contaminations from meat cutting surface, filling machine and cold room area; coliforms increase after filling, casing and wherever the workers' hands were introduced; enterococci counts incline with the addition of spices and also with workers' hands and equipment surfaces; spices, casing, batter vat and workers' hands contributed to increases in yeast and mould counts. Workers' hands were found to harbour staphylococci and were the primary contamination sources for this bacterium mainly in post-blending and kneading, and post-grinding samples.

For a high quality and safe sucuk, certified suppliers should be used to ensure the microbial safety in all raw materials. Additionally, plant personnel should continuously be trained and assure the followings before, during and after the processing, both for their safety and the product's wholesomeness: apply good hygiene practices for the plant, take actions in processing and storage areas for cross, post or recontamination. All given recommendations are to overcome the problems encountered in the production of heat treated Turkish style sucuk.

CURRENT EFFICACY OF CLEANING PROCEDURES USED IN POULTRY INDUSTRY

Tina Tušar
University of Ljubljana, Ljubljana, Slovenia

The hygiene of surfaces, instruments and equipment in the food industry have an important impact on quality and safety of final product. In our research cleaned and dried surfaces in poultry plant are compared before working on process line and after the production was started. For this case were used swabs moistened with peptone saline. The swab contact method is commonly used to sample food industry equipment surfaces. The conventional swab technique involves rubbing 100 cm^2 area of the surface. We used swabs in the first place because it is simple and the second reason was regarding to death corners, valves, pipes, corners, crevices, gaskets etc. which are better reachable with swab. On account of delicate attainability with cleaning tools and disinfectants is given good chance to microbial ecology to grow on certain places. Microbial issues in poultry industry are frequently in formation of biofilms, which build up on equipment. Biofilms develop when microbes attached to surfaces secrete extracellular polymers such as polysaccharides and glycoproteins.

In sampling procedure two samples were taken from the same surface, from which first one was used for examining *Salmonella* spp., Total count and Coliforms-*E. coli*. Second swab was intended for *Campylobacter* spp. and *Listeria* spp. Results from plates for Total count and Coliforms showed interesting results. Clean surfaces were sometimes with more microbes than dirty surfaces. Main reasons for that could be not efficient cleaning for instance: dirty cleaning tools used for all the surfaces on production line or in different production rooms (slaughter and packing room). Reason can be also not completely dry surface after cleaning and formation of biofilms on wet surfaces, which are relatively resistant to disinfectants. Problematic was also taking samples with choosing exactly the same surface specially on working process line. It was also close estimation of surface (10 x 10) cm^2 and different pressure on clean/dirty surface may be the cause for these results.

Antibacterial effects of three commercially available disinfectants and three cleaning agents used in factories were also studied. To evaluate efficacy of cleaning and disinfecting agents (recommended concentration and temperature by the manufacturer) in real environment we used pure colonies from *Salmonella* spp., *Campylobacter* and *Listeria* spp. isolated from both factories. In research studies were used two different methods for testing efficacy: suspension test and biofilm test. 5-5-5 suspension test corresponds to the European Standard Test and was modified for our case. The results were expressed as microbicidal effect (ME) based on survival relative to appropriate controls exposed in saline. Stainless-steel coupons were the surface to grow organisms for biofilm test. Agars with stainless steel were incubated for 3 days on 30°C, and then removed to disinfectant solution for 5 min. Disinfectant effect was inhibited with neutralizer solution. For both methods we calculate microbicidal effect: ME = logNc – LogNd (Nc is number of microbes in control sample ans Nd in disinfectant sample). Results shows how many log units the disinfectant reduces the microbial population during the exposure, compared with tap water or saline.

Review of disinfectant efficacy showed that disinfectants are quite effective in suspension test on room temperature. In practice the suspension tests do not indicate the efficiency of the cleaning agents on industrial plant surfaces. Results from biofilm test were more interesting. It is well known that bacteria attached to surfaces are generally more resistant to a range of antibacterial agents, including disinfectants.

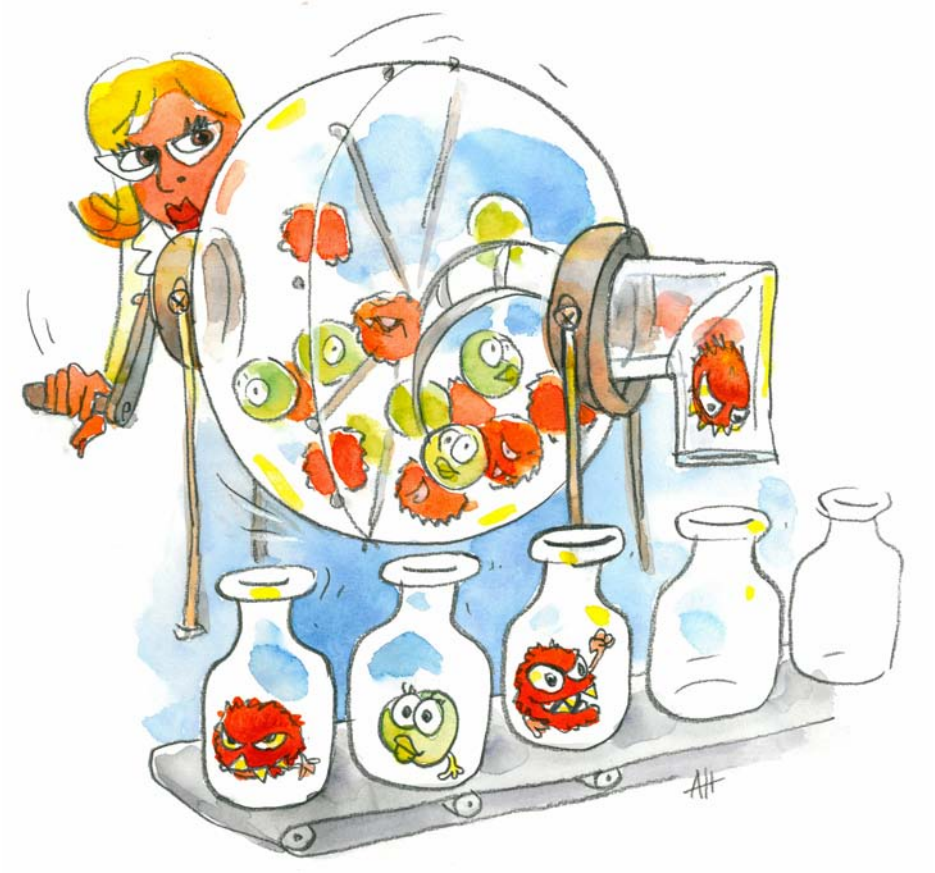
REAL TIME PCR VS. TRADITIONAL CULTIVATION METHODS

Vanja Uhan
University of Ljubljana, Ljubljana, Slovenia

The aim of the research was to compare the real time PCR results with results we gain by working with the standard cultivation method. For taking the samples we used swabs moistened with peptone saline, which are commonly used for sampling equipment surfaces in food industry. Sample was collected by swabbing approximately 100 cm^2 area of the surface. Samples were analysed on *Listeria monocytogenes, Salmonella spp.* and *Campylobacter spp.*. *Listeria monocytogenes* was analysed by modified ISO 11290-1 standard. The standard demands primary enrichment in a selective liquid enrichment medium Half Fraser broth and secondary enrichment in Fraser broth. After incubation the samples from both enrichment broths have to be plated on two selective solid media: Oxford and PALCAM agar, and put to incubation. The confirmation for *Listeria monocytogenes* was done with gram staining, catalase and oxidase test and with API test. Method for *Salmonella spp.* is based on ISO 19250. Samples were pre-enriched in Buffered peptone water and then subcultured in MKTTn (Muller Kauffmann tetrathionate novobiocin broth) and RVS (Rappaport Vassiliadis soya peptone). We used selective XLD and BGA solid agars for plating the enriched samples. For *Salmonella spp.* confirmation gram staining, catalase and oxidase test and ANI-test was done. For identification of *Campylobacter spp*. ISO 10272-1:2006 standard was modified. Samples were enriched in Bolton broth (Campylobacter Enrichment Broth, LAB 135). After microaerophilic incubation samples were subcultured on CCD (LAB 112 Campylobacter Selective Agar). For confirmation gram staining, oxidase and catalase tests were done. For our PCR research we used IQ-check ™ PCR kits from Bio-rad *Salmonella II*, *Listeria monocytogenes*, and a new kit for *Campylobacter*, which was used for the first time in our laboratory. For the PCR detection on *Listeria monocytogenes* we enriched our sample in Half Fraser broth as it was required in the instructions from Bio-rad. Samples analyzed on *Salmonella spp.* were enriched in Buffered peptone water, and for *Campylobacter spp.* Bolton broth was used. Further steps for PCR analyses were done as described by Bio-rad.

The PCR method is easy and quick, but you have to be really precise and careful so that the samples do not get contaminated. If that happens, you can get false positive results. We had some problems with *Listeria monocytogenes* PCR results. Among the latest results few were shown as inhibition. After the consultation with the expert from Bio-rad, we found out that the sample contains some problematic substance, which was inhibiting the tag polymerase and result is inhibition of PCR process. He suggested to do the dilution of a sample 1:10 and try again. Another problem, related to the negative control, was indicated right at the end of first preparation steps. We used both controls from the kit (negative and positive) at the beginning and again at the end of the sample queue. When doing the PCR test for the last samples the second negative control occurred as positive. The reason for that could be the contamination of the negative probe during the pipeting. The expert form Bio-rad suggested that we clean all the surfaces that came in contact with our DNA samples, especially the laminar, where the contamination most probably occurred. Among our samples few occurred positive on plates and negative on PCR, which should be more sensitive method. Usually are PCR analyses more comparable with standard cultivation on plates. So the main goal for the companies that are producing PCR kits, is to make the kit that will replace the cultivation on plates, because it takes less time and work and gives the same results.

GROUPWORKS



SAFETY MANAGEMENT IN PRODUCING HARD RAW MILK CHEESE

Alina Dobre[1], Reyhan Irkin[2], Annika Jürgens[3], Inesa Nielsen[4], Aura Suislep[5], Seran Temelli[6], Satu Salo[7] & Gun Wirtanen[7]

[1] Institutul de Bioresurse Alimentare, Bucharest, Romania

[2] Balikesir University Susurluk College, Susurluk, Balikesir, Turkey

[3] Greenclean OÜ, Greenclean Training, Tallinn, Estonia

[4] Technological University of Denmark, Lyngby, Denmark

[5] Tedex Eesti OÜ, Tallinn, Estonia

[6] Uludag University Faculty of Veterinary Medicine Department of Food Hygiene and Technology, Bursa, Turkey

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

INTRODUCTION

Cheese is the most diverse group of dairy products and is, arguably, academically interesting and challenging. While many dairy products, if properly manufactured and stored, are biologically, biochemically and chemically very stable, cheeses are, in contrast, biologically and biochemically dynamic, and consequently, inherently unstable. [P.F.Fox. "Cheese: chemistry, physics and microbiology". Vol. 1– General aspects. 2nd ed. 1993].

Safety of cheeses made from raw milk is a matter of great concern today. As a consequence of several outbreaks related to raw milk ripened cheeses, it was decided, that raw milk cheese has to undergo at least 60 days ripening period in order to eliminate pathogens. But many studies and results from some outbreaks show, that this ripening period alone cannot assure absence of pathogens as S*almonella spp.*, *Escherichia coli* O157:H7 and *Listeria monocytogenes*. Some of the critical factors, explaining the survival of pathogens during the aging process, were recovered: inadequate development of acidity during cheese making, a low salt level, and contamination by ill employees during the manufacture, temperature abuse of milk designed for cheese making and environmental contamination. For the control of these

and other factors affecting cheese quality and safety, many dairies apply the Hazard Analysis and Critical Control Point (HACCP) system as the most reliable tool for the successful food safety management. The main task of this group work was to: pretend being a food safety manager of a contrived dairy which produces a certain raw milk cheese; to choose and create a certain raw milk cheese product and to make a brief risk assessment covering of most important principles of HACCP system.

This group chose to work with the traditional Turkish cheese called “Manyas”. This group created the location of dairy and dairy plan itself. The cheese is made in a modern factory called “Pure Mount”, which is located in the green mountain area and has an access to a high quality drinking water. Milk supplying ecological farm is located only 10 km from the factory, away from railways, main roads and industrial area. This farm is famous with its high quality milk, excellent hygiene and animal care.

“Manyas“ cheese is made from quality raw milk and has a unique taste and aroma. Its production flow includes specific steps what are not common for other hard cheeses. Specifications of the cheese, quality requirements for the raw material, production flow diagram and description of process steps, generic HACCP plan, and other topics, concerning safety management of the cheese are described in this paper.

PRODUCT DESCRIPTION AND SPECIFICATIONS

“Manyas” cheese is tough cheese with eyes of 3–4 mM diameter decreasing from the centre to sides in its cross-section. It has own characteristic sharp taste and odour. The colour of the cheese is slightly bright cream to light yellow. “Manyas” cheese is quite salty and mostly made of raw unskimmed cow’s milk. Cheese is recommendated to store at the temperature 4°C. 100 g “Manyas” cheese contains 988 mg calcium, 518 mg of phosphorus, 1465 mg sodium, 131 mg of potassium, 43.8 mg of magnesium and 383 kcal of energy value. Other chemical quality characteristics of “Manyas” cheese is (Kamber, U., 2005; Traditional Anatolian Cheeses, Miki Edn., Ankara, 221 p):

moisture content	32.6–40.7%,
fat free dry matter	3.6–39.7%,
protein	24.8–26.5%,
fat	23.8–29%,
salt	7.5–9.3%
acidity (lactic acid)	1.43–1.68

RAW MATERIALS

The raw material for "Manyas" cheese production is milk. Milk in its natural state is a highly perishable material because it is susceptible to rapid spoilage by naturally occurring enzymes and contaminating microorganisms. [Richard K. Robinson. "Dairy microbiology handbook" 3rd ed. 2002]. To ensure the safety and quality of "Manyas" cheese quality control of raw milk has to be performed thoroughly and constantly. Quality parameters and limits are given in Table 1. The steps of raw milk from farm to dairy are following (Figure 1):

1. The raw milk is received from a cattle farm, which is close to the dairy. There are 250 Jersey cows, every one of them with veterinary certificate that assures their health and milk production. All the animals at the farm are kept in good conditions to minimize physical, chemical and microbial contamination.
2. The farm is equipped with automatic milking machines that reduce the risk of physical, chemical and microbial contamination of raw milk collected. After milking, an organoleptic and physico-chemical control is performed. There are special equipments for the control of physico–chemical parameters of the raw milk (temperature, density, freshness and cryoscopy point) and antibiotic residues. Milk showing any abnormality is not delivered for processing. The criteria are described in III Annex of CE no. 953/ 2004.
3. After quality control the milk goes through buffer tank into a delivery tank used for delivering milk to the dairy. The cleaning of the delivery tank is recorded.
4. Special equipped machines assure a proper transportation of the raw milk to the dairy. During the transportation milk has to be kept in constant temperature, and should not exceed 6–8°C at the arrival to the dairy. The transport tanks are equipped with registering temperature devices during the transportation. Further information on milk reception at the Pure Mount dairy is given in chapter 4.

Table 1. Quality requirements for the raw milk.

Quality parameters	Description/ Limits
Appearance	Cream-colored shade; no visible mechanical contaminants
Smell	Fresh and natural; foreign smell is not accepted
Temperature	6–8°C in the reception
Acidity- Freshness	6.0–7.5 °SH (titratable acidity)
pH measure	6.6–6.8
Bacteria total count/ml	100 000 cfu/ ml
Somatic cells/ ml	400 000 cfu/ ml
Antimicrobial substances	Not accepted

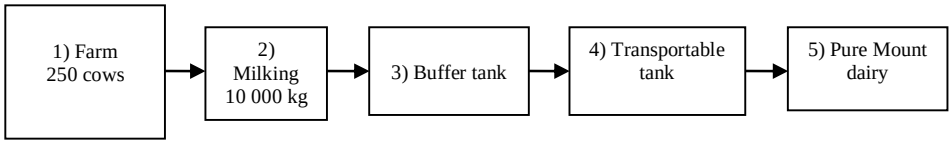


Figure 1. Raw milk flow from farm to dairy "Pure Mount".

Shelf-life is the time a product will remain acceptable in quality to the consumer. The shelf-life is dependent on the chemical, physical and microbial changes in the food. Intended shelf- life of "Manyas" cheese is one year and is assured by:

- high quality of raw milk from our trusted supplier farm;
- natural additive nisin to protect the cheese against pathogens (e.g., *Listeria monocytogenes*) and
- Good Manufacturing Practices and Good Hygiene Practices programs in processing.

SAFETY CONTROL OF "MANYAS" CHEESE AND HACCP

Requirements for Quality Control and Safety of "Manyas" Cheese

"Pure Mount" factory provides a high quality, safe and wholesome cheese "Manyas", which can be combined together with other products as macaroni, salads, sandwiches and/ grapes. It is not recommended for the elderly, children and persons with high blood pressure. Our quality management plan is including general requirements:

1) *A proper design and layout of factory*. Adequate space for all process stages and zoning of factory prevents external contamination and crosscontamination in process stages.
2) *Hygienic equipment design*. Optimising easy cleaning.
3) *Waste removal*. Continuous waste removal in production prevents cross-contamination.
4) *Water supply*. Potable water in all food contacts e.g. formulation, food washing, and cleaning of food contact surfaces prevents recontamination.
5) *Cleaning*. Provision of facilities for the cleaning of factory environment and equipment in order to avoid product contamination or cross-contamination. Clean-in-place (CIP) systems are professionally designed to ensure specified detergent concentration, temperature, and flow rates. That contributes in minimizing of possibility of cross contamination.
6) *Personnel*. Appropriate hygiene training to assure process requirements is obligatory. Streaming of suitably located toilets and hand-washing facilities.

7) *Air quality*. Ventilation e.g. air flow from clean toward dirty areas, ambient temperature control and air filtration is designed to prevent contamination.
8) *Pest and foreign-body control*. Application of special control programs and monitoring.
9) *Personal hygiene*. Procedures and guidelines for health screening, sickness/ injury reporting, working habits and protective clothing to prevent contamination.
10) *Training of personnel*. Obligatory courses concerning food processing hygiene requirements.
11) *Recall procedures*. It is very important to be able to have a recall plan and records from processing, which enables identification and recovery of any unacceptable product.
12) *Process control*. Detailed control plan for different process stages is essential in controlling hazards and in preventing consumer from buying unacceptable products. This factory has implemented an HACCP system and is using it successfully to control the production of "Manyas" cheese. HACCP is a systematic approach to identify and assess microbial hazards and risks associated with a food process.

HACCP Team

The first task in the application of HACCP is to assemble a team having the knowledge and expertise to develop an HACCP plan. Our HACCP team is multi-disciplinary and includes plant personnel from production/ sanitation, quality assurance, laboratory, engineering and inspection. It was very important for us to assemble the right blend of expertise and experience, as the team is collecting, collating and evaluating technical data and identifying hazards and critical control points. The team is also including personnel who are directly involved in daily processing activities, as they are more familiar with the specific variability and limitations of the operations. HACCP team composition, including responsibilities and number of involved people, is described in the Table 2. The HACCP team personnel should have basic understanding of: 1) technology and equipment used on the processing lines; 2) practical aspects of the food operations; 3) the flow and technology of the process; 4) applied aspects of food microbiology and 5) HACCP principles and techniques. The scope of the HACCP team is to: 1) limit the study to a specific product and process; 2) define the type(s) of hazards to be included (e.g. biological, chemical, physical) and define the food chain parts to be studied. The responsibilities of the HACCP team coordinator are: 1) ensure that the composition of the team meets the needs of the study; 2) suggest changes to the team if necessary; 3) coordinate the team's work; 4) ensure that the agreed established

plan is followed; 5) share the work and responsibilities; 6) ensure that a systematic approach is used; 7) ensure that the scope of the study is met; 8) chair meetings so that team members can freely express their ideas; 9) represent the team before management & 10) provide management with an estimate of the time, money and labour required for the study. The HACCP team members are trained on the Codex General Principles of Food Hygiene and the guidelines for the application of the HACCP system. They work together with a common focus and are using the same approach and terminology.

Table 2. HACCP Team composition.

TEAM MEMBER	RESPONSIBILITIES	PERSONS
HR director	personell, qualifications and training	1
Production director	management of the product, product elaboration	1
Supply person	establishment of contract with the farm for raw milk delivery; verification of quality certificates for raw milk; records of the nonconformitys and archives	1
Maintenece responsable	verifies tehnical stage of the equipment and sanitary performance of equipment; construction materials for production spaces and storage; ventilation equipments and the eficience of drain system	2
QC manager	identifies hazard in the dairy unit	1
Microbiologist	quality control of raw material and final product; hygienic stage of the equipments; determines the critical control points in the process flow based on the contamination level; writes procedures for hygiene practices in the unit	2
Delivery personal	contacts with beneficiary; tasks notes; complains and market information; takes measures respecting withdrawal from market of a product lot that is nonconform	1
HACCP expert	professional consultations from consultant company concerning implementation of HACCP system	1
Factory personnel	daily involvment in process activity	10

HR = Human resources & QC = Quakity control

Process Flow Diagram and Process Conditions

Figure 2 presents a general flow diagram for the manufacture of “Manyas” cheese. This figure shows numerated process steps and influence of environmental contamination to the one to ten (1–10) process steps from the reception of raw milk until the packing of final product. The process conditions are:

Raw milk held:	at 4°C, max 48 h
Heating:	58°C, 10 min
Cooling:	30–35°C
Addition of nisin:	1–25 ppm/100 l milk
Addition of rennet:	0.02%
Curd formation:	1 h
Addition of water:	50°C
Heating of curd	45°C, 30 min
Pressing:	4 h
Brining:	
1st salting	15% NaCl, for 2 d
2nd salting	8% NaCl from day 3
3rd salting	22% NaCl from day 10
Ripening:	15–25°C, 3 months
Slicing, packaging & storage:	4°C

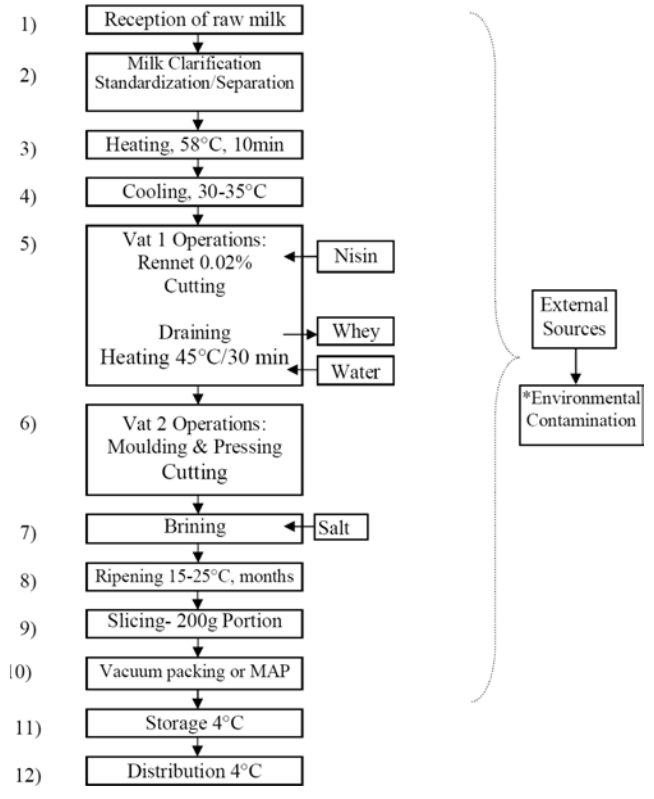


Figure 2. Process flow diagram of “Manyas” cheese production.

Potential Hazards in Raw Materials and in the Process

Hazard is biological, chemical, or physical agent in a food with the potential to cause an adverse health effect. Potential hazards in the manufacturing of the "Manyas" cheese are shown in the Table 3.

Table 3. Potential hazards in raw materials and in the process.

Hazard	Probability	Severity	Presence in raw milk	CC / S / P
Chemical				
Detergents & disinfectants	Low	High	?	P
Antibiotics			+	-
Pesticides			+	-
Mycotoxines, *Aspergillus, Fusarium & Penicillium*			+	P
Physical Technical spare parts & soil	Low	High	+	P
Microbial Somatic mastitis cells	Medium	High	+	CC, S
Pathogens: *L.monocytogenes, E. coli, B. cereus, S. aureus & Salmonella spp.*				
Total aerobic bacteria				

CC = Cross contamination; S = Survival & P = Processing

Table 3 shows the probability and severity of above mentioned hazards, their presence in raw milk and during the processing:

1) Chemical hazards (CH) - Probability of CH in raw milk is low, because we use milk from a trusted farm, which is also our business partner. This farm has a strict control on milking procedures and milk quality. Both cows and milk are being checked regularly to assure the absence of antibiotics and hormones. The farm is focusing on animal welfare and cleanliness of environment. The raw milk should also be free of moulds, which may produce hazardous mycotoxins. CH in the process is mostly detergents and disinfectants, which can contaminate product because of improper cleaning. The probability is low, because there are well developed and strict rules for cleaning procedures. Implementation of HACCP control system based on both GMP and GHP at both farm and factory level helps to avoid contamination of milk and product with CH. Severity of CH is high. Product contamination with CH can cause heavy allergic and poisoning reactions resulting in hospitalizing of consumer.
2) Physical hazards (PH) can occur in raw milk as well as during the manufacturing. In this case probability is low, because of the same reason as for CH. Severity is high,

because physical bodies in final product can hurt consumer and result in hospitalising of consumer e.g. caused by nails or studs.

3) Microbial hazards (MH) - Raw milk can contain mastitis cells, pathogens (*Bacillus cereus, L. monocytogenes, Yersinia enterocolitica, Salmonella* spp, *E. coli* O157:H7, *Campylobacter jejuni)* or an unacceptable amount of total aerobic bacteria. MH can be present in the raw milk and in the final product. Probability is at medium level, because it is not easy to assure total absence of pathogens, especially when there is no pasteurization step during the processing. Pathogens can get into the product during manufacturing, e.g. through cross-contamination from equipment, environment or personnel. The probability is lowered from high to medium by having a detailed HACCP plan based on GMP and GHP programs and training personnel constantly.

Process Steps and Description of CCP and CP's

A critical control point (CCP) is a step at which a hazard can be prevented or reduced to an acceptable level. A control point (CP) is a step in the processing of food on which the safety of the final product is not entirely dependent on but at which it is essential to maintain food safety. In processing of "Manyas" cheese, CCP and CP's were identified by using a decision tree for the determination of CCPs in the HACCP plan (Figure 3).

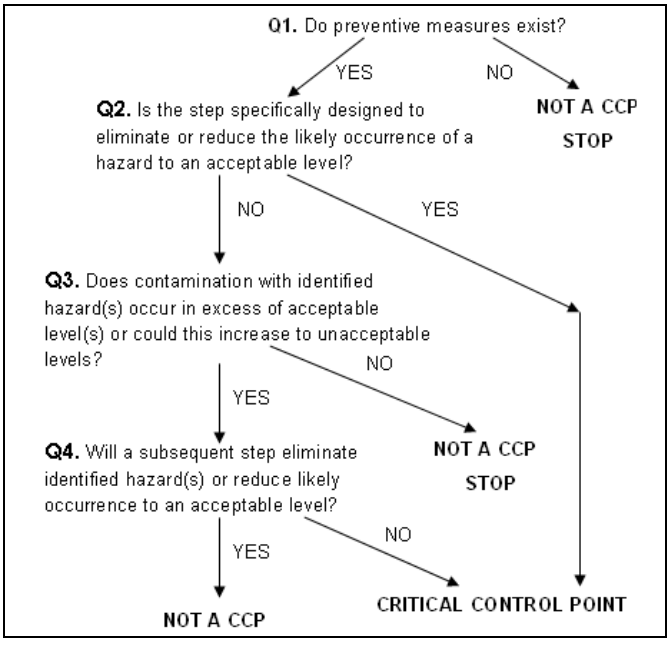


Figure 3. A CCP decision tree.

Process Steps

Step 1. Reception of raw milk

This step is identified as a CCP with respect to chemical and microbial hazards. Certain procedures have to be performed for the assurance of milk quality and safety. More information is added to the HACCP plan Table 5. Flow of milk through the milk reception is shown in the Figure 4:

1) Milk is being transported by the truck from a farm. Our supplier has to assure an immediate cooling of the milk after milking and a constant transportation temperature, which should not exceed 6–8°C.
2) At the reception all the milk is transported by pipes to the cooler and being kept at 4°C for max 48 h. It is because raw milk is in bacteriostatic phase; the cell development is stalled because of antimicrobial substances (lizozim, lactoferine). This phase can last 24–48 h at 1–4°C.
3) While milk is in the cooler, samples of milk are taken to determine the microbial contamination of raw milk. A technician is taking samples of milk in sterile sampling containers. At this stage, the laboratory is receiving samples of milk through a special designed cabinet for the further analysis The qualitative and quantitative tests are performed in a short time, with rapid tests for determination on total aerobic bacteria, somatic cells, mycotoxins and coliforms.
4) Through the balance tank raw milk goes through clarification step and other process steps.

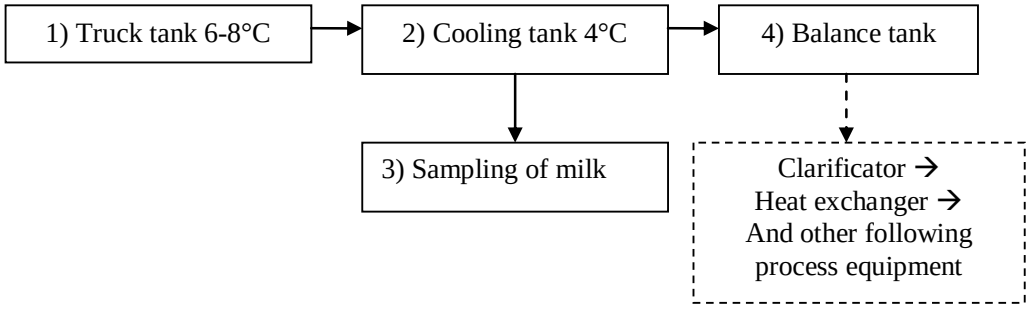


Figure 4. Raw milk flow at the reception point.

General requirements for this step are: 1) the raw milk is accepted in the production unit only with quality certificate and veterinary acceptance; 2) the reception of raw milk: must be equipped with equipment of corresponding size with the milk production; 3) a written procedure required, for the control of raw milk criteria in

accordance with CE nr. 853/2004 & 4) daily milk reception records are collected to ensure traceability of raw milk in the cheese unit.

Step 2. Clarification, standardization and separation of the milk

Clarification is a milk cleaning step, where solid impurities are being removed from milk. Separation and standardization are process parts where we adjust fat level in the milk to a desired fat content. It is done automatically in separators where microcomputer system separates the cream from the skim milk, relends part of the cream fat with the skim milk to the desired standardized content and eliminates the excess cream. Control focuses on hygiene of the process and equipment.

Step 3. Heating (58°C, 10 min)

This is a thermization step, which is replacing a pasteurization step in order to improve the bacteriological quality of the milk avoiding modification of serum proteins and impairment of factors active in cheese ripening. Thermization can inhibit only spoilage group bacteria and it can not prevent pathogenic microorganisms in the milk. Control is focused on heating temperature and time.

Step 4. Cooling (30–35°C)

Milk has to be cooled before following Vat operations. In this step a natural food preservative nisin is added.

Step 5. Vat 1 operations

This is a curd formation step. For milk coagulation and curd formation we add a natural complex of enzymes called rennet. When the curd is formed, it is being cut and stirred for a better whey separation. When curd gained enough dry matter, whey is drained out and these operations are finalized with the addition of 50°C water and gentle stirring of the curd in the water until the curd reaches 45°C. During 30 min of heating continuous mass of curd is formed. Afterwards blocks of curd are transported to Vat 2 by conveyor belts for the further operations. Control is focused on time, temperature, hygiene of vat, water (water must be free of chemicals and physical bodies and must be pasteurized at 80°C 15 min so pathogens will be killed).

Step 6. Vat 2 operations

Molding, pressing and cutting. The blocks of curd obtained are put into molds and pressed in high pressure for 4 hours at 25°C – room temperature. Molded curd blocks are being cut into loafs of smaller size: 40 x 20 x 20. This step is responsible for curd

fusion and a big part of moisture loss. Control is focused on pressing time, temperature and hygiene of the equipment also hygienic process conditions.

Step 7. Brining (Salting)

Addition of salt helps to control microbial growth and activity and various enzyme activities in cheese. The effect of salt cause syneresis of the curd resulting in whey expulsion and thus in the reduction of cheese moisture, which also influences on above mentioned things [Cheese: Chemistry, physics and microbiology, Vol. 1 – General aspects. Ed. P.F.Fox, second ed. 1993]. Though many pathogens will not grow in salty environment, some of them, for example *L. monocytogenes, S. aureus* and *Enterococcus* groups, are resistant and can contaminate the cheese. Therefore the brine solution is being sterilized by UVA treatment (320–400 nm). There are three salting steps during the brining of "Manyas" cheese and salt content is being slowly increased, in order to achieve an optimal flavor development. Control is focused on amount of salt, pH and hygiene of the process.

Step 8. Ripening

Agents involved in the ripening of cheese are: 1) rennet (influencing both curd formation and ripening); 2) indigenous milk enzymes (influencing ripening); 3) starter bacteria and their enzymes (influencing both curd formation and ripening); 4) enzymes form secondary starters (those who influencing only ripening) & 5) non-starter bacteria that is natural for raw milk or comes into milk during processing.

The ripening of "Manyas" cheese is special, because in this cheese making method no starter culture is added. Fermentation processes, formation of a special flavor, texture, structure and eyeholes are dependent on naturally present microorganisms, for example *Leuconostoc spp*. (eyeholes and flavour), propionic acid bacteria (eyeholes and flavour) and lactic acid bacteria (flavour). Propionic acid bacteria also form bacteriocins (propiocins) and contribute to safety of cheese by inhibiting the growth of some pathogens during the ripening period. The pH of the cheese is decreasing slowly during 3months of ripening and the pH of the final product is about pH 6–5.7. This is an important factor contributing to the inhibition of the growth of pathogenic bacteria. Control is focused on ripening temperature and environmental conditions (insects, rodents).

Step 9. Slicing

In size of 200 g cheese pieces are being sliced to thin diam. Control is focused on slicing room temperature (max 4°C) and slicing equipment.

Step 10. Packing

Vacuum packing is a step where the cheese is being placed in the airless environment in an airtight pack to prevent the growth of microorganisms. Absence of atmospheric oxygen protects food from spoiling by inhibiting the growth of aerobic bacteria or fungi. Pure Mount uses vacuum packing method for the packaging of Manyas cheese, so cheese (and all foods) maintain their freshness and flavour 3–5 times longer than with conventional storage methods. Packaging is adjusted in weight of 200 g of sliced product.

Table 4. Reasons for vacuum packaging.

Problem	Need	Explanation
Preservability	sliced cheese maintain its texture and appearance	microbes e.g. aerobic bacteria, mould and yeast cannot grow in a vacuum
	shelf life 4 to 8 months	when cheese is packed in vacuum and placed in a refrigerator its normal shelf life (1 to 2 weeks) turns longer (months)
Freezer burn	freezer burn is eliminated	cheese no longer become dehydrated from contact with cold, dry air
Drying	moist in cheese won't dry out	there's no air to absorb the moisture from the food
Foul odor	cheese will not become rancid	there's no oxygen coming in contact with the fats, which causes the rancid taste and smell
Pesticides	insect infestation is eliminated	insects require oxygen to survive and hatch
Food bill	reduced	cheese lasts longer; less spoiled food will need to be thrown away
Cross-contamination	eliminated	risk stays when packaking and storing (holes, sharp objects near by etc)

Vacuum system

Pure Mount is using electric pump system, which is expensive but effective and is using polyethylene (VLDPE) as a bag material. Electric pump systems are the only storage systems that eliminate exposure to oxygen. They utilize electric-powered piston pumps to extract air from the container, and seal with container to prevent air from re-entering. VLDPE is a substantially linear polymer with high levels of short-chain branches, commonly made by copolymerization of ethylene with short-chain alpha-olefins and defined by a density range of 0.880–0.915 g/cm^3. It is most commonly produced using metallocene catalysts due to the greater co-monomer incorporation exhibited by these catalysts. VLDPEs are used for hose and tubing, ice and frozen food bags, food packaging and stretch wrap as well as impact modifiers when blended with other polymers.

Labeling

Under control is that packing and packaging material are: 1) in accordance to the EU no 1272/2008 December 16th; 2) registered by authorized HACCP expert; 3) avoiding cross contamination towards packaging material (floor, food, dirt etc); 4) packing area and storage room needs to be cleaned, dry and with acceptable ventilation; 5) not allowed to store in wrong areas like process rooms, hallways, chemical storage room, rooms which are in overhauling or refurbished, etc; 6) stored so that there is possibility to access nearby (apart from floor, aloof of wall); 7) in hygienic process when looking zoning and aseptic & 8) protecting the "Manyas" as a final result, not contaminating. *Control*: packing room temperature should not exceed 4°C and PM should be kept in original packages until their use.

Step 11. Storage

The product should be kept at constant and stable temperature- 4°C. *Control*: storage temperature and pests.

Step 12. Distribution

The transportation of cheese to the wholesalers or retailers temperature should be 4°C.

Verification actions

We use production evaluation (control methods, procedures, product tests etc) to determine compliance with the HACCP plan. Our HACCP team has specified methods and frequency of verification procedures, which are following: 1) microbial examination; 2) review of complaints from consumers or regulatory bodies and outcomes of investigations into these complaints; 3) auditing all monitoring and corrective actions records & 4)a review of validation records, if necessary apply more selective tests at selected CCPs.

Documentation and record keeping

We have to be able to demonstrate that the seven HACCP principles have been correctly applied. We need following three (3) types of documentation: Documentation control in ISO 22000 – finalized HACCP plan; guidelines of good hygienic practice (GHP) & procedures state clearly what should be done, how equipment or materials should be used, and by whom and how defects should be recorded. The procedures are:

- Training for hygiene and operation (once a year hygiene courses at the factory from professional consultant);
- personal motivation program, (psychologists are invited for the private conversations about working atmosphere with employees. Reasons for that: 1) motivation & 2) improvement of working atmosphere);

- personnel hygiene and sickness reporting;
- on-site food services;
- use of protective clothing;
- inspection and maintenance of equipment, manufacturing services (water, compressed air, drainage), and the building/ site;
- raw materials/ ingredients- specification/ audit/ sourcing;
- waste disposal;
- cleaning equipment/ environment, CIP/ manual – work instructions give detailed instruction to operatives as to what has to be done at each process step. They are generated directly from the HACCP plan: 1) equipment manufacturer's instructions; 2) product recipe (ingredient quantities, process times and temperatures, routing of intermediate product and final product through the factory), and action & 3) record sheets.

Process Environment

Environmental process conditions have a big importance in food processing. For example airborne pollution is one of the major hazards, also accumulation of water or swirling dusts from the unpaved roads beside the factory should be avoided. In this case when we created a fantasy factory, we assumed that the factory Pure Mount is located far away from main roads in the green mountain area with the good access to fresh water. There will always be food contamination risks derived from environment.

Airborne problems

The air entering "Pure Mount" factory will contain only few viable dust partcles e.g. bacterial and fungal spores, because an appropriate ventilation system has been installed in the factory. In the "Pure Mount" factory risk of air contamination is minimized by ducting of incoming air through air filters. Because most of our processes are open, it is essential that air filters remove as small dirt particles as possible. In the factory air goes through the primary filter to remove gross contamination (5.0-to 10.0-µm diameter particles) and after that it goes through the filter capable of removing at least 99.9% of particles in the 0.1–0.2-µm range. Air quality acceptable limits are: total bacterial count of $< 1.0 \times 10^{2}$cfu m^{-3} and yeast and mould count of $< 1.0 \times 10^{1}$cfu m^{-3}. The testing and cleaning frequency of filters and routine for replacing filter holders is described in the HACCP plan for environmental contamination.The main sources of microflora are coming into the facilities from air movement, drains in the floor and the passage of personnel. Ventilation chambers,

filters and ducts should be cleaned of easily ignited dust and accumulated combustible material within the terms established by the person in possession of the site, but not less frequently than once a year. It is important that the building be able to breathe. The latter is ensured by a clean ventilation system, above all by cleaning hatches on ventilation ducts.

Water supplies

Water is a very important substance in the cheese processing. We use it, for example, for heating of cheese or cleaning the plant equipment etc. Our water supply is safe to drink. Though pathogens as *E. coli, L. monocytogenes*, or *Salmonella spp*. cannot grow in water and are sensitive to the levels of chlorine found in drinking water doesn't mean that they are unable to survive. In the factory Pure Mount there is installed a special water treatment system, where the water is being treated with ozone in order to assure the high water quality. According WHO (1993) suggestions, water which cones into direct contact with food should have a psychotropic count of $< 1.0 \times 10^2$ cfu ml^{-1} and no *E. coli* detected in 100 ml.

Animal vectors

Storage pests, insects and other animal vectors cause a big risk of infestation. The following animal vectors are controlled: 1) *Rodents* – a large amount of food can be contaminated with saliva, hairs, urine or faeces. Main controlling steps include careful observation of possible entry points. Rat's urine and hair fluoresce brightly in UVA, so that occasional inspections with "black" lamps are helping to confirm that rats have not found an entry point and rodent proofing of vulnerable points with sheets of metal or wire mesh and 2) *Insects* are particularly able to contaminate product. They transmit the dirt, infections and dying insects easy drops into food without being noticed. In this dairy the windows cannot be opened during normal processing. Futhermore, electrified grids backed by ultraviolet fluorescent tubes have been installed in the pre-treatment, processing and packaging areas.

Structural features

Seeking to avoid aerial contamination we put a lot of attention on physical separation of specific processes e.g., dry storage areas from wet processing areas. Factory was built with respect to hygienic requirements and suggestions of GMP and GHP. *Floors* are made of polyurethanes. This type of flooring in the production area is very suitable for us as it provides seamless covering, has an excellent durability and is resistant to chemicals, such as the acidic or alkaline detergents and sanitizing agents used for

cleaning of the plant and equipment. As with floors, we were also focusing on hygienic structure of *walls and ceilings*. Walls in the production area of our factory have polyurethane-based coating, which ensures seamless surface and together with the use of proper cleaning procedures ensures a high standards of hygiene. Some of the general requirements are: 1) walls must be free from crevices, where dirt, bacteria and fungi can harbour; 2) walls and ceilings must be resistant to hot water and chemical detergents/sanitizing agents; 3) the junctions between floors and walls must have smooth concave surfaces to aid cleaning and 4) all joints on the ceilings have to be sealed with a bonding material that does not crack or loosen during routine cleaning.

Factory layout and zoning

A proper zoning of the "Pure Mount" factory helps to avoid cross contamination during processing. It also helps to achieve an optimal process and personnel flow.

Process equipment

A number of different facilities are being used in the dairy Pure Mount during the cheese processing. Main equipment is: tanks, clarifiers, separators, heat exchangers, vats, mixers, slicing and packing equipment, etc. While buying an equipment for this factory, we were concerning on hygienic equipment design (rounded corners and edges, no welding in the corners, no crevices etc.), the durability, ability to sustain the impact of acid or chlorine and heat resistance. Every item in our factory is accessible for cleaning both inside and outside. Product contact surfaces are made of stainless steel and our cleaning regime allows thorough disinfection of all facilities used for Manyas cheese processing. Areas behind or beneath the items are also easy accessible for cleaning so the dirt is not collecting in any spaces or crevices. Our pipework was installed without dead ends, from which food residues are hard to be removed during cleaning.

Cleaning and sanitation program

Cleaning steps are generally documented steps that must be followed to ensure adequate cleaning of product contact and non- product surfaces. These training procedures must be detailed enough to make certain that adulteration of product will not occur. All HACCP plans require mentioned steps to be documented and reviewed periodically to incorporate changes to the physical plant by responsible management. The documented paper- a cleaning plan- is constructed of sanitary design. Manual cleaning requires total disassembly for cleaning and inspection, e.g. in packaging and cutting area. In manual cleaning dry or damp cleaning is preferred. The movements

during cleaning are directions from- window- to- door and from above downwards. Open surface cleaning (OSC) is for open surfaces (transport lines, floors etc.) are used foam-cleaning method. Medium pressure foam system is for pre-rinsing, foaming, rinsing and disinfection. Concentration is fixed by correct nozzles in the foam system satellite. Cleaning- in- place (CIP) method is cleaning of interior surfaces without disassembling equipment e.g. pipes, vessels and associated fittings. The process is fast, not labour intensive, is repeatable and poses less chemical exposure risks to people.

- **CIP-1** → Raw Milk Storage tank and line to the Machinery Room; Every Day → after empty one-step alkaline cleaning and once per week alkaline + acidic cleaning
- **CIP-2** →Machinery Room and Production Area close system objects; **c**losed system (e.g. Machinery Room objects, and Production areas objects) is cleaned by automated CIP-system and cleaning solution collecting back to the CIP-tanks and final rinsing water collect back to the recovery tank.

A useful mean of preventing cross-contamination is by the implementation of an effective colour code policy e.g. red for sanitary rooms, yellow for kitchen, white for contact areas (disinfection). The use of a printed microfibre cloth with the GPS-folding technique is based on a logical, useful and user-friendly pattern. The print is a guiding hand to have full control, full access and full speed during the most important cleaning operations in furniture and sanitary cleaning area's. All the surfaces from the cleaning cloth can be used correctly, safely and with a higher performance. It helps saving time, water, chemicals and energy. All cleaning equipments must be hygienically designed and washable in washing machine. Cleaning equipments must be cleaned after use. The cleaning is taking place from dirty towards cleaner place. Chemical Storage Room will hang instructions on colour coding and cleaning plans for each room and area.

Training and internal control

Obligatory training courses are usually held lecture-style, and many people attend such lectures only for the sake of receiving a piece of paper. Pure Mount adds some variety to food hygiene training, HACCP and self- checking and cleanout trainings; making even lectures interesting on microbiology level. Pure Mount also invites trainers to go thru among personnel of dairy motivation trainings etc. The trainings will be held periodically and the target group are all workers in the dairy, i.e. production director, supply person, maintenance responsible, quality control manager, microbiologist, delivery personal and factory personnel. Also dairy invites farmers to participate in trainings. Every person has therefore a perfect overview of what they are obliged to

follow, where restricted authorization are in zoning to avoid cross contamination, what equipments should be sanitized and how, etc. All trainings are headed in the name of welfare of Pure Mount, it means that all subjects are in accordance to real questions that one dairy faces- cross contamination, *B. cereus, S. aureus, E. coli* etc. Usually trainings will be led by Director of Human Resources who has passed exams by Trained HACCP Auditor. But facility periodically invites guest lecturers from both inside Europe and in third countries. Nevertheless, the trainings are pursuant to Pure Mount standards and are not delegated to any person without proper training. All trainings end with handing over a certificate over a person who participated in the training, successfully passed a control test. The renewed certificates are to be held in dairy. The European Parliament and Council of the European Union regulation no 852/2004 (29 Feb 2004) on foodstuff hygiene lists following topics to be discussed: 1) food hygiene and principles of food hygiene; 2) microbes and factors involved in their growth and reproduction; 3) food poisoning, diseases and infections which spread via foodstuffs; 4) physical, chemical and microbial contamination of food and prevention; 5) methods of and conditions for preservation and storage of food and temperature control; 6) structure of handling companies, technology and equipment; 7) food hygiene and cleaning and disinfecting; 8) food hygiene and pest control; 9) food hygiene and personal hygiene; 10) principles of self-checking; 11) legal acts pertaining to food and 12) obligations of food handling employees and responsibility for compliance with food hygiene requirements based on job duties.

HACCP, self-checking and plan

Topics to be discussed are: 1) threat, risk, critical surface, contact point, control criterion; 2) remedial actions; 3) preparations for implementing self-checking; 4) threats and identification of risks, threat and risk assessment; 5) critical contact and determination; 6) remedial action and determination; 7) self-check system and documentation; 8) supervision & 9) self-checking system and plan assessment. A self-checking plan and training contains: 1) description of the company's activity; 2) company location map and room layout; 3) description of the handling stages; 4) supply and receiving of food; 5) checking storage conditions; 6) description of cleaning and disinfection; 7) employee hygiene; 8) employees who come into contact with food have passed food hygiene training – briefing; 9) employees hold valid health certificates; 10) pest control; 11) waste handling; 12) water supply and sewerage; 13) results of drinking water analyses; 14) complaints and suspected food poisoning – how to resolve and what action to take; 15) briefing all employees on actions related to the

self-checking plan & 16) verifying the functioning of the self-checking plan and document archival.

Cleanout

In here, considered are two aspects. Firstly, food industry equipment is constructed of sanitary design. Secondly, some automated processing equipment by necessity is difficult to clean. An individual cleaning plan includes both as well as: 1) the equipment or affected area to be cleaned, identified by common name; 2) the tools necessary to prepare the equipment or area to be cleaned; 3) how to disassemble the area of equipment & 4) the method of cleaning and sanitizing. Professional cleaning service is an action through time, money and health is saved. Topics to be discussed are:

- cleaning and the fundamentals of cleaning;
- the cleaning process: cleaning, sanitizing and inspecting;
- cross contamination, risks;
- antiseptic;
- aseptic technique;
- cleaning service provider and necessity and responsibility;
- cleaning work and planning;
- ergonomics and ergonomic techniques;
- proper cleaning agents, accessories, supplies, chemicals, cleaning machines.

CONCLUSION

This group work was carried out as a training exercise in food risk management. The group were from different countries and had distinct educations and experiences in different fields of food production. During the work together we found out how important is to have a good communication, support and share of work. Making HACCP plan requires a lot of time and specialists with different competences. Though a generic HACCP plan can seem very detailed including a lot of information about control of different hazards, separate HACCP plans for separate hazards need must be made in order to have a fully control over all possible hazards. Factory, seeking to have the best quality and safety assurance of the product, has to link its HACCP to the other quality control systems e.g. Total Quality Management (TQM), which include good manufacturing practice (GMP), good hygiene practice (GHP), and document control e.g. ISO 9000 Quality systems. This would help to avoid a stand-alone approach, which usually leads to a failure of a successful implementation of HACCP.

RISK MANAGEMENT IN PRODUCTION OF EGG-PASTA

Ayse Handan Baysal[1], Savvas Gennaris[2], Primož Likar[3], Darja Peterka[4], Oana Popescu[5] & Tiina Veskus[6]
[1] Izmir Institute of Technology, Gulbahce, Izmır, Turkey
[2] Veterinary Services, Cyprus
[3] Mlinotest d. d. Adjovščina, Slovenia
[4] Pekarna Pecjak d. o. o. Škofljica, Slovenia
[5] S.C. Vel Pitar S.A., Romania
[6] Tallinn University of Technology, Tallinn, Estonia

Pasta (Italian for "dough") is a generic term for Italian variants of noodles, food made from a dough of flour, water and/or eggs. The word can also denote dishes in which pasta products are the primary ingredient, served with sauce or seasonings. There are approximately 350 different shapes of pasta. Examples include spaghetti (solid, thin cylinders), maccheroni (tubes or hollow cylinders), fusilli (swirls), and lasagne (sheets). Two other noodles, gnocchi and spätzle, are sometimes considered pasta. They are both traditional in parts of Italy. Pasta is categorized in two basic styles: Dried and fresh. Dried pasta can be stored for up to two years under ideal conditions, while fresh pasta can be kept for a couple of days in the refrigerator. In preparation for consumption, pasta is generally boiled. Pasta is made from a simple combination of flour and water. Pre-packed speciality pasta often includes spices, cheese or added colouring from spinach, tomatoes or food dye. Under Italian law, dry pasta (pasta secca) can only be made from durum wheat flour or durum wheat semolina. Durum flour and durum semolina have a yellow tinge in colour. Italian pasta is traditionally cooked al dente (Italian: "to the tooth", meaning not too soft). Outside Italy, dry pasta is frequently made from other types of flour (such as wheat flour), but this yields a softer product, which cannot be cooked al dente. Egg-pasta is a very popular food in world and its appreciation is growing in Europe and in the United States. Starting from very simple ingredients (semolina and eggs), the secret to obtain the best quality is to find the higher quality of the two base-materials (no water is added to the kneading) and the best drying process. A high percent content of eggs and good semolina are the

basic starting points to obtain egg-pasta of a good quality. However, the drying process is the final fundamental step to obtain a quality product. In fact, the right drying process assures the production of a safe, tasty, and nutritious product after the cooking process with the proper sensory characteristics.

The food chain has become more and more complex and that is why it now represents an important issue, which requires specific measures, meant to assure an acceptable standard for food security. The general legislative framework, which will enforce preparation and distribution of food fit for consumption must be strengthened by the organizations through specific regulations. These regulations require adequate standards concerning food safety, correct labelling that is easily read and understood, good manufacturing practice, good hygiene practice and also proper management systems concerning food quality. If there is an established system for controlling food risks, the authorities respond quickly if a food safety problem appears. A lot of effort is put into preventing food risks and food safety laws are stringently enforced by the authorities which results in an active consumer protection. Therefore the process of weighing policy alternatives in the light of the results of risk assessment (RA) and, if required, selecting and implementing appropriate control options, including regulatory measures which is defined as "Risk Management" by FAO (1997) meets these goals. The objectives of the present study were (1) to analyze probable microbial hazards in the dried egg-pasta production, (2) to establish the critical control points of the whole process, and (3) to evaluate the risk assessment (RA).

PASTA MANUFACTURING

General

Although pasta products were first introduced in Italy in the 13th century, efficient manufacturing equipment and high-quality ingredients have been available only since the 20th century. Prior to the industrial revolution, most pasta products were made by hand in small shops. Today, most pasta is manufactured by continuous, high capacity extruders, which operate on the auger extrusion principle in which kneading and extrusion are performed in a single operation. The manufacture of pasta includes dry macaroni, noodle, and spaghetti production.

Process Description

Pasta products are produced by mixing milled wheat, water, eggs (for egg noodles or egg spaghetti), and sometimes optional ingredients. These ingredients are typically

added to a continuous, high capacity auger extruder, which can be equipped with a variety of dies that determine the shape of the pasta. The pasta is then dried and packed for market.

Raw Materials

Pasta products contain milled wheat, water, and occasionally eggs and/or optional ingredients. Pasta manufacturers typically use milled durum wheat (semolina durum granules and durum flour) in pasta production, although farina and flour from common wheat are occasionally used. Most pasta manufacturers prefer semolina, which consists of fine particles of uniform size and produces the highest quality pasta product. The water used in pasta production should be pure, free from off flavours, and suitable for drinking. Also, since pasta is produced below pasteurization temperatures, water used should be of low bacterial count. Eggs (fresh eggs, frozen eggs, dry eggs, egg yolks, or dried egg solids) are added to pasta to make egg noodles or egg spaghetti and to improve the nutritional quality and richness of the pasta. Small amounts of optional ingredients, such as salt, celery, garlic, and bay leaves, may also be added to pasta to enhance flavour. Disodium phosphate may be used to shorten cooking time. Other ingredients, such as gum gluten, glyceryl monostearate, and egg whites, may also be added. All optional ingredients must be clearly labelled on the package. The kinds of wheat used to make pasta are two: durum wheat (*Triticum durum*) and wheat (*Triticum vulgare*). The first one is milled in order to produce durum wheat flour and is mainly used to make pasta. The second one is milled and the flour is used to make homemade egg-pasta, as well as other recipes. At first sight these two kinds of wheat do not show big differences, durum wheat's grain is a little longer and more opaque, whereas the grain of wheat is less opaque and rounder (Walsh & Gilles, 1977; Anonymous, 1992).

Wheat Milling

Durum wheat is milled into semolina, durum granular or durum flour using roll mills. Semolina milling is unique in that the objective is to prepare granular middling with a minimum of flour production. After the wheat is milled, it is mixed with water, eggs, and any other optional ingredients (Walsh & Gilles, 1977; Anonymous, 1992).

Mixing

In the mixing operation, water is added to the milled wheat in a mixing trough to produce dough with a moisture content of approximately 31%. Eggs and any optional ingredients may also be added. Most modern pasta presses are equipped with a vacuum chamber to remove air bubbles from the pasta before extruding. In case of the air is not

removed prior to extruding, small bubbles will form in the pasta, which diminish the mechanical strength and give the finished product a white, chalky appearance (Walsh & Gilles, 1977; Anonymous, 1992).

Extruding

After the dough is mixed, it is transferred to the extruder. The extrusion auger not only forces the dough through the die, but it also kneads the dough into a homogeneous mass, controls the rate of production, and influences the overall quality of the finished product. Although construction and dimension of extrusion augers vary by equipment manufacturers, most modern presses have sharp edged augers that have a uniform pitch over their entire length. The auger fits into a grooved extrusion barrel, which helps the dough move forward and reduces friction between the auger and the inside of the barrel. Extrusion barrels are equipped with a water-cooling jacket to dissipate the heat generated during the extrusion process. The cooling jacket also helps to maintain a constant extrusion temperature, which should be approximately 51°C. If the dough is too hot (above 74°C) the pasta will be damaged. Uniform flow rate of the dough through the extruder is also important. Variances in the flow rate of the dough through the die cause the pasta to be extruded at different rates. Products of non-uniform size must be discarded or reprocessed, which adds to the unit cost of the product. The inside surface of the die also influences the product appearance. Until recently, most dies were made of bronze, which was relatively soft and required repair or periodic replacement. Recently, dies have been improved by fitting the extruding surface of the die with Teflon® inserts to extend the life of the dies and improve the quality of the pasta (Walsh & Gilles, 1977; Anonymous, 1992). The low-temperature drying process is clearly a better way to obtain the best egg-pasta quality, even if the final cost for the consumer is higher because of both the longer time needed and the higher quality of the starting semolina and egg (Materazzi et al. 2008).

Drying

Drying is the most difficult and critical step to control in the pasta production process. The objective of drying is to lower the moisture content of the pasta from approximately 31% to 12–13% so that the finished product will be hard, retain its shape, and stored without spoiling. Most pasta drying operations use a preliminary drier immediately after extrusion to prevent the pasta from sticking together. Pre-drying hardens the outside surface of the pasta while keeping the inside soft and plastic. A final drier is then used to remove most of the moisture from the product. Drying temperature and relative humidity increments are important factors in drying.

Since the outside surface of the pasta dries more rapidly than the inside, moisture gradients develop across the surface to the interior of the pasta. If dried too quickly, the pasta will crack, giving the product a poor appearance and very low mechanical strength. Cracking can occur during the drying process or as long as several weeks after the product has left the drier. If the pasta is dried too slowly, it tends to spoil or become mouldy during the drying process. Therefore, it is essential that the drying cycle is tailored to meet the requirements of each type of product. If the drying cycle has been successful, the pasta will be firm but also flexible enough so that it can bend to a considerable degree before breaking (Walsh & Gilles, 1977; Anonymous, 1992). Times and methods for the drying process vary from six to eight hours, from 40° to 80°C. Currently there is the tendency to increase the drying temperature because it was observed that the overall structure of the product is improved and gets a better consistency during cooking. The drying process varies according to the style of pasta to be made, it is very important because it gives pasta higher preservation, moreover stabilizes the raw materials while exalting organoleptic properties, as well as optimizing its characteristics for a good cooking. The process consists of ventilating pasta with a hot air stream, followed by a cooling process in order to have pasta at a room temperature. At this point pasta is ready to be packed (Walsh & Gilles, 1977; Anonymous, 1992).

Packaging

Packaging keeps the product free from contamination, protects the pasta from damage during shipment and storage, and displays the product favourably. The principal packaging material for noodles is the cellophane bag, which provides moisture-proof protection for the product and is easily used on automatic packaging machines but is difficult to stack on grocery shelves. Many manufacturers utilize boxes instead of bags to pack pasta because boxes are easy to stack, provide good protection for fragile pasta products, and offer the opportunity to print advertising that is easier to read than on bags (Walsh & Gilles, 1977; Anonymous, 1992).

Emissions and Controls

Air emissions may arise from a variety of sources in pasta manufacturing. Particulate matter (PM) emissions result mainly from solids during handling and mixing. For pasta manufacturing, PM emissions occur during the wheat milling process, as the raw ingredients are mixed and possibly during packaging. Emission sources associated with wheat milling include grain receiving, pre-cleaning/handling, cleaning house, milling, and bulk loading. There are no data for PM emissions from mixing of ingredients or

packaging for pasta production. Volatile organic compound (VOC) emissions may potentially occur at almost any stage in the production of pasta, but most usually are associated with thermal processing steps, such as pasta extruding or drying. No information is available on any VOC emissions due to the heat generated during pasta extrusion or drying. Control of PM emissions from pasta manufacturing is similar to that Grain Elevators and Processes. Because of the operational similarities, emission control methods used in grain milling and processing plants are similar to those in grain elevators. Cyclones or fabric filters are often used to control emissions from the grain handling operations (e.g., unloading, legs, cleaners, etc.) and also from other processing operations. Fabric filters are used extensively in flourmills. However, certain operations within milling operations are not amenable to the use of these devices and alternatives are needed. Wet scrubbers, for example, may be applied where the effluent gas stream has high moisture content (Walsh & Gilles, 1977; Anonymous, 1992).

Quality characteristics of pasta

The qualities that pasta must have in order to meet the criteria and expectations of consumers are as follows: (i) a uniform, amber-yellow colour without shades of grey or red; (ii) clean surface appearance without brown, black or white spots or other signs indicating faulty milling; (iii) when cooked, pasta must not be glutinous on the surface i.e. stick together, but should have good ribbing and resistance to mastication; (iv) a pleasant aroma and taste typical to pasta; (v) practically zero contamination from chemical pesticides and preservatives. All these characteristics can be measured using instruments, organoleptic and taste tests and constitute the basic requirements for high quality pasta. To produce pasta with these characteristics requires: raw materials with the characteristics needed to guarantee the final quality required, suitable and modern technologies for processing raw materials, production and human resource management systems focused on quality and systems which therefore involve personnel at all levels in the attainment of quality objectives. This system is known as Integrated Production Process Management, which developed at the end of the eighties. In Table 1 the product specification and the quality parameters of egg-pasta are given.

Table 1. Product specification and the quality parameters of egg-pasta.

Name of the product	**Egg spaghetti**			
Description of the product	Dried egg pasta, which must be cooked before consumption			
Ingredients	Durum semolina and eggs 16,5% (3 egg per 1 kg semolina)			
Packaging	PE-PP, per 1 kg			
Parameters of quality	Length 30 cm			
Parameters of safety:	Moisture below 12,5%, water activity a_w 0.5			
Microbial parameters	Parameter	n	c	Criteria
	Salmonella spp	5	0	n. n. in 25 g
	Coagulase (+) *Staphylococcus* and *Staphylococcus aureus*	5	2	m = 10^2 cfu/g M = 10^3 cfu/g
	Bacillus cereus	5	2	m = 10^3 cfu/g M = 10^4 cfu/g
	*Enterobacteriaceae**	5	2	m = 10 cfu/g M = 10^2 cfu/g
	*Clostridium perfringens**	5	2	m = 10 cfu/g M = 10^2 cfu/g
	Moulds	5	2	m = 10^3 cfu/g M = 10^4 cfu/g
Storage and transport conditions	Storage in ambient conditions, dry and dark place			
Shelf life:	2 years			
Instructions for food preparation	Product must be cooked before consumption (10 min) “al dente”			
Target population	Healthy population without limits in food consumption – exception are individuals with gluten intolerance, egg allergy			
Properties of cooked product	Cooked product has to be non sticky, without any other untypical smell and taste, typical yellow colour without discolorations. During cooking the product should not fall apart.			

SUPPLIERS AND DEMANDS FOR RAW MATERIALS

Raw materials for the production of egg-pasta include eggs (fresh eggs, frozen eggs, dry eggs, egg yolks, or dried egg solids), durum semolina and potable water. All raw materials should be of high quality. This can only be assured by the careful selection of suppliers who fulfil the necessary requirements set by the producing company. The suitability of water should also be monitored both from the supplier and company with the relevant microbial and physico-chemical analysis. Special quality and safety

criteria should also apply for the packaging material supplier. Here below follows some of the main requirements for raw material.

Liquid eggs:

1. Statement about sanitary and suitability that raw material satisfies the requirements EU acts and directives, which define the domain of foodstuff safety.
2. Statement from supplier that, in accordance with safety requirements for foodstuffs, they carry out an internal supervision on HACCP principles during all phases of foodstuff production, and that they have a system for internal supervision, an elaborate plan and a procedure in place for the recall of unsuitable foodstuffs from the market.
3. Product specification of raw materials
4. Statement GMO free – Raw materials do not contain genetically modified organisms as stated in Regulation EC 1829/2003.
5. Statement about suitability of primary package in accordance with Regulation EC 1935/2004.
6. Microbial analysis from an accredited laboratory regarding microbial parameters of safety issued in accordance with the specifications for raw material or in accordance with the Guidelines for microbial safety of foodstuffs or Regulation EC 2073/2005.
7. Statement about registration of establishments engaged in food related activity as per Regulation EC 852/2004.
8. For all establishments which are under veterinary supervision-Provision of veterinary inspection.

Durum semolina:

1. Statement about sanitary and suitability that raw material satisfies the requirements EU acts and directives, which define the domain of foodstuff safety.
2. Statement from supplier that, in accordance with safety requirements for foodstuffs, they carry out an internal supervision on HACCP principles during all phases of foodstuff production, and that they have a system for internal supervision, an elaborate plan and a procedure in place for the recall of unsuitable foodstuffs from the market.
3. Product specification of raw materials
4. Statement GMO free – Raw materials do not contain genetically modified organisms as stated in Regulation EC 1829/2003.

5. Statement about suitability of primary package in accordance with Regulation EC 1935/2004.
6. Microbial analysis from an accredited laboratory regarding microbial parameters of safety issued in accordance with the specifications for raw material or in accordance with the guidelines for microbial safety of foods.
7. Statement about the registration establishments engaged in food related activity as per Regulation EC 852/2004.
8. Analysis is required as per the Regulation EC 1881/2006 about definition of limited values of some pollutants in foodstuffs and regarding the presence of pesticides in accordance with Regulation EC 396/2005 about the limits of pesticide remains in or on food and fodder of vegetal and animal origin (Directive of Council 91/414/EEC amended by Regulation EU 839/2008 and Regulation EC 149/2008).

- Packaging material:

    - Declaration of a good production practice for materials and products that might come in contact with foodstuffs as per provision of Regulation EC 2023/2006.
    - Declaration by the holder of activity engaged in the process that they do / do not use recycled materials and products in their production process.
    - Declaration that the holder of activity engaged in the process for the production of material or product coming in contact with foodstuffs, which is not in accordance with Article 3 of Regulation 1935/2004, have an elaborate plan for the recall of these products and have nominated an authorized person for carrying out this procedure.
    - Statement about the registration of establisments engaged in food related activity as per Regulation EC No. 852/2004.
    - Labelling the material with the following information (name, description of material, structure, dimensions, parameters of quality, parameters of safety, testing methods, labelling of material-traceability, storage conditions and expiry date of these materials).

A systematic approach to safety is an important step towards food quality, safety and sustainable practices. Food safety begins at the farm and must be ensured throughout the manufacturing. In recent years, “Supply Chain Risk Management” has gone through rapid developments. Food manufacturers are no longer judged only by their own actions, but also by those of their suppliers or partners. A sound, corporate and responsible approach helps manufacturers in improving the management of

environmental, social and economic impacts on their business. Corporate responsibility helps the manufacturer maintain long-term profitability. By improving supply chain management and food chain traceability facilitates both in tracing problems of food safety and quality. Upstream it helps differentiate and provide credibility to foods with undetectable quality attributes and downstream it can make a product recall more efficient during an incident. Food supply chain is overdue in investment, in cost-effective quality processes and supporting technology that will address these problems and assure consumers that their food supply remains safe. Continued incidents of tainted lettuce, spinach, peanut butter, seafood, and now peppers and tomatoes should be a call for action to address supply chain risk factors among food producers and all other participants of the food supply chain (Jöhr & Ware, 2007).

RISK ASSESSMENT COMPONENTS

These risk assessments (RAs) reflect, to the extent practicable, a full range of current practices, behaviours, and conditions in the farm-to-table continuum. Major components of the assessments are Hazard Identification, Exposure Assessment, Hazard Characterization and Risk Characterization. Hazard Identification discusses the characteristics of the hazard of concern, the vehicle of human exposure, and host characteristics such as human susceptibility to illness. Exposure Assessment describes consumer exposure to pathogens from food ingredients. It estimates the prevalence and level of pathogens in food ingredients produced on the farm and translates that to the level of pathogens in foods consumed directly or as an ingredient in a meal. This translation involves taking into consideration the change in the level of pathogens in food during distribution, storage, and preparation. The effects of food handling and pasteurization are also evaluated. Similarly, the prevalence and level of the pathogen in food products before pasteurization are estimated and used to estimate the number of pathogens in food products consumed directly or as an ingredient in a meal. The output of the Exposure Assessment is the prevalence and level of pathogens in food products that consumers are exposed to as a function of pasteurization and refrigeration of food ingredients during distribution from farm to processor. Hazard Characterization estimates the likelihood of illness based on the levels of pathogens in a serving of food eaten. These estimates are based on the dose-response relationship developed by FAO/WHO. Risk Characterization estimates illnesses, hospitalizations, and deaths on per serving and per annum basis. Answers are provided to the specific risk management questions and also a sensitivity analysis is included to identify areas to consider.

Risk Assessment Outputs

Hazard Analysis The purpose of the Hazard Analysis and Critical Control Points (HACCPs) in food production is to analyze the potential hazards associated with each processing step to evaluate their risk and, thereby, to identify those operations in which corrective actions will be required. Experience has demonstrated that this approach improves food safety. Hazards vary from one product to another, depending on: (1) the raw material used (2) the particular process employed (3) the commercialization system and (4) the ultimate use of the product. The manufacturing process for dried egg-pasta includes steps ensuring the elimination of microorganisms. However, the primary sources of contamination are raw ingredients and contamination during processing. Heat-resistant toxins produced by *Bacillus cereus* and *Staphylococcus aureus* are not eliminated by pasta cooking, this being the main cause of the reported outbreaks attributed to pasta consumption. These foods have also been associated with outbreaks of salmonellosis (Grady et al. 1986; Woolaway et al. 1986). These data show the pressing need for the application of HACCPs in the manufacturing process of these products. The hazard analysis consists of the following steps: (1) inspection of both preparation and storage practices to identify sources and modes of contamination (2) measuring temperatures of internal region of food during preparation, storage, cooking and holding (3) collecting samples in the preparation stages and testing them for microorganisms of concern and (4) collecting samples after cooking and after overnight holding to evaluate survival, destruction and growth of microorganisms and the decrease of risks associated with the operations.

Application of HACCP Concept in Pasta Manufacturing

The flow chart of the process is shown in Fig. 1. The diagram also indicates the hazards and critical points of the process. In dough preparation, the use of fresh eggs could be dangerous, since they may contain *Salmonella* spp., particularly *S. enteritidis*. The water used in the process should comply with local regulations for drinking water. In the finished product microbial criteria that should be taken in consideration are total microbial counts, moulds, *Enterobacteriaceae*, coagulase positive *S. aureus*, *Salmonella* spp., *Clostridium perfringens* and *B. cereus* counts. The data obtained provides evidence whether the food hygienic procedures are correctly applied or not. The water activity (0.5 with a moisture content of 12.5%) makes these foods not a good substrate for microbial growth, including pathogens. Consumer practices like cooking and holding foods after preparation should also be evaluated since the industrial manufacturing process does not ensure the elimination of all microorganisms. While vegetative forms of pathogenic bacteria would have been killed during cooking, heat-resistant

endospores might have survived. Therefore, *B. cereus* and sulfite reducing *Clostridium*, (endospore-forming pathogens), are of primary concern.

Therefore there is a considerable responsibility on the manufacturer to ensure that *Salmonella* does not gain access to the product, in particular by using only pasteurized egg in pasta dough. *Cl. perfringens* has also been reported in dried pasta. However, pasta is unlikely to be a significant source of this organism under normal circumstances. *S. aureus* can grow well on cooked pasta. For example in cooked egg-pasta inoculated with approximately 100 *S. aureus* g^{-1}, production of staphylococal enterotoxin occurred in 3 d at 15°C and only 1 d at 25°C. Hence, cooked pasta needs to be treated with some care to minimize the opportunities for contamination with S. aureus and should be maintained in chilled conditions to retard its growth. Holding hot (> 63°C) prior to serving would be an alternative in a catering situation (Lee et al. 1975; Legan 2000). Dry pasta is extruded and dried below pasteurization temperatures, therefore pathogens may survive in the final product. Salmonellae from egg ingredient have been especially troublesome. Although levels of salmonellae are reduced during processing, significant numbers survive if the original contamination is high (Hsieh et al. 1976b). Once the pasta is fully dry, salmonellae may remain viable for several months (Walsh et al. 1974; Lee et al. 1975). *Salmonella* Infantis and *S.* Typhiurium have been detected in pasta after 360 days storage at room temperature (Rayman et al. 1979). Boiling will destroy the bacteria, but the presence of salmonellae in dried pasta is considered a basis for legal action under the laws of many countries.

Mixing equipment in pasta factories is often not cleaned more frequently than once a week, and in the early stages of drying, *S. aureus* can grow because conditions are nearly ideal (35–40°C, a_w 0.90–0.95, pH~ 6). Inadequate or inefficient mixing will add to this hazard. As with *Salmonella*, drying increases the stability of the bacteria, and death during storage may be incomplete (Walsh & Funke, 1975). In a survey of dried pasta products in retail stores in the US, *S. aureus* was found in 42 of 1533 packages of macaroni, and in related factory investigations, was found in 179 of 350 samples (Walsh & Funke, 1975). Dried spaghetti has been reported to be a source of *Cl. perfringens* endospores (Keoseyan, 1971), and this should be taken into consideration in the handling of prepared foods that contain pasta, and which could act as a favourable growth medium.

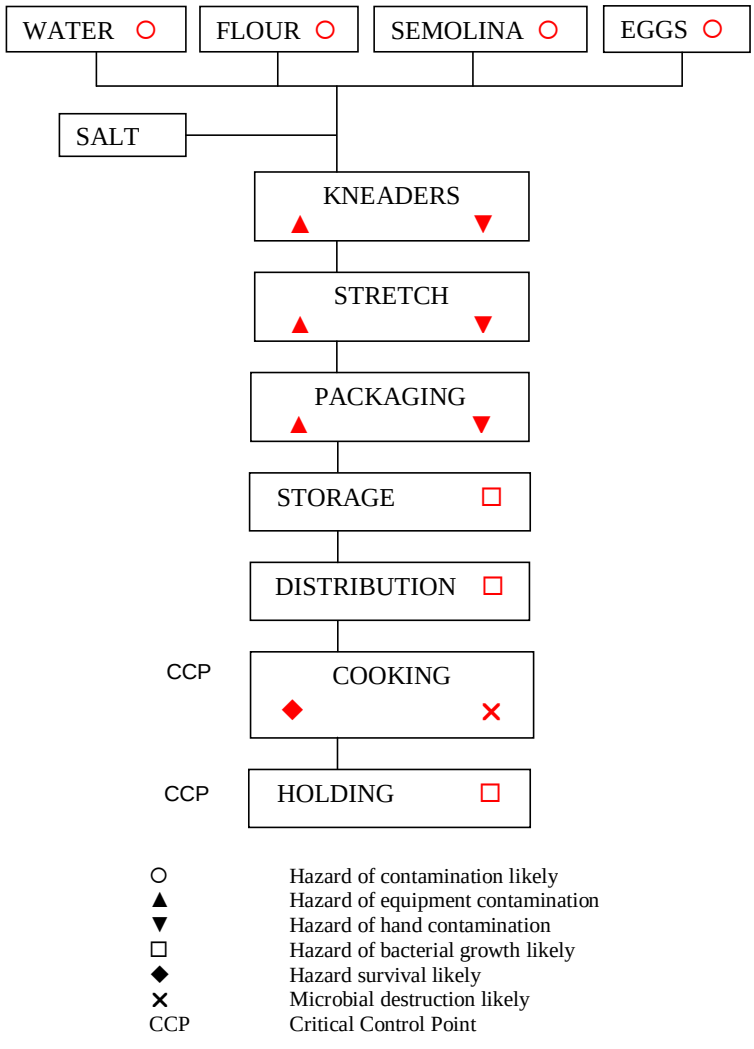


Figure 1. Flow chart for the pasta production.

Improvements of Pasta Manufacturing Process

From the HACCP application some drawbacks concerning sanitary conditions are corrected. At the end of the working day, both the mixer and the kneader should be thoroughly washed with a solution of sodium hypochlorite (0.02% active chlorine) for 30 min and then rinsed several times with drinking water. Another action should be taken is the incorporation of basins with liquid soap allowing periodic handwashing of operators. Both the machine used to rub and stretch the dough and that used to form the pasta should be dry-cleaned by high power vacuum cleaners to remove dry residues. Periodically, additional swabbing of the equipment should be done. In addition, the use

of separate cold stores for raw materials and final product to avoid cross-contamination can be suggested. It is recommended that products made first should be removed first from the store, i.e. “first in first out”. After all corrections suggested are implemented, the process is inspected again. Considerable improvements are obtained in microbial quality of the finished product, owing to the use of raw materials of better quality and to the adopted corrections regarding process hygiene. People in countries are routinely buying pasta with more than 1×10^8 CFU/g with no substantial health problems. But, it should be remembered that these products are always eaten cooked; people boil pasta in water for more than 7 min. However, the pasta industry is qualified to produce better quality products, provided good manufacturing practices, critical control point’s analysis and raw materials of adequate quality are considered.

Hazard Identification for Pasta Manufacturing

Salmonellae are susceptible to heat and killed at temperatures ≥55°C. Ordinary cooking is sufficient to destroy salmonellae provided sufficient time. Because of *Salmonella’s* thermal susceptibility, food borne infection is frequently associated with consuming foods containing raw eggs such as egg-pasta. However, warm temperatures provide an environment in which Salmonella can grow during the processes of production, transport, and storage. The age of patients with Salmonella infections follows a bimodal distribution, with most infections occurring in those at the extremes of age. The highest number of cases is seen among children. The association between salmonellosis and age, however, may be due to reporting bias because children and the elderly with diarrhoea may be more frequently cultured than other age groups (Aureli et al. 1986).

Exposure Assessment for Pasta Manufacturing

The first part of the exposure assessment estimates the frequency with which people are exposed to different doses of pathogen (e.g. *Salmonella* Enteritidis) in servings prepared from egg-pasta. The second part estimates exposures to all *Salmonella* spp. in servings of egg-pasta products. This exposure assessment should follow eggs from the farm to the pasteurizer, from the egg-pasta production (cooking step) and from the production to consumption (holding). Pasteurization of raw egg and the cooking step in pasta production has the special prominence in this assessment because it is the principal risk management measure being evaluated by this RA.

HACCP for Egg-Pasta Production

The food industry is currently working with two management systems, namely, HACCP and ISO 9001:2000. In September 2005 a new ISO standard has been

published ISO 22000:2005, which for the first time provides a framework for the harmonized approach of quality and safety issues applicable in the entire food chain (Rotaru & Borda, 2007). HACCP on the other hand remains an extremely flexible approach to safety management issues concerning products intended to be consumed as food. HACCP systems rely on a series of preliminary measures programs called prerequisites (Wallace & Williams, 2001). The prerequisites programs may contain Hygiene programs, Good Manufacturing Practices (GMPs), Supplier Quality Assurance (SQA) and statistic process control (SPCs) programs. Thus, the managerial strategy can be oriented in three directions, depending on the specific and the resources of the organizations in food industry: (1) the implementation of the ISO 22000:2005 standard; (2) the implementation of HACCP where ISO 9001:2000 already exists; (3) organisation of an ISO 9001:2000 where HACCP has been implemented. It is extremely important to understand that HACCP does not replace the hygiene programs or other prerequisites programs, but if correctly implemented these programs represent its support network (Rotaru & Borda, 2007).

It is necessary that each producer develops specific procedures for good hygiene practices. It is necessary to classify the products and the ingredients regarding the magnitude and the frequency of the risk like: high risk products, medium risk products low risk products. The hygiene practices are a solid base line for the accomplishment of the HACCP system (Jouve, 1999). Good communication is the heart of any successful system. Communication in this case is not just about dialogue between the “HACCP expert” and the “person from the food business”, it also involves many other people – the legislator, the enforcer, the food worker, the various people in the food chain from producer to retailer, the consumer and the media. The HACCP team should be in contact with competent authorities, chamber of commerce, and research institutions. The team should have meetings regularly at least once per month. The topics discussed in the meetings frequently should be the following: non-conformities at the reception of raw material, consumer complaints, monitoring CCPs, hygiene in production room and microbial tests’ results both of raw materials and final products, monitoring and changes in food legislation. At the end of the year HACCP team leader should prepare an annual report, which is going to be the base of planing for next year.

HACCP Study

Hazard analysis: Hazard analysis is made by evaluating each process step and possible hazards. Results of assessment are made by stating probability of the hazard (points between 1 and 5) and by severity of the hazard (points between 1 and 5). Point 1 means

very low probability – very rarely implicated. Points 2–3 mean that is probable to have a certain hazard. Points 4–5 mean very high probability and indicate that the hazard has occurred again in the past. Point 1 means very low severity – no effect on consumers' health, points 2–3 indicate medium severity on health – or chronic effect on health and points 4–5 indicate high severity problems like digestion problems or vomiting due to the hazard or even cases where the health of the consumers might be in danger. The result of each assessment is between 1 and 25. Process step which has 15 points or more is considered as CCP and preventative measures have to be implemented (Fig 2.). The Master plan is given in Fig. 3.

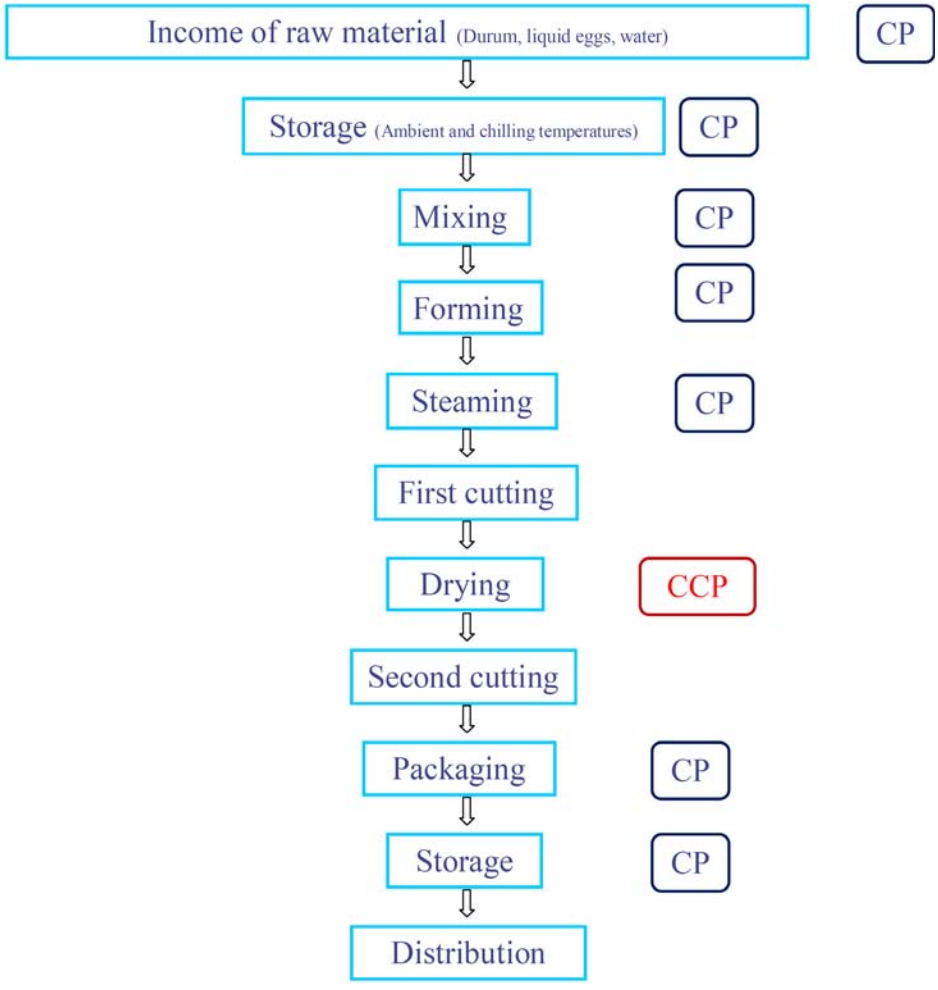


Figure 2. The production process of the egg pasta product is shown in the process flow diagram. CCP's location has been marked on the relevant diagram.

Table 2. Hazard Analysis Chart.

Process Step	Description of Hazard	Preventative Measures	Probability	Severity	Result	CCP/ CP
R&S – Durum Semolina	(M)Moulds-Mycotoxins,	Quality specifications & control; Emptying elevators totally,	2	4	8	
	(C)Pesticides and heavy metals	Frequency of delivery is 1/14 d; Durum semolina is sowed	2	4	8	CP
	(P)Foreign body	through control sieve before dosing into mixing vessel	2	2	4	
R&S – Liquid eggs	(M) *Salmonella* spp.	Control of temperature at reception, microbial analysis according to the plan, control of storage temperature	3	4	12	CP
R&S-Pack. materials	(C) Chemical residues	Approved suppliers	1	3	3	
	(P) Foreign body	Clearly set specifications	1	2	2	CP
Mixing	(M)Cross contam. (*S. aureus*)	Sanitation programme in place	2	2	4	CP
	(C) Residues of cleaning agents	Good rinsing after cleaning				
Forming	(M) Cross contam. (*S. aureus* Moulds)	Sanitation programme in place Daily cleaning	2	3	6	CP
	(C) Residues of cleaning agents	Good rinsing after cleaning	2	3	6	
Steaming	(M)Cross contam.	Control of steam quality	2	3	6	
1st cutting	No hazard identified					
No hazard identified	(M)Cross contam. (*Salmonella* spp, *S. aureus*) - survival & growth	Control of time, humidity, temperature of drying cell, a_w, survival of *Salmonella* spp. and other microbes	3	5	15	CCP
2nd cutting	No hazard identified					
Packaging	(M)Cross contam. (C) Residues of cleaning agents	Visual control for foreign body contamination, quality control of packaging	2	4	8	CP
Storage	(C) Residues from packaging materials	First in first out, control of batch number	1	4	4	CP
Distribution	No hazard identified					

R&S = Reception & storage (M) Microbial hazard; (C) Chemical hazard & (P) Physical hazard

Verification Activities

Verification activities such as random sampling and analysis, auditing and verification procedures are used to ensure that a system is working correctly. Verification activities are shown in Table 3. Monitoring programs for the cleaning and sanitation, pest control, health certificates, records of inspection of premises and equipment in the plant are also important and should be in place. A plan of annual training activities with data and the main topics of training activities should be prepared. Topics of interest are: personal hygiene (hand washing, health certificates, and reporting health problems), good manufacturing practice, good hygiene practice, general information about hazards and HACCP systems, principles of cleaning and sanitation, basics of microbiology and consumer complaints. Implementation of training activities should take place at least once per year (annual training) for whole company and then monthly training activities should be performed according to the problems which occur in production plant – for smaller groups of people. Basic themes for all training activities are: personal hygiene, good manufacturing practice, and good hygiene practice.

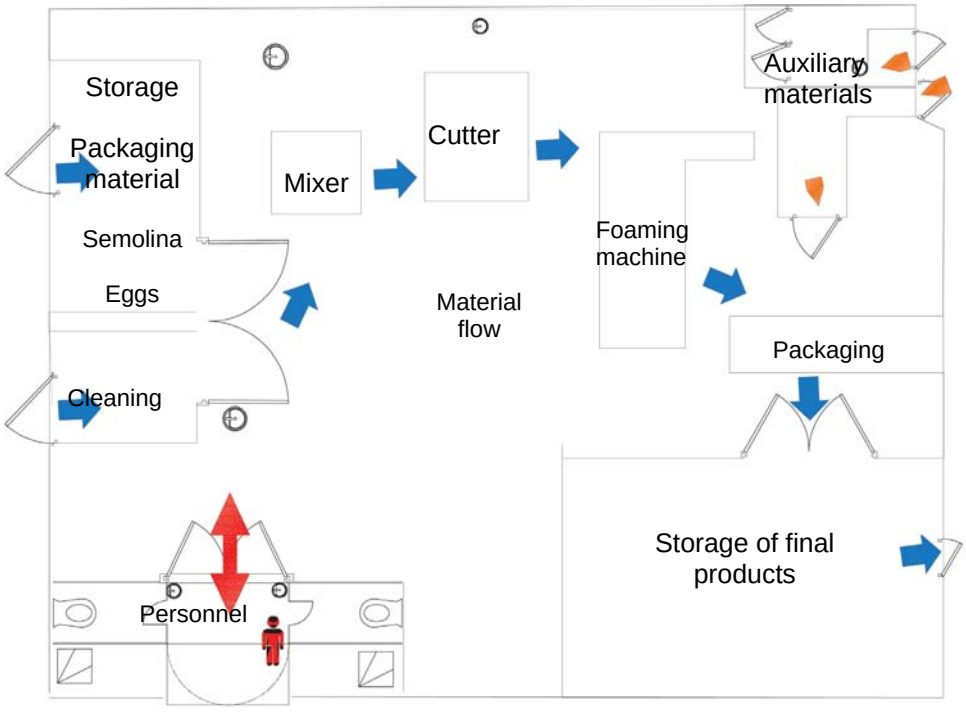


Figure 3. Master plan of the production site/No zoning required.

Table 3. Verification activities for egg-pasta production.

Verification activity	Frequency	Who is responsible	Report
Calibrated thermometer/ hygrometer	1 / a	Maintenance department	Certificate of calibration
MA & CA – liquid egg, durum & semolina	-1 / month (water – 1/a)	Microbiologist & 1/a in external accred. lab	HACCP team meeting
MA – water	4 / a	External accredited lab	HACCP team meeting
MA & CA – final products	2 / a & 1/a in external lab	Microbiologist & Ext. accredited lab	HACCP team meeting
Sensory testing (smell, taste, texture, colour)	1 per 14 days	Production technologist	Monthly to QM
Hygiene sampling for surfaces	1 / week 5 contact plates from surfaces	Microbiologist	HACCP team meeting
Physico-chemical anal. – a_w of end products	1 / a	External accredited lab	HACCP team meeting

MA = microbial analysis & CA = chemical analysis; QM = quality manager

RESEARCH NEEDS

In egg pasta, cholesterol can undergo oxidation depending on the raw material quality, the drying cycle or the storage of the finished product. However, the drying cycle used for egg-containing pasta should be milder with respect to those used for pasta without eggs, as shown by the determination of furosine in these products (Boselli et al. 2004). In a later study, Zunin et al. (1996) examined cholesterol oxidation in dried egg-pasta. Their data offered yet another critical point in the processing of cholesterol–containing foods materials. In this experiment, ten batches of commercially-prepared egg-pasta were selected as reference samples because the researchers knew the pastas were prepared with four eggs/kg semolina flour and that the drying conditions were well-controlled (75°C for 20 min and then at 65°C for 8 h). Other dried egg-pastas prepared with either fresh or powdered eggs were purchased from local markets. Commercially prepared egg-pastas containing powdered eggs tend to have a wide range of cholesterol

oxidation product (COP) concentrations. In the study, the researchers quantified both native and oxidized cholesterol and this additional information helped to evaluate pasta samples containing low to medium levels of COP and low levels of native cholesterol. Among the 32 dried egg-pastas examined, no correlation between native cholesterol and 7-ketocholesterol (the major COP found) was seen. This finding confirms that, within a reasonable range, the initial cholesterol level has no effect on the COP level of the finished product. The manufacturing protocols on the other hand have a significant impact on the extent of cholesterol oxidation in the product. In order to produce less cholesterol oxidation product in the product drying and heating conditions need to be carefully evaluated (Yan, 1999).

Unfortunately, the determination of cholesterol oxidation products (COPs) is not yet a routine analysis, due to the time-consuming purification of the analytes, the possibility of artefact formation during sample preparation, the interferences due to plant sterols, and plant sterol oxidation products, as happens when egg-pasta is analyzed. For these reasons, high resolution techniques, such as high performance liquid chromatography and gas chromatography have been coupled with mass spectrometry in order to confirm the identification of oxycholesterols (Boselli et al. 2004). The strength of RA modelling is its iterative nature. Models can be built with incomplete data and assumptions, and updated as new scientific studies are completed. Good RA differentiates what is known from what is not known, so that future research initiatives can be directed toward filling the data gaps that would most enhance the scientific basis for food safety regulations. Filling the research needs would improve understanding of the farm-to-table system modelled in the assessment by identifying the variability of the variables that affect risk reducing the uncertainty in the model. Some studies already in progress that might fill research needs but there should be additional research needs for Exposure Assessment and Hazard and Risk Characterization as well as from sensitivity analysis of the draft simulation model. Some research needs are likely to require long-term commitments from multi-disciplinary teams and may require expert consultations before more explicit applications in RA modelling are possible. The potential usefulness of new research initiatives to RA and public health protection is addressed.

Fast and sensitive methodologies of food analysis, especially for industrial purposes, are useful tools to determine the quality of final commercial products. Moreover, the possibility to use always easier tools to analyse the authenticity of the certificated food is a daily challenge. Thermal analysis is recognized as an instrumental method of food

analysis able to give unique information regarding the nature of the sample or the modifications induced by industrial processing. Moreover, thermal analysis combined with evolved gas analysis techniques (EGA) allows the optimisation of heating treatments as well as the recognition of alterations, caused by the heat during evaporation of water (to be avoided as much as possible in the field of pasta processing). This very important aspect is in fact strictly related to the more rapid industrial production but also to the possible decrease of the final product quality because of the loss of nutrients (i.e. proteins) which is more important at high temperatures. Thermogravimetry (TG) and derivative thermogravimetry (DTG) are fast and accurate useful tools to check the industrial drying process and to determine the spaghetti pasta quality (Materazzi et al. 2005). The bound water can be the discriminant to relate the egg-pasta quality, either before or after the cooking process. The low-temperature drying process is clearly a better way to obtain the best egg-pasta quality, even if the final cost for the consumer is higher because of both the longer time needed and the higher quality of the starting semolina and egg (Materazzi et al. 2008).

IMPROVEMENTS

Future improvements in the production include:

- vacuum cleaning which will aid in a more efficient and faster cleaning,
- use of rapid tests which will increase productivity and improve the controls upon the product,
- better training.

CONCLUSIONS

The RAs for dry egg-pasta is based on the best of the knowledge of the authors available from universities and pasta manufacturing companies. Pasteurization and after that rapid cooling of eggs used as ingredients and cooking during the pasta manufacturing are predicted to be effective for reducing/preventing illnesses from pathogens (Salmonella enteritidis and Salmonella spp. in eggs and egg-pasta products, respectively). Technology for processing and storing egg-pasta give to the products a good record, so good overall that their safety is taken for granted by the consumer. This compares favourably with the other foods particularly meat, poultry, eggs and dairy products: all perishable foods that readily support microbial growth and have a history of causing food poisoning when not stored or prepared correctly. Dry egg-pasta

can be considered as a very low risk product because of its low a_w, and because is has to be cooked before consumption. It would be unwise, however, to assume that there are no microbial hazards associated with dried pasta products.

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EVALUATION AND PRODUCTION OF FUNCTIONAL FOODS IN TERMS OF FOOD SAFETY IN ESTONIA, SLOVENIA AND TURKEY

Metin Guldas[1], Alexander Maher[2], Kadrin Meremäe[3], Kristi Praakle-Amin[3], Liina Kutsar[4], Tina Tusar[5] & Raivo Vokk[6]
[1]Department of Food Technology, Karacabey VS, Uludag University, Karacabey, Bursa, Turkey
[2]Mlekarna Celeia Dairy Factory, Petrovce, Slovenia
[3]Department of Food Science and Hygiene, Institute of Veterinary Medicine and Animal Sciences, Estonian University of Life Science, Tartu, Estonia
[4] Department of Product Development, Dairy factory Tere AS, Estonia
[5]University of Ljubljana, Ljubljana, Slovenia
[6]Tallinn University of Technology, Tallinn, Estonia

In this study the production of functional foods in terms of food safety in Estonia, Slovenia and Turkey were evaluated. The definition, classification and health benefits of functional foods were investigated. The legal aspects concerning functional foods were also considered. The probiotic yoghurt production was chosen as model production in this study. The properties of probiotic bacterial cultures, selection criteria and benefits of probiotic bacteria were given. The production steps for the probiotic yoghurt production in terms of food safety were also evaluated.

INTRODUCTION

Nowadays, many functional foods are aimed at boosting intakes of phytochemicals to reduce risk for chronic diseases like cancer and heart disease. Modern consumers are very interested in their personal health and expect the food that they eat to be healthy or capable of preventing illness (Mattila-Sandholm *et al.*, 2002). Foods that actually promote health as evidenced by clinical trials are commonly referred to as functional foods. Such foods contain physiologically active components effective in preventing or treating disease and aid in promoting optimal health (Chandan, 1997). The component that makes the food functional can be either an important macronutrient if it has

specific physiologic effects or an essential micronutrient if its intake is above the daily recommendations (Roberfroid, 1999). The main groups of functional foods in Estonia, Slovenia and Turkey are as follows: dairy products (kefirs, yoghurt, milk, curd products, cheese), bakery products (bread with fibers) and drinks (juices, smoothies, whey drinks). Commonly used functional ingredients are probiotics, vitamins, minerals, coenzym Q 10, unsaturated fatty acids omega 3, dietary fibers, L-carnitine, different fruits etc.

The term “functional foods” was first introduced in Japan in the 1980s and refers to processed foods containing ingredients that aid specific functions in addition to being nutritious (Hasler, 1998). Japan is the only country that has formulated a specific regulatory approval process for functional foods. Known as Foods for Specified Health Use (FOSHU), these foods are eligible to bear a seal of approval from the Japanese Ministry of Health and Welfare (Arai, 1996). In the United States and Europe, the functional foods category is not recognized legally. The safety of functional foods is governed by general food law, no specific legislation covers the safety evaluation of functional foods (O’Brien, 2004). The EFSA regulations of European Parliament (1924/2006) have started the registration of the health claims made on foods referred either in Article 13 or Article 14. A natural food can be made functional in the following ways (Roberfroid, 1999):

- By increasing the concentration of a natural component to reach a concentration that is more likely to induce the expected effects.
- By adding a component that is not normally present in most foods.
- By replacing a component with a component for which beneficial effects have been demonstrated.
- By improving the bioavailability of food components.

In recent years, there has been a significant increase in the popularity of yogurt, accentuating the relevance of incorporating *Lactobacillus acidophilus* and *Bifidobacterium bifidum* into yogurt to add extra nutritional-physiological value. The conventional yogurt starter bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, lack the ability to survive passage through the intestinal tract and consequently do not play a role in the human gut (Gilliland, 1979, Lourens-Hattingh and Viljoen, 2001). In this publication the importance, classification, health benefits and legal issues related with functional foods were explained. As a sample of functional food the probiotic yoghurt production and its functional properties were described.

CLASSIFICATION OF FUNCTIONAL FOODS

A functional food is any food that exerts health properties beyond the traditional nutrients it contains. There are two categories of functional foods: a) Foods that naturally contain biologically active, non-nutrient compounds that provides health benefits. These compounds are *phytochemicals*, which are also sometimes called *nutraceuticals.* Based on this definition, all plant foods could be considered functional foods since they are all rich in phytochemicals or nutraceuticals; and b) b. Food products specifically formulated to have higher amounts of nutrients or phytochemicals than would naturally occur in that food. These are also called "designer foods."

Defining nutraceuticals or phytochemicals

Phytochemicals are plant chemicals that differ from nutrients in some important ways. Essential nutrients which include protein, fats, minerals, and vitamins are essential for life. Without them, people develop acute deficiency disease symptoms that can eventually cause death. Nutrients are found in all of the food groups. Phytochemicals are not necessary for life but they help to promote optimal health by lowering risk for chronic diseases, such as cancer and heart disease. They are found *only* in plant foods. Fruits and vegetables are among the best sources of these compounds. Phytochemicals are believed to have many health benefits. Some groups of phytochemicals that have been linked to decreased cancer risk include:

- Allyl sulfides**,** which may stimulate activity of enzymes that help to eliminate toxic compounds and are found in onions, scallions, and leeks.
- Dithiolthiones and isothiocyanates**,** which may increase activity of enzymes that help to detoxify carcinogens and are found in the cruciferous family of vegetables (broccoli, cauliflower, Brussels sprouts, cabbage, turnips, and others).
- Indoles**,** which may interfere with estrogen metabolism and therefore could, reduce risk for some estrogen-related cancers such as breast cancer. They are also found in cruciferous vegetables.
- Isoflavones, which are found in soy foods and have a number of effects that may affect cancer risk.
- Lignans**,** which are found in rye and flaxseed and may reduce breast cancer risk because they act as anti-estrogens.
- Flavonoids are a special class of phytochemicals that includes hundreds of different compounds. Most are excellent antioxidants and some have hormonal

properties. Among some of the most studied flavonoids are quercetin, which is found in tomatoes, potatoes, broccoli, and onions and kaempferol, which is found in kale and endive.

- Carotenoids are a group of phytochemicals that act as pigments, giving plants their bright green, orange, yellow, red, and blue colors.

Carotenoids include:

- Beta-carotene, found in carrots, sweet potatoes, leafy green vegetables, red peppers, and pumpkin. Beta-carotene from foods has been linked to a reduced risk for lung cancer.
- Lycopene, found in tomatoes and strongly linked to reduced risk for prostate cancer.
- Lutein, found in leafy green vegetables and linked to reduced risk for cancer and macular degeneration.

The key focus of the functional foods market in Europe has been the development of probiotic dairy foods, whereas in the United States, vitamin and mineral fortification of foods in general has been the key area of development (Stanton *et al*., 2001). Biologically active compounds found in the functional foods can be categorized in three main groups as inorganic compounds (minerals), organic compounds (vitamins, organic nitrogen compounds, organic acids, aromatic compounds, antioxidants, lipids, carbohydrates and proteins) and microorganisms (probiotic microorganisms).

Probiotics

According to the currently adopted definition by FAO/WHO (2001) probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host". Most of the strains are from strains *Lactobacillus, Bifidobacterium, S.thermophilus* and *Bacillus*. At a minimum, probiotic products should be safe, effective, and should maintain their effectiveness and potency through the end of product shelf life. This requires a responsible approach both by the producer and the consumer (FAO/WHO, 2002). Live cultures are used to make fermented products as cheese, kefir, yoghurt, fermented milks, bread, beer, wine and etc. In some foods these stays in products as alive cultures prior consumption but not all live cultures are probiotics. These microbial strains have to be attributed to the individual strain tested. Testing of a supplement does not indicate benefit from any other strain of the same species, and testing does not indicate benefit from the whole group of LAB (or other probiotics).

Health benefits of probiotics

Probiotic bacteria use the same mechanisms to enhance or stabilize normal colonizing microbes. Protective functions of probiotics are: displace of pathogens; compete for receptor sites with pathogens; nutrient competition and production of antimicrobial substances. Structural functions of probiotics are: immune system development; reinforce intestinal barrier effects and intestinal cell health and development. Metabolic functions of probiotics are: aid in digestion; production of organic acids which inhibit pathogens; synthesize vitamins; increase mineral absorption; detoxify carcinogens and salvage energy. Benefits attributed to probiotic bacteria are (Ziemer and Gibson, 1998; Wisler and Ho, 1999): stabilization of intestinal microbiota; prevention of pathogenic and autogenous diarrhoea/treatment of some diarrhoeas; reduction of toxic metabolites; reduction of serum cholesterol; reduction of blood pressure; antitumorigenic activity and aid in absorption of calcium.

Probiotic yoghurt

Probiotics are live microbial food supplements, which benefit the health of consumers by maintaining or improving their intestinal microbial balance (Fuller, 1989). Most common probiotic bacteria are lactobacilli such as *Lactobacillus acidophilus*, *Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnosus*, and bifidobacteria such as *Bifidobacterium bifidum, Bifidobacterium longum, Bifidobacterium animalis, Bifidobacterium breve, Bifidobacterium lactis* (Daly and Davis, 1998). Suggested minimum numbers of probiotic bacteria at the time of consumption of a probiotic product, for example yoghurt, are 10^7 cells per milliliter or gram of product (De Vuyst, 2000). Daily ingestion in minimum doses of 10^9–10^{10} bacteria has often been the minimum required to show a health effect (Sanders and Huis in't Veld, 1999). The criteria developed by the „National Yoghurt Association" of the United States specifies 10^8 cells per milliliter or gram of product to place special logo on the packing of product (De Vuyst, 2000).

Cases of loss of viability in dairy products have been reported for probiotic bacteria during storage (Vinderola *et al*., 2000; Akalin *et al*., 2004), the need for careful strain selection. Before probiotic strain can be delivered to consumers, they must first be able to be manufactured under industrial conditions, and then survive and retain their functionality during storage as starter culture and also in the food products (Saarela *et al*., 2000). All probiotic strains are unique and therefore their properties and characteristics should be well defined. Each strain must be clearly identified using modern methodology. The assessment of the health-promoting potential of a probiotic

must be based on a valid scientific hypothesis and realistic studies supporting the hypothesis (Salminen *et al*., 2004). It is also important to know that the effect of a given species on the health of an individual within a specific probiotic species appear to be strain or host-dependent (Sellars, 1991). That's why, a strain of *Lactobacillus acidophilus* isolated in America may well be genetically different from a species discovered in Europe and properties of *Lactobacillus acidophilus* may also be different. Therefore, in many countries are isolated their probiotic strain, which have clinically proved effect to the human, and what is used in the dairy products technology (for example *Lactobacillus fermentum* ME-3 in Estonia; *Lactobacillus plantarum* 299v in Sweden). Safety issues relating to new and novel probiotics and fermented milks should be assessed according to the EU novel food regulations (for example Regulation (EC) No 258/97; Commission Recommendation 97/618/EC).

Selection criteria of probiotic bacteria include the following specifications (Klaenhammer & Kullen, 1999; Saarela *et al*., 2000): 1) Strain for human use is of human origin; 2) Strain is isolated from healthy human gastrointestinal tract; 3) Strain is non-pathogenic and non-toxic; 4) Strain is able to exert one or more clinically documented health benefits; 5) Strain is acid tolerant and tolerant to human gastric juice; 6) Strain is able to compete with the normal microflora; 7) Strain is adherent to epithelial surfaces and persistant in the human gastrointestinal tract; 8) Strain have antagonistic activity against pathogenic bacteria such as Helicobacter pylori, Salmonella sp., Listeria monocytogenes and Clostridium difficile; 9) Strain is antimutagenic and anticarcinogenic; 10) Strain is able to produce antimicrobial substances (bacteriocins, hydrogen peroxide, organic acids, or other inhibitory compounds); 11) Strain is amenable to mass production and storage: adequate growth, concentration; 12) Strain has high viability in the probiotic products, prefarably 10^7 cfu / ml or g; 13) Strain has desirable organoleptic qualities (or no undesirable qualities) & 14) Strain is genetically stable and amenable.

In the **production of probiotic yoghurt** selected probiotic culture should be used to (Mattila-Sandholm *et al*., 2002): 1) produce concentrated cultures of each specific strain in level above 10^{10} with good storage properties at low temperature; 2) produce yoghurts with the help of a supporter culture such as a yoghurt culture; 3) ferment milk together with some supporter cultures without inhibition of the growth of any of the added strains; 4) produce yoghurt with levels of the specified probiotic strain up to 10^7 cells/g product; 5) produce yoghurts with high and constant levels of thee probiotic strain when stored at low temperature for three weeks & 6) produce yoghurts with an acceptable taste and flavour throughout the storage time.

Yoghurt is a fermented milk product which made by lactic acid fermentation. The responsible microorganisms for the fermentation are mainly *Lactobacillus bulgaricus* and *Streptococcus thermophillus*. *Lactobacillus acidophilus* and *Bifidobacterium bifidus* can be used to produce probiotic yoghurt together with *Streptococcus thermophilus*. In general, the probiotic yoghurt production is made according to the following procedure (Figure 1): 1) Homogenized and pasteurized milk is heated to 45°C and skim milk powder (SMP) is added with high-speed stirring to make 180 g/l total solids in yoghurt; 2) Heating is continued to 80…85°C, and the mixture is held at this temperature for 15 min; 3) It is then cooled to 45°C. The yoghurt starter culture and the probiotic cultures are added. Then yoghurt mix is poured into the plastic cups & 4) Incubation process is carried out at 42…43°C until a pH of 4.5 is reached. Then, the yoghurt is cooled in an ice water-bath and stored at 4°C for 7 weeks (Kailasapathy, 2006).

According to our group trial probiotic yoghurt is made from standardized milk containing 1.6% fat, 6% milk proteins, 4.6% lactose, 1% minerals, 86.8% water & 10^{10} CFU/ml probiotics bacteria (can be added more). The raw materials used in the production of probiotic yoghurt are raw milk, skimmed milk powder and starter culture (deep frozen –45C°). Polypropylen (PP) or polystyren (PS) pots with aluminium lid is used for yoghurt packaging. The expected shelf life of the product is 4 weeks at 2…8°C. Termination and pasteurization, aseptic fermentation and filling and following continued cold chain are needed for the final product.

The probiotic yoghurt is used for the following health issues: 1) for intestinal problems (constipation, ulcer and etc.); 2) for tissue regeneration (antioxidant activity); 3) for increased absorption of minerals; 4) for protection from pathogenic bacteria; 5) for anti-tumoral activity; 6) for adjusting blood pressure; 7) for lowering blood cholesterol level & 8) for strengthening the immune system.

The potential hazards in the production of probiotic yoghurt are: 1) biological hazards (microorganisms); 2) chemical hazards in raw milk (cleaning agents, disinfectants, antibiotics) & physical hazards (uncontrolled heating process or pasteurisation).

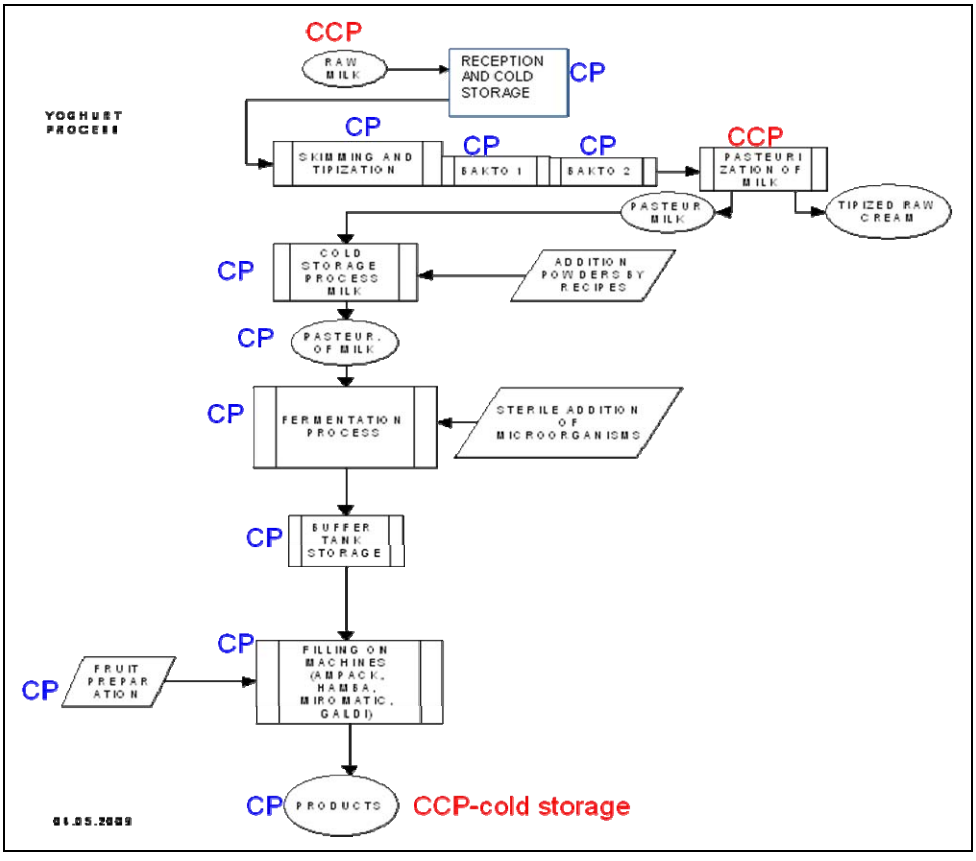

Figure 1. The production line of probiotic yoghurt – during the yoghurt production CCP (critical control points) and (CP) control points are defined.

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EVALUATION AND PRODUCTION OF READY-TO-EAT MEALS

Taner Baysal[1], Terje Tamme[2], Helen Ehavald[3], Figen Korel[1], Maarten Overes[4], Ülle Hödrejärv[3], Aet Kala[5] & Mati Roasto[2]
[1]Izmir Institute of Technology, Urla, Izmir, Turkey
[2]Estonian University of Life Sciences, Tartu, Estonia
[3]Fazer Eesti AS, Tallinn, Estonia
[4]Wageningen University, Wageningen, The Netherlands
[5]Rimi Baltic, Estonia

Ready-to-eat (RTE) is defined as the status of the food being ready for immediate consumption at the point of sale or serve. It could be served as raw or cooked, hot or chilled, and can be consumed without further heat-treatment including reheating (Anon., 2001). RTE vegetables and salads, also called minimally-processed products, are raw products that must preserve as much as possible the nutritional, sensorial and microbial qualities of fresh products. A very wide range of vegetables are used, both cut and whole. Even during refrigerated storage, the fresh fruits and vegetables are characterized by active metabolism. Brecht (1995) indicated that some of the factors affecting the intensity of wounding are species, variety, maturity index, temperature, oxygen (O_2) and carbon dioxide (CO_2) concentrations and water vapor pressure. In general the problems with whole fruits and vegetables are rapid quality deterioration of the fruit and enzymatic browning, moisture loss, weight loss, shriveling, flaccidity (due to water loss), fungal attack, loss of flavor, decay and textural changes, physiological disorders, physical injuries and limited shelf life (Ben-Yehoshua, 1966; El Ghaouth *et al.*,1992b; Lerdthanangkul and Krochta, 1996; Miller *et al.*, 1983; Motlagh and Quantick, 1998; Taşdelen and Bayındırlı, 1998; Zhang and Quantick, 1997).

The production process of RTE salad is generally very simple: the vegetables are selected, washed, cut, dried and packaged in sealed pouches or in plastic trays wrapped with an extensible high permeability polymeric film. The lack of heat treatment does not ensure microbial destruction, while physical damages, determined by cutting, increase the metabolic activity of the plant tissues bringing about oxidative reactions,

dehydration, senescence and ethylene production. Consequently, the products show modifications of sensorial properties such as necrosis, browning, loss of texture, and the discharge of cellular liquids favours microbial proliferation faster than that in fresh unprocessed vegetables. An improvement of shelf-life can be obtained with special cares during processing and packaging and along the chain trade, using good quality raw products and a control of relative humidity and temperature so as to reduce microbial hazards and physical damages. The stability of these products depends on many factors, but temperature is certainly the most important parameter especially during the marketing process, when the control of this parameter is more difficult. Under the same thermal conditions shelf-life can vary according to the type of vegetables, the production process, the packing and the quality of raw materials used (Riva *et al*., 2001). In the Table 1 there is shown the Q_{10} values for some vegetables. Q_{10} is a unit less quantity. It is the factor by which the rate increases when the temperature is raised by ten degrees. For many biological processes, particularly those that involve large-scale protein conformational changes, Q_{10} values are greater than two.

Table 1. Parameters for commercial shelf-life of different ready-to-eat vegetables.

Vegetables	Q_{10} value	Stability time at 5 °C (days)
Cut cicorino	4.51	6.5
Lettuce	5.16	6.7
Carrots	2.66	4.5

Q_{10} – temperature coefficient

There are number of studies about the improving quality of RTE lettuce. Recent emerging technologies to reduce the initial microbial load and browning during storage of minimally processed lettuce were the use of ozone, electrolyzed water, ultrasound, irradiation, as well as warm water treatment, warm water treatment in combination with chlorine in combination with hydrogen peroxide or with irradiation. Most studies on minimally processed lettuce were performed on laboratory scale or have dealt with samples obtained at the retail level. Up to now, only few studies on the effect of different washing treatments have been conducted on pilot plant or industrial scale Therefore, detailed process relevant data are scarce. According to the microbial criteria recommended by the German Society for Hygiene and Microbiology (DGHM, 2002), the load of aerobic mesophilic microorganisms on mixed, packaged salads at the consumer level should not exceed 7.7 log (5×10^7) colony forming units per gram

(cfu/g). Additionally, the use-by date should not exceed 6 days, and the direction for use should advise to keep the product below 6 °C (Baura *et al*., 2004).

It is also well known that microbial safety has main importance for RTE salads. This type of food can be dangerous to consumers' health if safety measures are not strictly complied with at the time of preparation. In these cases, contamination can be caused by animal manure and contaminated irrigation water. Several authors make reference to the presence of bacteria *(E. coli, Listeria monocytogenes)* and illness outbreak have been associated to the consumption of raw vegetables such as cabbage, lettuce, tomato, etc. (Pellicer *et al*., 2002). *Stafylococcus aureus* is detected on fresh produce and RTE salads, most likely because of contamination by food handlers (Beuchat, 1996). *L. monocytogenes* has been detected on lettuce between 1–2% of incidence. (Heisick *et al*., 1989).

Although various studies deal with the growth of *L. monocytogenes* on lettuce, no growth data on RTE lettuce under MAP initially flushed with a mixture of CO_2, O_2 and N_2 gases are available. Studying the behaviour of *L. monocytogenes* on RTE lettuce (and in general, on RTE vegetables) becomes highly relevant, as it does not require decontamination practices such as washing at retail, foodservice or consumer stage, which could reduce a potential contamination of the microorganism (Carrasco, *et al*., 2008). To minimize the risk of infection or intoxication associated with raw fruits and vegetables, potential sources of contamination from the environment to the table should be identified and specific measures and interventions to prevent and/or minimize the risk of contamination should be considered and correctly implemented. Where the possibility of contamination cannot be excluded, the application of the most effective decontamination processes should be considered. Application of good hygienic practices during production, transport and processing, combined with the Hazard Analysis Critical Control Point (HACCP) system, will certainly minimize the contamination of fruits and vegetables and reduce the risk of illness associated with these foods. However, food handlers and consumers also need to observe good hygienic practice during processing and preparation of these foods for consumption including treatments for reducing the number of pathogens. The simple practice of washing raw fruits and vegetables in hot water or water containing detergent or permanganate salts removes a portion of the pathogenic and spoilage microorganisms that may be present, but studies showing the efficacy of these treatments are few. Even washing fruits and vegetables in potable water, then again washing or rinsing in potable water would aid in removing microorganisms. Additional 10-fold to 100-fold

reductions can sometimes be achieved by treatment with disinfectants. Viruses and protozoan cysts on fruits and vegetables generally exhibit higher resistance to disinfectants than do bacteria or fungi. However, relative resistance varies greatly with the type and pH of disinfectant, contact time, temperature and the chemical and physical properties of the fruit or vegetable surface. Little is known about the efficacy of disinfectants in relation to the roughness of fruit and vegetable surfaces, although higher amounts of cuticle material may protect against embedding of cells, thereby increasing the need for exposure to chemical treatments. Several types of treatment are known to be partially effective in removing disease-causing organisms from the surface of whole and cut raw fruits and vegetables or from contact surfaces during handling. Perhaps with the exception of irradiation, none of these treatments can be relied upon to totally disinfect raw produce, at least when administered at levels that will not cause deterioration in sensory quality. Even irradiation may not be completely effective in killing viruses on fruits and vegetables. Rather, these treatments should be considered as methods of disinfection, causing reductions in populations of microorganisms but not always yielding fruits and vegetables free of pathogens.

Each type of disinfectant has its own efficacy in killing microbial cells. Effectiveness depends on the nature of the cells as well as the characteristics of fruit and vegetable tissues and juices. Some types of disinfectants are appropriate for use in direct contact washes, while others are suitable only for equipment or containers used to process, store or transport fruits and vegetables. The mechanism of action of many disinfectants on microbial cells and the influence of factors associated with plant materials is poorly understood. The legal use of various treatments differs from country to country. Available information on the effect of a number of disinfecting agents on *L. monocytogenes* was summarized in the review published by the WHO in 1998 (WHO, 1998) *L. monocytogenes* was stated to be generally more resistant to disinfectants than *Salmonella*, pathogenic *Escherichia coli* and *Shigella* (Beuchat, 1998).

At 200 ppm chlorine reduces the count of *L. monocytogenes* on brussel sprouts, shredded lettuce and cabbage by about 1–2 $\log_{10}$ units. However, simply dipping inoculated sprouts in sterile water reduced *L. monocytogenes* on sprouts by 1 $\log_{10}$ unit. The action of chlorine appears to occur during the first 30 seconds of exposure, so longer periods did not affect the reduction. However, the effectiveness of chlorine is increased if the temperature of the treatment solution is higher than the temperature of the fruit or vegetable (Beuchat, 1998).

On the other hand hurdle technology has been around for many years already as a concept for the production of safe, stable, nutritious, tasty and economical foods. It advocates the intelligent use of combinations of different preservation factors (hurdles) in order to achieve multi-target, mild preservation effects. The spoilage and poisoning of foods by microorganisms is a problem that is not yet under adequate control despite the range of preservation techniques available (*e.g.* freezing, blanching, pasteurizing, canning, drying). Because food manufacturers increasingly rely on mild preservation techniques (*e.g.* refrigeration, modified atmosphere packaging, biopreservation) in order to meet the consumer demand for fresh-like foods, this problem could even increase. According to the hurdle technology concept the desired safety and durability of fresh-like foods may be obtained by using combinations of mild preservation techniques. Hurdle technology (or combined processes) advocates the deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors that no microorganism present should be able to overcome. These hurdles may be temperature, water activity (aw), pH, redox potential, preservatives, etc. It requires a certain amount of effort from a microorganism to overcome each hurdle. The higher a hurdle, the greater this effort is (*i.e.* the larger the number of organisms needed to overcome it). Some hurdles, like pasteurization, can be high for a large number of different types of microorganisms, whereas others, like salt content, have a less strong effect or the effect is limited in the range of types of microorganisms that it affects (Gorris, 1995).

The purposes of this group work are to establish a RTE lettuce salad producing factory and to describe the needs for safe production. RTE lettuce salad consisting of fresh cut lettuce, naturally fermented olive, RTE tomato cubes, and salad sauce (vinegar-olive oil) was chosen as product and needs were described in this study.

HACCP TEAM

The HACCP team should have a competent and experienced team leader. HACCP team selection should be done by the team leader. HACCP team should be multi-disciplinary and consists of different specialists – quality manager, technical manager, warehouse manager, production manager, logistics manager and maybe of some additional specialists. It means that the members of the team have wide range and specific knowledge of HACCP, production processes, food safety and hazards, machinery, storage, transport etc (Table 2). The team should not be larger than 6 people. When the team has assembled all the members should participate in the

HACCP training to understand of HACCP, critical control points (CCP), prerequisite programs (PRP), hazards etc. Company's senior management should demonstrate commitment and support to the HACCP team (Dillon & Griffith, 1997; Anon., 2008, www.codexalimentarius.net).

Table 2. HACCP team members and their competences.

Team member	Field of competence
Quality manager	Product description, product contamination and hazards, hygiene, cleaning and disinfection, product inspection and laboratory tests
Production manager	Production processes, product design and development, pest control, garbage and waste
Technical manager	Machinery, maintenance, hygienic design, calibration
Logistics manager	Dispatch, picking, transport
Warehouse manager	Raw material storage and specifications, control of raw material

HACCP DOCUMENTATION

Documented HACCP procedures should be assembled. Efficient and accurate documentation provides the manufacturer a confidence that product is safe and helps HACCP auditors to do their work. All the prerequisite programs (PRP-s) should be documented: factory layout with machinery, movement of personnel, raw material, garbage, product; cleaning and sanitation procedures; garbage plan; pest control procedures; hygiene rules; plan of product inspection and laboratory test; storage and transportation procedures, maintenance, product flow diagram and product description, raw material description, complaints, training procedures. All the identified and relevant hazards, critical control points and critical limits, preventive and corrective actions should be documented. Monitoring system consists of records about the control of process, critical control points and prerequisite programs. Verification process and procedures and all the results of HACCP audit should be documented. Company should record all the consumer complaints and corrective actions (Dillon and Griffith, 1997, www.codexalimentarius.net).

PRODUCT DESCRIPTION

RTE lettuce salad including 2 pieces of naturally fermented black olive, RTE canned tomato cubes and pre-packed olive oil-vinegar sauce product for airplane passengers

was chosen for this study. The detailed description for product and production is given below. Potential hazards in raw materials and in the process are given in Table 3.

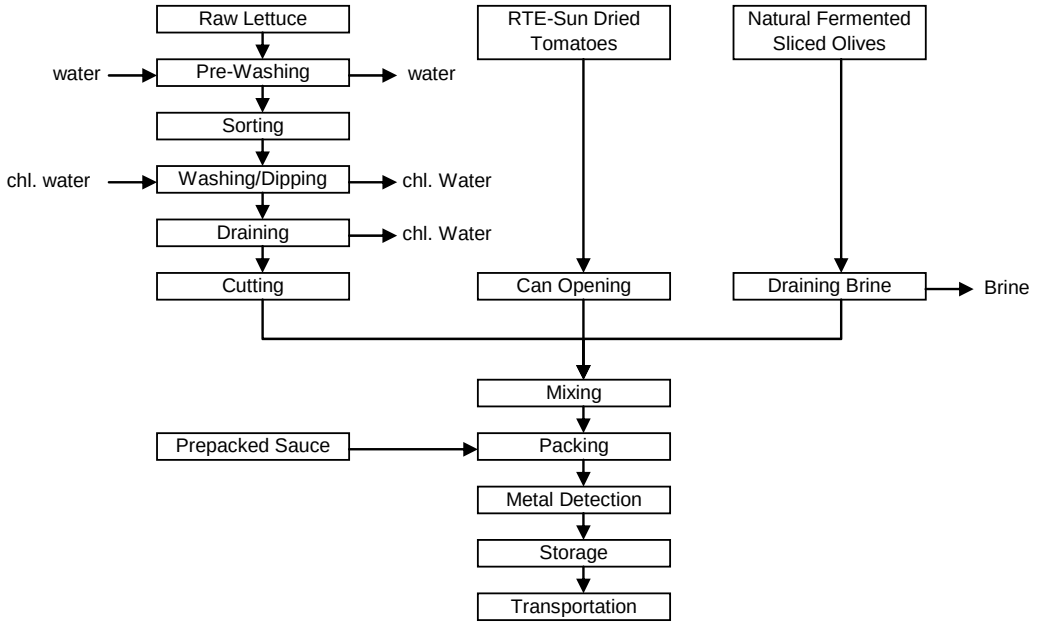

Figure 1. Flow chart of RTE lettuce salad production.

Table 3. Potential hazards.

Material / process	Biological hazard	Chemical hazard	Physical hazard
Raw material	Bacteria Yeast Mould Pests	Pesticides	Soil & stones Pieces of packaging Not hermetically sealed dressing packaging
Packaging materials	Bacteria		Broken packaging materials
Process	Microbes by cross contamination & employees	Wrong conc. of dipping solution Cleaning agents and disinfectants	Wrong temperature Pieces of equipment Materials from employees Pieces of packaging materials

Raw material

Main ingredient in salad is lettuce. It should be fresh, not physically damaged. Short shelf-life and strict control of refrigeration (it should be +2 – +5 C°) limit the growth of pathogenic and spoilage microorganisms (Francis, Thomas & O'Beirne, 1999). Sources of microorganisms are soil (*L. monocytogenes, Cl. botulinum* etc), water e.g. *A. hydrophila, Salmonella,* faeces e.g. *E. coli, Salmonella, L. monocytogenes, Cl. botulinum* etc) (Francis, 1999). Because of contaminated irrigation water and poor hygiene practices parasites like *Giardia lamblia, Entamoeba histolytica* and *Ascaris* spp can be present in vegetables (Francis, 1999). Lettuce should be bought as often as possible, temperatures should be strictly controlled. Quality of lettuce should be checked before taking it to production. During fermentation of olives lactic and acetic acid are produced which increase the acidity level of the brine and lower its pH. Low pH in combination of high salt content is reducing the risk of overgrowth of harmful microbes. Polyphenols in olives provide some degree of protection against microorganisms. Olives should be produced by GHP. (Zervakis, 2005). Naturally fermented black olives should be stored at temperatures under +15 C°, kept from contamination.

Process and Zoning

In production it is needed to follow: 1) Temperature in rooms – if it is too high, the growth of microorganisms will increase; 2) Producing time – as there are raw materials with short shelf life, it is necessary to wash, cut, mix and pack as quickly as possible; 3) Personal hygiene of employees – if hygiene is poor, product may be contaminated with big quantities of extra microorganisms. If personnel is not following the rules and wears jewelers, artificial fingernails etc in production, don't cover hair correctly, those can drop into the product. Also washing and disinfesting hands and wearing sanitary clothes must be strictly followed. Personnel should be well trained about HACCP and their work; 4) Cleanliness of machines – shredding and mixing destroy surface cells, bruise under laying layers and allow juices to leak from inner tissues on to equipment and on to fresh-cut products. Moisture and exudates on cut surfaces and on surfaces of utensils and equipment provide excellent media for rapid growth of microorganisms. (Francis, 1999); 5) Clean water used in washing, dipping – unclean water can cause extra contamination. Ultraviolet radiation may be useful in inactivating vegetative bacterial cells in wash water (Francis, 1999); 6) Cleanliness of the rooms – if not well cleaned, product can be contaminated by extra microorganisms, pests, rodents and their excrements. Cleaning solutions should be with correct concentrations and rinsed off

well & 7) Pest control – if system is not working, there is a risk of contamination with pests, rodents and their excrements.

HACCP plan for RTE lettuce salad is given in Table 4. RTE tomatoes, naturally fermented sliced olives and salad sauce are purchased according to the specifications which are set by the quality manager. Raw lettuce is purchased from a supplier who guarantied that there are no herbicides and pesticides used in the product. They are organically grown lettuces. The processing steps for the RTE lettuce salad is shown in Figure 1. Washing/dipping step is considered as critical control point since if the microbial hazards cannot be inhibited in this step, the product could cause a severe risk to the consumers. The metal detection process is considered as the second critical control point in this process. The temperature in packaging and storage areas need to be controlled an hourly basis and corrected if a deviation occurs. The distribution of the products needs to be performed under cold-chain to inhibit product spoilage.

Before workers enter the production area they need to wash and disinfect their hands. The disinfection of the hands will open the door to the production area (leaving one person through), thereby preventing workers without disinfected hands entering the production area. The toilets are placed outside the production area to prevent workers who went to the toilet to continue work without washing their hands. Suppliers are only allowed to bring supplies to the storage room, and the shipping agent is only allowed to take the end product from the storage room. Both the suppliers and shipping agent are not allowed to enter the production area to prevent contamination.

TRAINING ACTIVITIES NEEDED FOR STAFF

Every food manufacturer has to make sure that their staff has sufficient knowledge about personal hygiene and food handling. It is important to have regular food hygiene training to ensure the adequate level of competence. According to EU regulation 852/2004 food handler has to ensure that: 1) food handlers have had enough training in food hygiene to perform required working tasks & 2) trainees themselves have had adequate training in HACCP and food hygiene.

Table 4. HAACCP Plan for RTE lettuce salad (modified from Hentges, 2003).

Process Step/ CCP	Potential Hazard	Critical Limits	Monitoring				Corrective Action(s)	Recording Keeping	Verification
			What	How	Frequency	Who			
Washing / dipping	*L. mono-cytogenes, E. coli* O157:H7, *Salmonella S.aureus*	Potable water @ pH 7.0	pH	pH meter	before pro-cessing; 2 times / shift	QA[a], test kits/ meters evaluated by QA regularly	Preprocessing batch adjustment; manually adjust water	Recording charts monitored and procedures reviewed	Random sampling; QA[a] audit; HACCP plan validated & review annually
		Potable water. ~1 ppm free residual Cl* for min 30 s	Free chlorine	Test kit/ auto-mated	Continuous		Hold product from last correct reading for rewashing; record incident in deviation log	Continuous strip chart	
Metal detection	Metal pieces	3.5-mm stainless steel **	Metal	Sample run through detector	Hourly	Line operator	Keep correctly calibrated product and rerun it; record incident and product status in deviation log; identify source of metal and investigate line; add to maintenance program	Calibrated metal detector records by QA every shift; records monitored by QA every shift	Random sampling for metal analysis; QA audit; HACCP plan validated every year
Storage[#]	Microbial	+7 °C	Temp.	Thermo-meter	Continuous	QA personnel of the storage rooms	Mechanical regulation of cooling equipment. Continue with cooling until the required temperature is achieved.	Recording sheets of temperature control at storage rooms by QA in every shift	Random temp. control of the final products with calibrated thermometers. Regular data analysis

[a] QA: quality assurance personnel

* or other appropriate concentration of approved antimicrobial solution for wash water.

** or according to manufacturer's guidelines or customer specifications.

[#] Previously storage room temperatures were the part of prerequisite programs in present food enterprise. According to HACCP team decision the Control Points related with storage rooms temperature control were determined to Critical Control Points because of frequent not compliances registered in this area.

The quality manager has to organize food hygiene training that personnel would understand the importance of the means to anticipate the contamination of food products. Staff should be trained so that they can handle food according to good hygiene practice and has knowledge of the HACCP procedures at least in their working area. This requirement applies also to those working part time and staff dealing with working equipment. In spite of training, quality manager has to check regularly the level of understanding and following of the food hygiene principles. It is advisable to have information about food hygiene at their working place (e.g. on the corridor wall) but not directly in the production area. A new staff member should not work before he or she has passed the hygiene training and testing. Afterwards training frequency should be at least once in every two years but in some cases more often. For example if changes in technology or working procedures are made it would always include some additional training.

A plan of the food hygiene training is put together by the quality manager. The purpose, frequency and scope are determined in the plan. According to the plan quality manager organizes food training and measures and tests knowledge in food hygiene. If there are some deficiency or if food hygiene principles are not followed correctly, quality manager will increase the extent or/and frequency of the training. All training and testing should be documented. To ensure a high quality production process staff should, besides having good competence in food hygiene, be able to work together as a team. Also they should be on top of new trends in salad producing technology. Twice a year staff should have training in food technology were new trends of salad making are studied. At least once a year staff including the management will go out to travel to evolve their team working abilities and clarify their goals. As a result we will have an excellent, hard working and innovative team of professionals.

HACCP VERIFICATION

HACCP plan should be reviewed at least once a year. Review of HACCP plan by the team is needed when changing some raw material, recipe, production conditions, equipment, packaging, distribution conditions, consumer use etc. For the verification processes a procedure and audit checklist is needed. Competent auditors should carry out the audits and they should be independent from the audited field. During HACCP audit critical control points (CCPs), hazard analysis, prerequisite programs, documentation and records, non-conformance and corrective action should be assessed. The auditor should focus to the food safety and critical control points, but the full

production should be viewed (Dillon and Griffith, 1997; www.codexalimentarius.net, Anon., 2008).

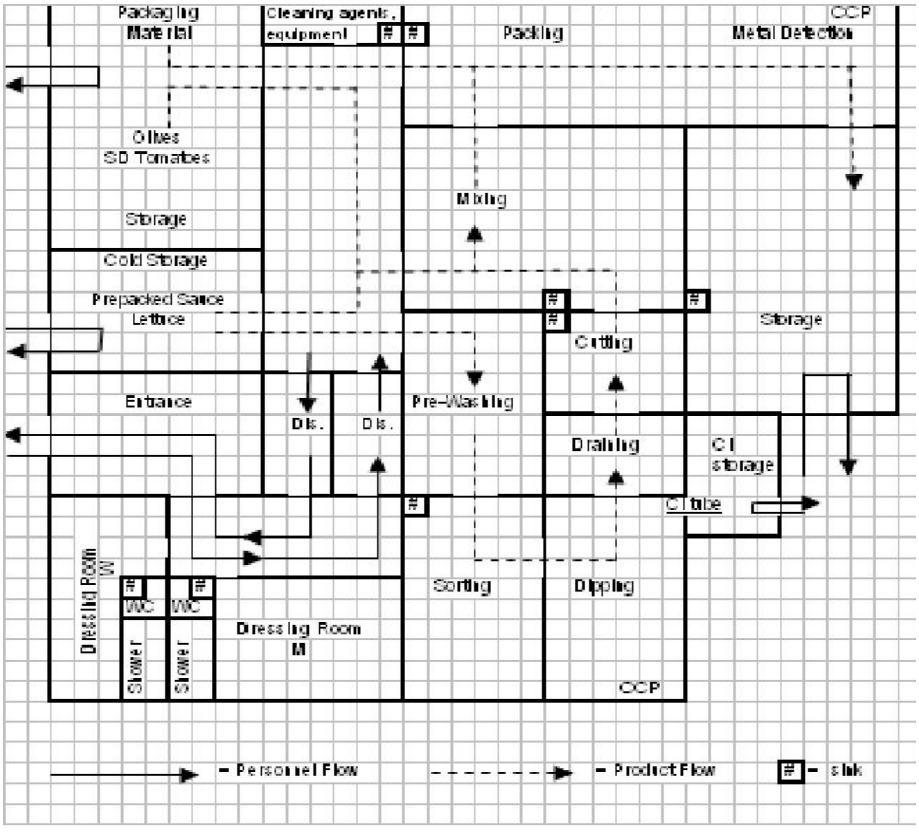

Figure 2. Floor plan for RTE lettuce salad factory.

RESEARCH NEEDS FOR TO IMPROVE FOOD QUALITY

There has been rapidly growing interest produce to ready to eat or ready to use foods and to prolong the shelf life and improve the quality. Specifically for RTE lettuce salad production one of the most important step is to trainee of farmers and stuff. Hygiene and microbial knowledge can be improving product safety. Cleaning of raw material and machinery are also important this kind of production. According to researches there are number of different disinfectants, detergents and cleaning agents could be use in production for to improve hygienic conditions. According to the hurdle technology concept, the desired safety and durability of fresh-like foods may be obtained by using

combinations of mild preservation techniques. Hurdle technology (or combined processes) advocates the deliberate combination of existing and novel preservation techniques in order to establish a series of preservative factors that no microorganism present should be able to overcome. These hurdles may be temperature, water activity (aw), pH, redox potential, preservatives, etc.

„There are theoretical concerns regarding the safety of the use of antimicrobial dips. For example, pathogens if present on raw vegetables may not be fully eliminated by disinfection procedures, while at the same time the effects of disinfection on the indigenous microflora may be to reduce or remove natural competitive organisms. As a result, disinfection may produce conditions which favor survival/growth of the pathogen (Francis, 1999). Chlorine can be replaced with organic acids as lactic and acetic acid. To remove soil and reduce the number and growth of surface microorganisms, fresh produce is washed in potable water and cooled as quickly as possible after harvest. For storage is used modified-atmosphere packaging (MAP), perishable products are packaged in atmospheric gas compositions different than that of air (Larson *et al*., 1997) MAP extents the self life of minimally processed fruits and vegetables by suppressing the growth of aerobic spoilage microorganisms, reducing the rate of oxidation and enzymatic degradation and reducing the loss of water (Austin *et al*., 1998). Optimal oxygen (at least 2%) and carbon dioxide (no more than 20%) concentrations are generally maintained within the package to prevent anaerobic respiration, which accelerates senescence accompanied by development of off flavors and microbial growth (Watada *et al*., 1996). Refrigerated storage (5 °C) is a primary method used to suppress microbial growth and ensure safety of fresh cut produce. Psyhrotrophic pathogens such as *Listeria monocytogenes*, *Aeromonas hydrophila*, and *Yersinia enterocolitica*, however, can survive and may reproduce at temperatures below 7 °C (Callister and Agger, 1989). Bacteria can survive and grow in the pH range of 4.0 to 9.0; however, specific genera need a narrower pH range, with pathogenic bacteria being the most fastidious. The optimum growth pH of many pathogens is within the pH range of vegetables (pH 4.2–7.3).

In addition TTI (Time Temperature Indicators) may be used and improve to monitoring existing of chilled condition after production. Furthermore, research can be done for to build new biosensors for *L. monocytogenes* and salmonella. Research can also performed to using natural antimicrobials such as lysoyme, edible coatings and films incorporated antimicrobials and intelligent packaging materials.

CURRENT AND FUTURE IMPLICATIONS

Although MAP, acidity, antimicrobials and surface disinfection practices are methods frequently used to inhibit pathogen growth, maintaining refrigeration and decreasing storage time before consumption are the most efficient ways to ensure the safety of fresh-cut produce (Nguyen-the and Carlin, 1994). Opportunities for marketing fresh cut salads will increase as consumers continue to demand freshness and convenience. But, pathogenic microorganisms are capable of growing on fresh-cut salads subjected to packaging and distribution practices common to the produce industry. To ensure the safety of salads, the further exploration is needed to identify safe, effective and affordable alternatives to chlorine for surface disinfection. Several non-thermal physical techniques, such as oscillating magnetic fields, high-intensity pulsed light, ultrasonics and hydrostatic pressure, are being developed that may offer alternative treatments for improving the quality and safety of minimally processed salads (Hentges, 2003).

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RISK MANAGEMENT IN CATERING

Ayşegül Eyigör[1], Mati Roasto[2], Asli Kisikkaya Öncü[3], Kärt Kontram[4], Aija Melngaile[5], František Šišak[6], Vanja Uhan[7] & Gun Wirtanen[8]
[1] Uludağ University, Faculty of Veterinary Medicine, Bursa, Turkey
[2] Estonian University of Life Sciences, Tartu Estonia
[3] Tübitak, Gebze, Kocaeli, Turkey
[4] Fazer Bakeries Estonia, Tallinn, Estonia
[5] Latvian University of Agriculture, Latvia
[6] Veterinary Research Institute, Brno, Czech Republic
[7] University of Ljubljana, Ljubljana, Slovenia
[8] VTT Technical Research Centre of Finland, Espoo, Finland

Catering is the act of providing food and services or it may be defined as preparing or providing foods for someone else to serve; or preparing, delivering and serving food at the premises of another person or event. The aim of catering systems developed over recent years is to overcome problems of the shortage of skilled labor and reducing operational costs by industrializing the catering operation. The basis for developing these systems has been the industrialization of the catering operation through adopting methods of food processing technology such as centralized production, large-scale equipment, consistent heat treatments and sophisticated packaging (Kahraman *et al.*, 2004). There are two types of catering: on-premises catering and off-premises catering. In on-premises catering, all the food preparation and serving is done in a facility that is owned, leased, or rented by the caterer. This type of catering is often referred to as “banquet” or “hall” catering. On-premises catering are dominated by the hotel and motel industry along with the thousands of freestanding privately owned banquet halls. In off-premises catering, the food is prepared in a licensed commissary, transported to a location selected by the client, and served often without the support of and available kitchen (Kahraman *et al.*, 2004).

In 1993 the European Union issued a food hygiene directive (EU, 1993) establishing a general requirement for all food business to adopt a risk based food safety management system with the principles of the internationally accepted system hazard analysis critical control point (HACCP) recommended. However, each country in the EU

interpreted the Directive into their national regulations in different ways – some requiring all the principles of HACCP others only some of them. This led to widely differing levels of interpretation. As a consequence members of the EU, as part of a wide consolidation of food safety legislation, negotiated legal requirements that could be applied to all businesses across the food industry. This Regulation, with no option for national amendment, came into force across Europe in January 2006 (EU, 2004). It requires all food businesses to implement a system based on HACCP principles' (Taylor, 2008). Risk management in catering includes lot of different aspects. What are the main things one has to think in catering? Probably the main object is the raw material – basically its quality. But we should not forget laborer – their knowledge of catering, skills, hygiene etc. The aim of this group work is to review the main aspects of risk management in catering.

RISK ANALYSIS IN CATERING ESTABLISHMENTS

Risk Analysis

The Regulation EC 178/2002 establishes the principles of risk analysis in relation to food and establishes the structures and mechanisms for the scientific and technical evaluations which are undertaken by the European Food Safety Authority (EFSA). Depending on the nature of the measure, food law, and in particular measures relating to food safety must be underpinned by strong science. The EU has been at the forefront of the development of the risk analysis principles and their subsequent international acceptance. Regulation EC 178/2002 establishes in EU law that the three inter-related components of risk analysis (risk assessment, risk management and risk communication) provide the basis for food law as appropriate to the measure under consideration. Clearly not all food law has a scientific basis, e.g. food law relating to consumer information or the prevention of misleading practices does not need a scientific foundation. Scientific assessment of risk must be undertaken in an independent, objective and transparent manner based on the best available science. Risk management is the process of weighing policy alternatives in the light of results of a risk assessment and, if required, selecting the appropriate actions necessary to prevent, reduce or eliminate the risk to ensure the high level of health protection determined as appropriate in the EU. In the risk management phase, the decision makers need to consider a range of information in addition to the scientific risk assessment. These include, for example, the feasibility of controlling a risk, the most effective risk reduction actions depending on the part of the food supply chain where

the problem occurs, the practical arrangements needed, the socio-economic effects and the environmental impact. Regulation EC/178/2002 establishes the principle that risk management actions are not just based on a scientific assessment of risk but also take into consideration a wide range of other factors legitimate to the matter under consideration.

Transparency

Food safety and the protection of consumer interests are of increasing concern to the general public, non-governmental organizations, professional associations, international trading partners and trade organizations. Therefore, the Regulation establishes a framework for the greater involvement of stakeholders at all stages in the development of food law and establishes the mechanisms necessary to increase consumer confidence in food law. This consumer confidence is an essential outcome of a successful food policy and is therefore a primary goal of EU action related to food. Transparency of legislation and effective public consultation are essential elements of building this greater confidence. Better communication about food safety and the evaluation and explanation of potential risks, including full transparency of scientific opinions, are of key importance.

Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbial 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 microbial criteria should form an integral part of the implementation of HACCP-based procedures and other hygiene control measures. According to Article 4 of Regulation (EC) No 852/2004, food business operators are to comply with microbial 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 microbial criteria for foodstuff.

The basic principles of food hygiene introduce a certain level of flexibility that is believed essential in order to take account of particular situations. This is in particular the case with regard to the implementation of the HACCP-system, traditional ways of preparing certain food, and for certain small enterprises. It is clearly stated that this must not compromise food safety. Finally, all the legislation alone is not able to guarantee the quality and safety of the food. Food hygiene legislation and detailed microbial standards are meaningless if the legislation is impossible to apply in practice. Most important, however, is the care taken in the whole chain of food handling operations. The quality of raw materials, the hygienic environment within the food processing enterprise, the processing standards applied and the attitude of food enterprise personnel are all of crucial importance.

Risk Management

Risk management arrangements in public catering establishments are tightly connected with implementation of HACCP procedures in course of certain technological processes. Results of food safety practical assurance depend on application of adequate methods and resources during implementation process of good manufacturing practice and good hygiene practice. Provision of food safety nowadays is based on introduction of HACCP principles. Development of preventive food safety assurance systems comprises both the identification of food hazards and the introduction of regular monitoring measures in critical control points of technological processes. Management of technological processes in food establishments should be associated with detailed analysis of food products, processes and process conditions to evaluate their impact on quality and safety to the end products. To make the adequate decision about control measures that are necessary within stages of the technological process, application of risk analysis is needed to perform.

Incorrect food preparation and storage regime in catering establishments, can promote outbreak of food-borne diseases due to low infective dose of many pathogenic foodborne microorganisms. Foods that are prepared only with methods of cold processing comprise the highest risk of microbial contamination. Although foods after thermal processing are usually considered comparatively safe, the observance of good hygiene practice guidelines is of crucial importance. The information which is available in the literature sources suggests that growth of microorganisms in foods is governed by both the factors of external media (e.g. temperature) and internal media (e.g. pH, a_w) that can eliminate or promote growth of microorganisms, as well as by other factors that are related to growth of certain microbes (specific growth rate,

physiological status of cells, characteristic features and adoption capacity of family of microbes). Final level of microbial contamination of foods depends on interactions of several factors like working environment, including presence of microbial contamination in raw materials, hygiene level in food handling area, observance of personnel hygiene during preparation of salads, and storage regime of salads. Therefore, improvement of hygiene procedures is not possible without validation and verification of hygiene measures. Assurance of adequate hygiene measures is a critical process to ensure safety of end products; however, the efficiency of hygiene procedures is not often adequately controlled. It could be concluded that regular microbial risk assessment activities regarding food products and food environment safety help to improve both the risk management procedures and the communication with food service personnel. Taking into account the actual trends in food microbial contamination, it is possible to ensure purposeful hygiene control measures in the frame of HACCP procedures (Melngaile, 2008).

HACCP Applications in Catering

HACCP is the system of the hazard analysis and determination of the critical control points (CCP-s) related with food production, storage and food distribution. It is aimed to prevent of the contamination, instead of end-product evaluation. HACCP shifts the responsibility to the food producer to ensure that the food product is safe and fulfilling all requirements set by the legislative acts. Catering establishments such as restaurants, cafes and canteens are a major source of food poisoning outbreaks Principles of HACCP: The National advisory committee on microbial criteria for foods, developed seven principles that serve as the foundation for a HACCP system. They are:

1. Conduct a hazard analysis to identify potential hazards that could occur in the food production process. The risk should be determined for every component in every production phases. First we have to examine the relevant literature, to identify the food safety hazards associated with catering establishments. In our case – salad – the potential hazards could be as following: 1) biological risks *Salmonella spp., Listeria monocytogenes, Escherichia coli, Yersinia enterocolitica;* 2) No chemical risks because of organic farming and the use of biodegradable cleaning agents & 3) a low physical risks was noted due to pieces of metal that could get in at shredding carrots.

2. Identify the critical control points (CCPs) i.e. step or procedure in a food process at which the potential hazard could occur and control can be applied. As a result, a food safety hazard can be prevented, eliminated, or reduced to an acceptable level. A

food safety hazard is any biological, chemical, or physical property that may cause a food to be unsafe for human consumption. In our salad preparing process we have two CCPs. The first CCP is in process where the salad components are cut, sliced and shredded, this last process is most critical because of possibility of physical hazards (metal pieces from the shredder could came in the salad). Our solution is the metal detector which detects those parts and removes. The other CCP was the transportation container related temperatures. We control this CCP by regular monitoring of cooling equipment and calibration of thermometers.

3. Establish critical limits for preventive measures at each CCP. These limits are either the maximum or minimum value in which a physical, biological, or chemical hazard must be to prevent and/or eliminate the hazard, or at least reduce it to an acceptable level. The critical limit for the microbial hazards is being measured as colony forming unit per 1 ml, which muss not exceed the concentration that could be harmful. The harmful concentration varies according to the microorganism. The critical limit for the chemical hazards is determined to the chemicals that are not harmful and they must be in lowest possible concentrations. Physical hazards like pieces of equipment (metal, plastic pieces) muss not get into the food. E.g. in our case we can not have any metal pieces in the salad. The temperature in the transporting container has to be within the limit from 4 to 8°C and the salad has to be consumed within 24 h (shelf life of the product).

4. Establish CCP monitoring requirements to ensure that the process is under control at each critical control point. Monitoring may require materials or devices to measure or otherwise evaluate the process at CCPs. Chemical and physical hazards are being controlled all the time. A microbial survey is done by taking samples (swabs) from different places in food factories, that have direct or indirect contact with the product (worktops, cutting boards, knives, refrigerators, aprons and personnel (hands)) These samples are then taken to the laboratory and examine for Total Viable Counts (TVC), Total Enteric Counts (TEC), *Salmonella*, *Campylobacter*, *E. coli*, *S. aureus* and *Listeria*. A technical survey is also wery important in catering. Most catering activities extend shelflife of prepared or semi-prepared menu items by "coldholding" (refrigerated storage), or "hot-holding" in *bain-marie* and other devices. However neither of these methods are likely to be effectively and/or consistently applied. Thus some of refrigerators operate under conditions which are "cool" rather than "cold" and are therefore likely to support undesirably significant rates of growth of important food borne pathogens. On the other hand "hotholding" devices are being operated as "warm" rather than "hotholding" systems, and will

therefore also support undesirable pathogen growth. Both of these holding conditions are also subject to significant rapid temperature changes as part of normal catering operations, for instance by adding/removing products we have to openen/close containers. The equipment must be regulary checked and we should pay attention to the temperature floaculations during the opening/closing containers, so that the temperature changes are as low as possible.

5. Establish corrective actions are necessary when monitoring indicates a deviation from an established critical limit. The rules require a HACCP plan to identify the corrective actions to be taken if a critical limit is not met. Corrective actions are intended to ensure that all products are wholesome.

6. Establishment of effective recordkeeping procedures is required according to the HACCP system along with archiving documents including a written HACCP plan with information on monitoring of critical control points, critical limits, verification activities, and handling of the deviations.

7. Establishment of verification procedures to ensure the HACCP plan is working properly. Verification procedures may include such activities as review of HACCP plan, CCP records, critical limits and microbial sampling and analysis. HACCP plan must include verification tasks to be performed by plant personnel. Verification tasks would also be performed by inspectors undertaking microbial testing as one of theverification activities.

Personel hygiene in catering sector

In the catering sector humans are important and therefore it is important to understand how people and environment contaminate food. Every single person throughout the chain can play a very important part in preventing food being contaminated. If every person who handles food could achieve personal hygiene to its highest standards, food contamination could be kept to a minimum. In the food industry the term ‘personnel’ includes operatives employed on the factory floor as well as managers, engineers, contractors and visitors. Ill persons should not handle food. In a Catering plant, high standards should be set. There are certain activities that will help a person to achieve this goal of personal hygiene. Regular bathing at least once a day is essential; otherwise germs can be transferred onto the clothes and so onto food. If foods are being contaminated, people who consume the food will become sick and might even die from consuming contaminated foods.

Hands must be washed frequently i.e. before commencing work and whenever food is handled. They should be washed thoroughly in hot water with the aid of brush and soap; rinsed; and dried using clean, disposable towels. Hands and fingernails must be kept clean when dealing with food. Many Catering industries are now providing gloves to their employees. For many of the workers, if not properly trained, this might give them a false sense of security. Sometimes, they can forget to change their gloves after touching the nose, scratching the heads, or picking up things that are dropped on the floor. Therefore, workers who use gloves should be aware of this point. Rings, watches, and jewelry should not be worn when food is handled. Particles of food may be caught under a ring, and germs can multiply and be transferred into food. Watches and jewelries should not be worn in food handling, since they may fall off into food, unknown. Fingernails should always be kept clean and short as dirt can easily lodge under the nail and be discharged when, for example, handling and processing food, thereby introducing bacteria into food. Fingernails should be cleaned with a nailbrush, and nail varnish should not be worn when one has to process food at a food processing plant. Hair should be washed regularly, cut properly and covered and if needed also tied back when food is being handled. The hair should never be scratched, combed, or touched while handling food, because hair and dandruff with microbes could be transferred to the food.

The nose should not be touched when food is being handled. If a disposable handkerchief is used it should be discarded directly afterward and the hands should not be washed and disinfected. The nose d amouth area is harbouring vast numbers of harmful bacteria. To avoid microbes spreading it is very important that neither food, people nor working surfaces are sneezed over or coughed at. Neither cooking utensils nor fingers should be used for tasting food, to reduce food contamination. A clean teaspoon should be used for tasting and washed well after each tasting. As food handlers stand for many hours during a working day, care of the feet is important. They should be washed regularly, and the toenails kept short and clean. Tired feet can cause general tiredness, which leads to carelessness, which often results in a lowering the hygiene level in the food handling. It is also important to keep all cuts, burns, scratches, and wounds covered with a waterproof, coloured dressing or plasters.

Clean white protective clothing with clean underclothes should be worn at all times. Cloths used for wiping clean utensils or used for holding hot cooking utensils or used to wipe working surfaces in case of spills should also be kept clean and stored in the sanitizing solutions when not in use. Be sure to change the sanitizing solutions at

regular intervals because the organic materials accumulating will compromise the strength of the sanitizing solution. Outdoor clothing and other clothng taken off before wearing white working clothes should be kept in a locker away from the food processing area.

The importance of training employees to meet company standards, industry standards, regulatory standards, and consumer expectations cannot be overemphasized; this is one of the key elements to help a company survive and be competitive. The traditional ways of training employees such as in a classroom setting, using textbooks, and showing videos may still be a viable way to train a company's employees.An effective induction training and a program of ongoing training are the best ways to educate and reinforce good personal hygiene practices. In one study of food handlers' attitudes, 62% admitted to sometimes not carrying out all food safety procedures on every occasion, with 6% admitting that they often did not (Clayton *et al*. 2000). Lack of time was the most quoted reason for failure to implement agreed procedures. Management must establish appropriate procedures to ensure hygienic practices for employees. Supervisors and managers should set an example for employees by their own high levels of hygiene and good health while conveying the importance of these practices to the employees. In general, hygienic practices are more likely to be implemented if they are properly integrated into the organization's culture. If management takes good hygiene practices seriously, provides the time and resources needed and rewards good performance, employees will take their responsibilities more seriously.

Perhaps the most effective way to carry this out is to present all new employees with a comprehensive induction program, then reinforce it through means of posters, clear instructions in toilet blocks, changing rooms and hand washing facilities in the plant. An induction program should include: personal responsibilities, protective clothing requirements and use, hand washing requirements and prevention of cross-contamination from raw materials to finished product areas. Regular group sessions, which can include videos, are also helpful. Additionally, there must be sufficient on-going supervision of personal hygiene procedures in production departments to ensure that everyone complies with these procedures. Good hygiene practice should be part of any appraisal system of employees, supervisors and managers and violations of practices should be handled as disciplinary violations. Incentives for superior hygiene and sanitary practices should also be provided. Involving staff in developing and monitoring hygiene procedures is an effective way of winning commitment.

In order to ensure a personal hygiene training program works for organization, it is important for management to have a continued evaluation program to ensure effective hygiene practices are incorporated in an organization. A periodic inspection is a useful tool to determine its effectiveness. Supervisors should always function as examples through showing own, good habits. They should also observe how employees perform their hygiene practices to identify if there is a need to retrain employees. Statistics show that most people forget what they learn after a period of time. It is suggested to have an annual or biannual retraining if the company can afford the time to retrain their employees.

As a result, bacteria are present all around us. Some of them are beneficial, but some are not. It is important for individuals who work in a catering plant to break the chain of contamination by following sound hygiene practices. Everyone is important in keeping the food safe from farm to table. It is true that the government has set rules and regulations for different food processing plants to follow, but these rules and regulations will not be effective unless enforced and followed by the workers. Remember that consumers are counting on food processors to safeguard this process. And the most essential thing is that products are produced in processing plants under sound hygienic conditions.

WATER HYGIENE IN CATERING ESTABLISHMENTS

This part refers to three basic documents, which relate to water hygiene, and can be used as reliable guidelines in establishing hygienic water requirements in catering. Codex Alimentarius Comission Recommended International Code of Practice General Principles of Food Hygiene (CAC/RCP 1-1969, rev. 4-20031) section 5.5 indicates that only potable water should be used in food handling and processing in contact with food with the following exceptions:

- for steam production, fire control and other similar purposes not connected with food; and
- in certain food processes, e.g. chilling, and in food handling areas, provided this does not constitute a hazard to the safety and suitability of food (e.g. the use of clean sea water).

Water recirculated for reuse should be treated and maintained in such a condition that no risk to the safety and suitability of food results from its use. The treatment process should be effectively monitored. Recirculated water which has received no further

treatment and water recovered from processing of food by evaporation or drying may be used, provided its use does not constitute a risk to the safety and suitability of food. Also in 5.5.2 As an ingredient; Potable water should be used wherever necessary to avoid food contamination.

In Codex Alimentarius Comission Code of Hygienic Practice for Precooked and Cooked Foods in Mass Catering (CAC/RCP 39-1993) 4.3.12, water supply is indicated as an ample supply of water, in compliance with the WHO "Guidelines for Drinking Water Quality" under adequate pressure and of suitable temperature should be available with adequate facilities for its storage, where necessary, and distribution, and with adequate protection against contamination. It is also noted that samples should be taken regularly, but the frequency should depend upon the origin and the usage of the water, e.g. more frequent from private supplies than from public supplies. Chlorine or other suitable disinfectants may be used. If chlorination has been employed checks should be made daily by chemical tests for available chlorine. The point of sampling should preferably be at the point of usage, but occasionally it would be useful to sample at the point of entry of the water to the establishment. In the same CAC/RCP, section 4.3.12.2 indicates that there should be a system to ensure an adequate supply of hot potable water, and ice shall be made from potable water and should be manufactured, handled and stored so as to protect it from contamination. 4.3.12.4 of the same document dictates that steam used in direct contact with food or food contact surfaces should contain no substance which may be hazardous to health or may contaminate the food, and the 4.3.12.5 indicates non-potable water used for steam production, refrigeration, fire control and other similar purposes not connected with food should be carried in completely separate lines, identifiable preferably by color, and with no cross-connection with or back-siphon age into the system carrying potable water. In 4.3.13 of the same document relates to effluent and waste disposal and indicate that establishments should have an efficient effluent and waste disposal system which should at all times be maintained in good order and repair. All effluent lines (including sewer systems) should be constructed to avoid contamination of potable water supplies. All waste pipes should be properly trapped and lead to a drain.

Third Edition of the WHO Guidelines for drinking-water quality implies that access to safe drinking-water is essential to health, a basic human right and a component of effective policy for health protection. The importance of water, sanitation and hygiene for health and development has been reflected in the outcomes of a series of previous international policy forums. Most recently, the UN General Assembly declared the

period from 2005 to 2015 as the International Decade for Action, “Water for Life.” This published guideline provides information on the assessment and management of risks associated with microbial hazards and by internationally peer-reviewed risk assessments for specific chemicals; guidance on good practice in surveillance, monitoring and assessment of drinking-water quality in community supplies. The Guidelines are also accompanied by other publications explaining the scientific basis of their development and providing guidance on good practice in implementation. This volume of the Guidelines for Drinking-water Quality explains requirements to ensure drinking-water safety, including minimum procedures and specific guideline values, and how those requirements are intended to be used. The volume also describes the approaches used in deriving the guidelines, including guideline values. It includes fact sheets on significant microbial and chemical hazards. The development of this third edition of the Guidelines for Drinking-water Quality includes a substantive revision of approaches to ensuring microbial safety. This takes account of important developments in microbial risk assessment and its linkages to risk management.

FOODBORNE PATHOGENS AND CATERING

Related with production hygiene and food safety there are many foodborne pathogens which has to be taken into account within self-control activities of any kind of food industry as well as in catering establishments. Causative agents of foodborne diseases are transmitted to humans via different vectors and it is not an easy task to guarantee the safe food at maximum achievable level for all the people who consume it. Therefore, good hygiene practices, good production practices and the effective HACCP systems play very important role to achieve the best food hygiene level in any kind of food enterprise. Taking into account the negative effect to human health as well as the causatives for sanitation program failures in the food industries, by following the overview about the some of the most important foodborne pathogens in EU is given.

Listeria monocytogenes

Listeriosis and *Listeria monocytogenes* continue to be of worldwide interest to the food industry and regulatory agencies, scientists in various diciplines, and consumers of food. Such intrest is prompted by the occasional appearance of *L. monocytogenes* in ready-to-eat foods, leading to the removal of these products from the marketplace. Furthermore, sporadic cases of listeriosis continue to occur and several food-associated outbreaks of the disease have occurred (Ryser and Marth 2007). The bacterial genus

Listeria currently comprises six species, but human cases of listeriosis are almost exclusively caused by the species *Listeria monocytogenes*. *L. monocytogenes* is a small (0.5 µm in diameter and 1 to 2 µm in length), regular Gram-positive and motile rod with rounded ends that is commonly present in the environment and occurs in almost all raw food materials sporadically (Ryser and Marth 2007). *L. monocytogenes* has been associated with food sources such as raw milk, unreliably pasteurized milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (all types) as well with raw and smoked fish. *Listeria* is able to grow at temperatures as low as 3°C and this permits it's multiplication in refrigerated foods and makes the occurrence in ready-to-eat foods with a relatively long shelf life of particular concern. It can survive or even grow at pH values as low as 4.4 and at salt concentrations of up to 14% (Roasto *et al*., 2004). It is recognized that presence of *L. monocytogenes* in raw foods cannot be completely eliminated, but through the application of effective hygienic measures, it is possible to reduce its occurrence and level 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 that are able to reliably control the growth of *L. monocytogenes* and to prevent from the multiplication to potentially harmful level of > 100/g (Commission Regulation (EC) No 1441/2007; Berziņš *et al*., 2007).

Raw fruits and vegetables would normally be expected to be free of most human and animal enteric pathogens unless somehow contaminated by human or animal waste. Although few cases of foodborne disease had traditionally been associated with consumption of fresh produce, such outbreaks have been recognized in recent years to occur with greater frequency than previously thought. *L. monocytogenes* is among the foodborne pathogens most often associated with these foods. This may result, in part, from the variety of ways in which *L. monocytogenes* can contaminate fresh vegetables. Consequently, the fact that *L. monocytogenes* can find its way into fresh produce makes its potential presence on such products a public health issue. Recent *Listeria monocytogenes* risk assessment ranked fruits as carrying a slightly higher risk than vegetables, although still ranking it as a low predicted relative risk. Although the exact reasons for the higher risk are somewhat unclear, one important factor was the higher percentage of samples with detectable contamination. In addition, the ability of certain fruits, such as melons, to support rapid growth of *L. monocytogenes* together with the absence of data required for more precise calculation of risk lead to a calculated high risk for fruits (Brackett, 2007).

Salmonella

Among the Gram-negative rods that cause foodborne gastroenteritis, the most important are the members of the genus *Salmonella*. *Salmonella* is a member of the family *Enterobacteriaceae* that comprises a large and diverse group of Gram-negative rods (Jay *et al.*, 2005a). Members of the genus *Salmonella* are zoonotic and can be pathogenic in man and animals. Salmonellae are facultatively anaerobic, Gram-negative, straight, small rods, which are usually motile with peritrichous flagella. They are nonlactose fermenting and nonspore forming. There are currently well over 2400 serovars. Epidemiologic classification of *Salmonella* is based on host preference. One group includes serotypes that infect only humans, for example, *S.* Typhi and *S.* Paratyphi (Jeffrey *et al.*, 2002).

Salmonellosis, one of the most common and widely occurring foodborne disease, constitutes a major public health burden and represents a massive cost to society in many countries. Millions of cases are reported worldwide every year resulting in thousands of deaths. A *Salmonella* control program in food animal production has been in place for several years in Denmark and the annual estimated cost of this control program is 10.8 million euros. It is estimated that this program saves annually 19.6 million euros to the Danish society. Some countries have managed to limit and even reverse salmonellosis but the spread of two strains of *Salmonella*, namely *Salmonella* Enteritidis and *Salmonella* Typhimurium are causing increased concern (European Commission, 2004). Multiresistant strains of *Salmonella* are now encountered frequently. The occurrence of multiresistance has increased considerably in recent years owing to the global spread of multiresistant *Salmonella* Typhimurium DT104. While the spread of DT104 may have been facilitated by the use of antimicrobials, international and national trade of infected animals is thought to play a major role in dissemination (INFOSAN, 2005).

A wide variety of foods have been identified in outbreaks caused by several serotypes of S*almonella*: 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 gelatin, peanut butter, cocoa, and chocolate. Various *Salmonella* serotypes have long been isolated from the outer surface of egg shells. The present status 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* is still 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 the case especially considering the most important meats from pig and poultry. In areas such as Scandinavia measures against this pathogen have been traditionally more thoroughly endeavored, finally resulting in a lower prevalence of *Salmonella* in these countries compared to Central Europe (Roasto *et al*., 2006). Whatever the *Salmonella* serotype, effective controls for minimizing/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 and consumer handling (Roasto *et al*., 2006).

Escherichia coli

E. coli is short, typically motile, facultative anaerobic, non spore forming, Gram-negative rods (1.1–1.5 μm and 2.0–6.0 μm) within the family *Enterobacteriaceae.* The optimum growth temperature is 37°C. The combination of O and H antigens defines the *E. coli* serotype, and serotyping of isolates is useful for foodborne outbreak and epidemiological investigations. *E. coli* form a part of the natural gastro-intestinal microflora of man and warm-blooded animals. 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* strains are harmless commensal organisms, there are many pathogenic strains capable to cause a variety of illness in humans.

There are six recognized groups of pathogenic *E. coli* (EAEC, EPEC, ETEC, EIEC, EHEC, VTEC). Each group has different virulence features and mechanisms of pathogenity (Duffy, 2006; Fratamico *et al*., 2002). The enteroagregative *E. coli* (EAEC) are associated with persistant diarrhea in young children especially in developing countries. These strains produce three toxins which stimulate intestinal secretion. The enteropathogenic *E. coli* (EPEC) cause severe diarrhea in infants. Certain EPEC strains produce one or more cytotoxins. The enterotoxigenic *E. coli* (ETEC) cause also diarrhea in humans, both in infants and adults, and for the latter world-wide illness known as traveller´s diarrhea. ETEC strains produce enterotoxins. The enteroinvasive *E. coli* (EIEC) produce a cytotoxin and often induce rather severe illness like colitis and a form of dysentery, accompanied by fever and bloody stools. The enterohaemorrhagic *E. coli* (EHEC) produce cytotoxins which give more severe

symptoms. These toxins (verotoxin 1 and verotoxin 2) are closely related or identical to the toxin produced by *Shigella dysenteriae*. They have the same biological activity but can be distinguished immunologically. The toxins are lethal to Vero cells and hens are known as Vero cytotoxin producing *Echerichia coli* or VTEC. The toxins destroy the intestinal cells of the human colon causing haemorrhagic colitis (HC) which is characterized by severe abdominal pain and diarrhea. About 15% of HC cases, notably children, develop haemolytic ureamic syndrome (HUS). This is renal failure and haemolytic anaemia and may result in permanent kidney damage. *E. coli* serotype O157:H7 is the most well-known EHEC strain. Due to current detection procedures *E. coli* O157:H7 is the only serotype routinely identified, however, other verotoxigenic *E. coli* serotypes such as *E. coli* O26:H11 are known (Forsythe and Hayes, 1998; Fratamico *et al*., 2002).

Strains of EAEC have been isolated from the contents of infant feeding bottles, and outbreaks have been associated with food. Disease due to EPEC occurs predominantly in developing countries with vehicles including different foods or formula, contaminated hands of nurses and contaminated utensils such as linen, scales, toys etc. ETEC strains have been implicated in several large outbreaks of infection with water as well as a wide range of foods (various meats and poultry, mashed potatoes, milk products) as sources. Many outbreaks have been attributed to EIEC strains, with cheeses, milk and meats, potato salads and other foods. The most well-known EHEC strain, *E. coli* O157:H7, outbreaks have been mostly implicated with undercooked or raw hamburger (ground beef). However, *E. coli* O157:H7 outbreaks have implicated also from 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 (Forsythe and Hayes, 1998; Fratamico *et al*., 2002).

Yersinia enterocolitica

In the genus *Yersinia*, which belongs to the family *Enterobacteriaceae*, 11 species and 5 biovars are recognized, including *Y. pestis*, the cause of plague. *Y. enterocolitica*, a small rod-shaped, Gram-negative bacterium, is often isolated from clinical specimens such as wounds, feces, sputum and mesenteric lymph nodes. The species of primary interest in foods is *Y. entreocolitica*. Only *Y. enterocolitica* has been detected in environmental and food sources, such as ponds, lakes, meats, ice cream, and milk. Most isolates have been found not to be pathogenic. This Gram-negative rod is often present in the environment (Jay et al., 2005b).

Yersiniosis is the third most common bacterial enteric disease in Europe (Anonymous, 2006). The number of yersiniosis cases per 100.000 individuals reported in Estonia, Latvia and the Leningrad Region of Russia, oscillated from 2005 to 2006 in 2.3–3.1 (Estonia), 2.43–4.1 (Latvia) and 0.2–4.2 (Leningrad Region) (Anonymous, 2007). Signs of yersiniosis are gastroenteritis, terminal ileitis, and mesenteric lymphadenitis. Symptoms manifested by the patient are diarrhea, vomiting, fever and abdominal pain. Post-infection consequences like reactive arthritis, erythema nodosum and uveitis may occur (Smego *et al*., 1999; Fredriksson-Ahomaa *et al*., 2008). Although sporadic cases due to *Yersinia enterocolitica* in Europe are common, outbreaks related to *Y. enterocolitica* are scarce (Babic-Erceg *et al.*, 2003; Grahek-Ogden *et al*., 2007). In Europe, large foodborne outbreaks due to *Yersinia pseudotuberculosis* have been reported in Finland and Russia (Anonymous, 2002–2007; Jalava *et al*., 2004; Rimhanen-Finne *et al*., 2008).

Yersinia enterocolitica has been isolated from cakes, vacuum-packaged meats, seafood, vegetables, milk, and other food products. Of all sources, swine appears to be major source of strains pathogenic for humans (Jay *et al*., 2005b). Yersiniosis is also considered as an occupational disease among butchers (Kelesidis *et al*., 2008). There are many possibilities for enteropathogenic *Yersinia* to access to the food chain and to multiply in foods due to its psychotropic properties (Fredriksson-Ahomaa *et al*., 2001). As previously, mentioned the strains of *Y. enterocolitica* can be found in meats (pork, beef, lamb, etc.), oysters, fish, and raw milk. The exact cause of the food contamination is unknown. However, the prevalence of this organism in the soil and water and in animals such as beavers, pigs, and squirrels, offers ample opportunities for it to enter our food supply. Poor sanitation and improper sterilization techniques by food handlers, including improper storage, cannot be overlooked as contributing to contamination.

CONCLUSIONS

There are many foodborne, pathogens which have to be taken into account, when establishing of the effective self-control system in the food enterprise but good hygiene and production practices are most important preliminary conditions which have to be introduced before the HACCP system. In more and more cases the use of experts in various fields of expertise is necessary, different value systems are to be taken into account, etc. The main critical task in risk management in catering establishments are to perform to perform the adequate risk analysis – different microorganisms, which are

responsible of different foodborne diseases (such as listeriosis, salmonellosis, *coli* infections etc); water hygiene – one of the main thing, what can provide us safety food; personnel hygiene – seems easy to provide good hygiene, but there are many "dark corners", what leads to another problems. Personnel is sometimes the hardest part of risk management, to make them realize how important are to follow the enterprise specific hygiene and food safety guidelines and instructions. Finally, there are no easy solutions and golden standards of operating systems to all kind of catering establishments. Self-control systems have to be created separately in each individual food enterprise taking into account the real hazards and fixing the real critical control points.

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RISK MANAGEMENT DURING THE PRODUCTION OF NATURAL MINERAL WATER

Ibrahim Mehmeti[1], Mehlica Borackli[2], Urban Wiik[3],
Kristian Cernelek[4] & Ivan Rychlik[5],
[1] Norwegian University of life Science, Ås, Norway
[2] Tübitak, Marmara Research Center, Food Institute, Gebze, Turkey
[3] JohnsonDiversey, Helsinki, Finland
[4] Pivcap, Košana, Slovenia
[6] Veterinary Research Institute, Brno, Czech Republic

In order to get a better understand on risk management, we make one case study on the production of natural mineral water. The strategy is made based on HACCP system, EU role and EU regulations. The conclusion shows that in every production the primary consideration is to protect the human health. If we need to work in properly way and right the inspection should be totally transparent and the inspector should be in touch with all groups of workers and also with the consumers. From a biological standpoint, water has many distinct properties that are critical for the proliferation of life. However, the world every day is hearing that is missing the water. Based on the importance of the water is very useful to see and to be strict in hygiene and to make a good strategy for potable water – from the tap to the table. The most important way of having the good water quality is to protect it from outer contaminants. These contaminants are biological, chemical or physical. The biological contaminants are bacteria e.g. *Clostridium*, *E. coli*, *Salmonella* spp., *Aeromonas*, *Pseudomonas* spp. *Yersenia*, *Vibrio* spp., moulds, yeast, virus e.g. enteroviruses, hepatitis A, noroviruses and protozoa e.g. Amoeba, Giardia, Toxoplasma ect). The chemical contaminants are e.g. pesticides, minerals salts, nitrate, nitrite and heavy metals[1].

Risk analysis is widely recognized as the fundamental methodology underlying the development of food safety standards. As recognized in the 1995 the risk analysis is divided in three groups namely risk assessment, risk management and risk communication. The risk management is defined within Codex and has the possibility in development of standards, guidelines and other recommendations for food safety. The main goal of risk management in food is to protect public health by controlling such us risk in the

production. The Hazard Analysis and Critical Control Point, (HACCP), is a systematic preventive approach to food safety that addresses physical, chemical, and biological hazards as a means of prevention rather than finished product inspection. Critical control point (CCPs) can be taken to reduce or eliminate the risk of the hazardous being realized. The system is used in all stages of food production and also is including the preparation processes. In general the HACCP system has seven general principles but also they have the second's principles which are used to prevent the contaminants. In order to get e better understanding on HACCP system we have make one case study that if we have chance to work on company which criteria we have to follow in inspection.

The natural mineral water is our production in this case. Analyzing the water quality will help us in the future to have good and stable business. Before we start to check the inspection inside the building we have to check some important criteria e.g. the quality of the water before entering the production, conformity of water quality with EU legislation and risk evaluation of any recontaminants in the system. When we have drawn the conclusion that the physical, chemical and microbial quality of water confirms with ISO standards we have to inspect the building with pipings so that the processing including disinfection is correct. The design of the building for natural mineral water bottling with instruments in-place are shown in Figure 1. It shows the lines of the water and bottles how they enter into the system. The first step us you see from the Figure 1 is the filtration when the water come in to the factory. The filter has the function to minimize the dirty thinks and contamination of source water from large thinks. The UV light has a disinfecting function in details us a best disinfection for the moment. On the other side is line from where they come dirty bottles and enter into the washing system and process of the fill up with water. The distribution system is the separate place when we will distribute the final product. When we have the design of the factory is inspector's job to make the control points and critical controls pints. In Figure 2 are shown the control points (CPs). The CPs are: before the water enters into the building, before the bottles enter into the line, after washing steps and in the end of the system before living the building. The workers has to inspect these points very carefully and every time during production.

The Figure 3 showed the critical control points (CCPs). CCPs have been pinpointed at water disinfection process using UV light and inspection ofbottles after washing system before entering into the filler capper. To obtain a good understanding on hazard system we made an inspection in which we marked the hazard, type of the hazard control points and preventive and correlation action. Table 1 shows explanations on

hazard types. Firstly, if the incoming water is of bad quality we should check its physical, chemical and microbial aspects. A preventive action is re-inspection of the filters and UV light. The water analysis results are very important and the water should be analysed, frequently i.e. in the beginning every week in a reference laboratory. On other hand if the problems come from dirty water in the production the problem will be of microbial aspects because the microorganisms have possibilities to grow in dirty area. To avoid such problems we should stop the work and re-check all the washing line. When such problems have been fixed the production should restarted. Another important thing for inspectors based on the HACCP system is to use zoing. Figure 4 show two cleaning zones and one dirty zone. The dirty zone is the part of the factory in which the bottles are kept before washing.The cleaning zone have been marked from the place where the water enters the factory until the end of the bottle cleaning system. In the clean zones the workers must be careful with hygiene and they must stop every external person who does not have proper documentation.

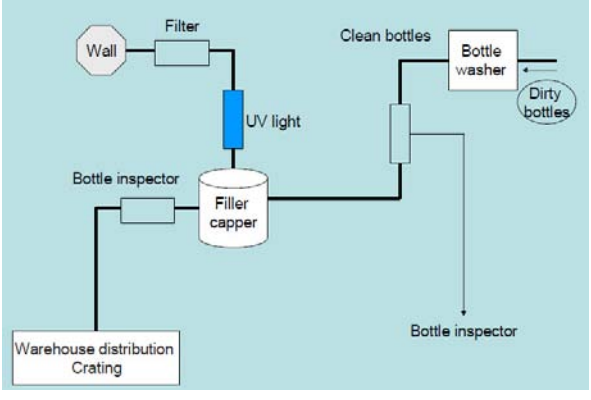

Figure 1. General design of water bottling company.

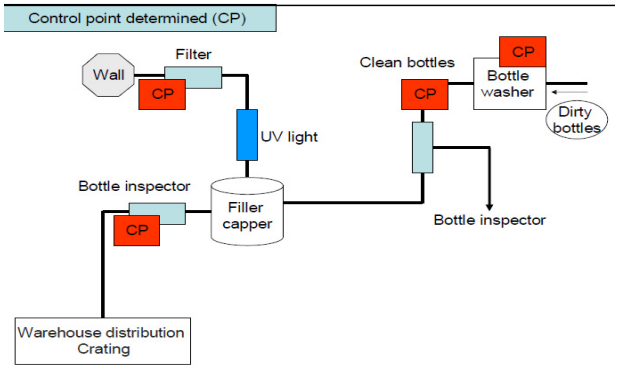

Figure 2. Determination of control points in the water bottling process.

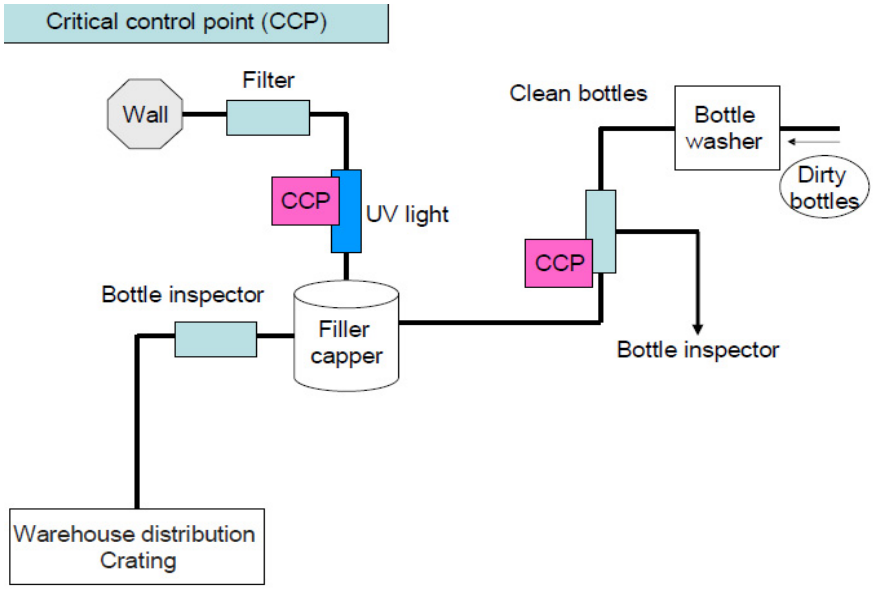

Figure 3. Critical control points (CCPs) in the water bottling process.

Table 1. Hazard system in details.

Hazard	Hazard type	Control point	Preventive action	Corrective action
Bad quality incoming water	Microbiology, Chemical , physical contamination	Water analysis once a week after the UV	Follow UV maintenance procedures	Exchange UV lamp have spare on stock
Probability low		Filter condition		
Severity high		UV condition		
Dirty bottles	Microbiological	Microbiological monitoring of bottles once a week	Monitor temperature, caustic content, rinse water chlorine once every shift, empty and clean the bottle washer every week	Stop production bring values back into specification
Probability high	Dirt foreign objects	Bottle inspecting machine	Run sample bottles every shift	
Severity low		Monitoring		
Cap quality	Microbiology	Monitoring in house and manufacturer	Store caps in proper warehouse	Take another batch of caps
Probability low				
Severity Medium				
Filling machine	Microbiological	Filling machine surfaces product quality	Propr cleaning and regular disinfection. Staff training	Intensify cleaning program
Probability high				
Severity high				

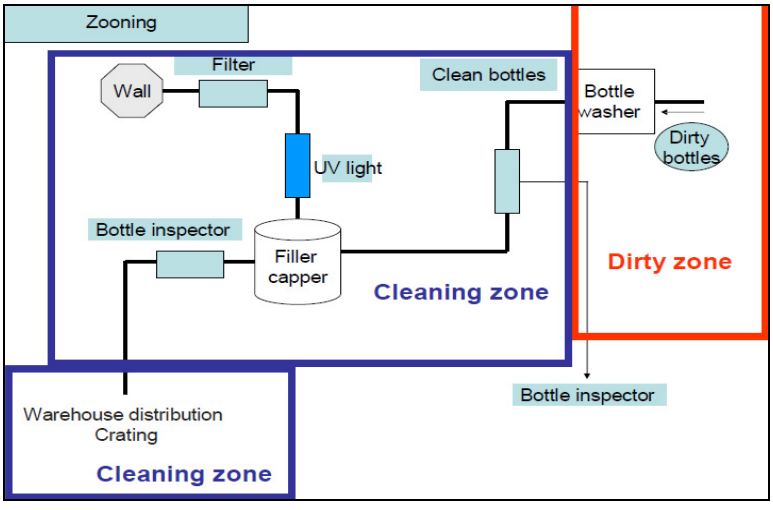

Figure 4. Zoning system.

CONCLUSION AND SUGGESTION

The inspections show some suggestion that they will help the company in the future. It is important that: 1) every worker must know his/her responsibility; 2) information is available in written form at every machine, door, etc; 3) the company rules clarely state the role of hygiene rules and that they are based on regulation; 4) specialists are invited to teach/train the workers; 5) every person without proper documentation is stopped at the company entrance before entering the clean zones; 6) workers and approved visitors should wear clean protective clothes; 7) there are proper, strict rules for inspecting water, process surfaces, personnel, etc and 8) persons working with documentation should be aware of changes in roles and regulation e.g. EU and WHO regulations.

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APPENDIX 1: PARTICIPANT LIST

1. Ayse Handan Baysal, Izmir Institute of Technology, Urla, İzmir, Turkey
2. Taner Baysal, Ege University, Bornova, Izmir, Turkey
3. Kristijan Črnelec, Pivkap, Košana, Slovenia
4. Alina Dobre, Institutul de Bioresurse Alimentare, Bucharest, Romania
5. Helen Ehavald, AS Fazer Bakeries Estonia, Tallinn, Estonia
6. Aysegül Eyigör, Uludag University, Gorukle Campus, Bursa, Turkey
7. Savvas Gennaris, Veterinary Services, Lefkosia, Cyprus
8. Metin Guldas, Uludag University Karacabey VHS, Bursa, Turkey
9. Ülle Hödrejärv, AS Kalev Chocolate Factory, Tallinn, Estonia
10. Reyhan Irkin, Balikesir University, Susurluk, Balikesir, Turkey
11. Aet Kala, Rimi Eesti Food AS, Tallinn, Estonia
12. Kärt Kontram, AS Fazer Bakeries Estonia, Tallinn, Estonia
13. Figen Korel, Izmir Institute of Technology, Urla, Izmir, Turkey
14. Risto Kuisma, University of Helsinki, Helsinki, FInland
15. Liina Kutsar, Tere AS, Tallinn, Estonia
16. Marja Lehto, MTT, Helsinki, Finland
17. Primož Likar, Mlinotest d.d, Ajdovščina, Slovenia
18. Aleksander Maher, Mlekarna Celeia d.o.o., Velenje, Slovenia
19. Ibrahim Mehmeti, Norwegian University of Life Science, Ås, Norway
20. Kadrin Meremäe, Estonian University of Life Sciences, Tartu, Estonia
21. Camilla Nielsen, DTU National Food Institute, Lyngby, Denmark
22. Maarten Overes, Wageningen University, The Netherlands
23. Darja Peterka, Pekarna Pečjak, d.o.o., Ljubljana, Slovenia
24. Oana Popescu, S.C. Vel Pitar S.A., Bucharest, Romania
25. Kristi Praakle-Amin, Estonian University of Life Sciences, Tartu, Estonia
26. Mati Roasto, Estonian University of Life Sciences, Tartu, Estonia

27. Tiina Tarkiainen, Scanegg Suomi Oy, Piispanristi, Finland
28. Terje Tamme, Estonian University of Life Sciences, Tartu, Estonia
29. Seran Temelli, Uludag University, Gorukle Campus, Bursa, Turkey
30. Tina Tusar, University of Ljubljana, Ljubljana, Slovenia
31. Vanja Uhan, University of Ljubljana, Ljubljana, Slovenia
32. Anne Villem, AS Eesti Munatooted, Tallinn, Estonia
33. Rijkelt Beumer, Wageningen University, The Netherlands
34. Mehlika Borcakli, Tübitak, Istanbul, Turkey
35. Rene Elgaard, Alfa Laval Tank Equipment A/S, Denmark
36. Alan Friis, DTU, Lyngby, Denmark
37. Annika Jürgens, Greenclean OÜ, Tallinn, Estonia
38. Asli Kisikkaya, Tübitak, Istanbul, Turkey
39. Hanne Løje, DTU National Food Institute, Lyngby, Denmark
40. Aija Melngaile, Latvia University of Agriculture, Jelgava, Latvia
41. Marika Mikelsaar, University of Tartu, Tartu, Estonia
42. Helvi Mustonen, Orion Diagnostica Oy, Espoo, Finland
43. Jana Ramus, CCIS, Ljubljana, Slovenia
44. Ivan Rychlik, Veterinary Research Institute, Brno, Czech Republic
45. Satu Salo, VTT, Espoo, Finland
46. Tiina Saron, Piimaliit, Tallinn, Estonia
47. Frantisek Sisak Veterinary Research Institute, Brno, Czech Republic
48. Sonja Smole Možina, University of Ljubljana, Ljubljana, Slovenia
49. Tiina Veskus, Tallinn University of Technology, Tallinn, Estonia
50. Raivo Vokk, Tallinn University of Technology, Tallinn, Estonia
51. Urban Wiik, JohnsonDiversey, Helsinki, Finland
52. Gun Wirtanen, VTT, Espoo, Finland

APPENDIX 2: PROGRAMME

Arrival on 3rd of May Sunday evening – information and Get-together event at the St Olav's Hotel (Lai 5) in Tallinn, Estonia

Monday, May 4, 2009

8.40 – 9.00	Registration
9.00 – 9.20	Welcome and general information on the seminar, Dr. Gun Wirtanen, VTT Technical Research Centre of Finland & Prof. Raivo Vokk, Tallinn University of Technology
9.20 – 9.45	Food legislation in the EU, Külli Rae, Ministry of Agriculture, Estonia
9.45 – 10.10	Food chain management from the perspective of sustainability, product safety and quality, Tiina Saron, Piimaliit, Estonia
10.10 – 10.35	International (microbiological) standardization – part I, Rijkelt Beumer, Wageningen University, the Netherlands
10.35 – 11.00	Coffee break
11.00 – 11.25	International (microbiological) standardization – part II, Rijkelt Beumer, Wageningen University, the Netherlands
11.25 – 11.50	Does evidence based research in functional food area avoid risks for health: different regulations, Prof. Marika Mikelsaar, University of Tartu, Estonia
11.50 – 12.15	Hygienic engineering guidelines in closed equipment, Prof. Alan Friis, National Food Institute, DTU, Denmark
12.15 – 13.15	Lunch
13.15 – 13.40	Hygienic engineering guidelines in open equipment, Dr. Satu Salo, VTT Technical Research Centre of Finland
13.40 – 14.05	Zoning and hygienic integration, Prof. Alan Friis, National Food Institute, DTU, Denmark
14.05 – 14.30	Coffee/tea break
14.30 – 15.00	Hygiene control methods in food prosessing, Helvi Mustonen, Orion Diagnostica Oy, Finland
15.00 – 15.30	*Campylobacter* spp. detection in risk management, Mati Roasto, Estonian University of Life Sciences, Estonia

15.30 – 16.00	Coffee/tea break
16.00 – 16.30	In-place cleaning systems, Urban Wiik, JohnsonDiversey, Finland
16.30 – 17.00	The power of in-place cleaning tools in tank systems – Tank cleaning technology, Rene Elgaard, Alfa Laval Tank Equipment, Denmark
17.00 – 17.30	Cleaning agents & disinfectants in practice, Annika Jürgens, Greenclean OÜ, Estonia
18.00 – 19.30	Guided walking tour in old town & Dinner (at Restoran-Õlletehas Beer House (Dunkri 5))

Tuesday, May 5, 2009

8.40 – 9.00	Registration
9.00 – 9.25	Effectiveness of HACCP systems in egg production and distribution, Jana Ramus, CCIS, Slovenia
9.25 – 9.50	Food safety risk management in bakeries, Helen Ehavald, Fazer Bakeries AS, Estonia
9.50 – 10.15	Risk management in public catering establishments, Aija Melngaile, Latvian University of Agriculture, Latvia
10.15 – 10.45	Coffee/tea break
10.45 – 11.10	Risk management in a ready-to-eat meal factory, Asli Kisikkaya, Tübitak Marmara Research Centre, Turkey
11.10 – 11.35	Risk assessment of microbial contamination in carcass surfaces, František Šišák, VRI, Czech Republic
11.35 – 12.45	Introduction to group works on risk management - raw milk cheese by Gun Wirtanen - functional foods by Raivo Vokk - food production water supplies by Mehlika Borcakli - ready-to-eat food by Alan Friis
12.45 – 13.45	Lunch
13.45 – 16.30	Group works (coffee/tea served at 2.30–3.00 pm)
16.30 – 18.00	Preparation of presentations
19.00 – 22.00	Dinner

Wednesday, May 6, 2009

8.10 – 8.30	Registration
8.30 – 8.50	Risk management of functional foods, group 1
8.50 – 9.10	Risk management of ready-to-eat meals, group 2
9.10 – 9.30	Risk management of egg in pasta products, group 3
9.30 – 9.50	Risk management in catering, group 4
9.50 – 10.10	Coffee/tea break
10.10 – 10.30	Risk management of raw milk cheese, group 5
10.30 – 10.50	Risk management in food processing water supplies, group 6
10.50 – 11.00	Concluding remarks on the seminar
11.15 – 11.30	Introduction to the expert group meeting
11.30 – 12.00	Pilot case I – Cypriot food factories, Savvas Gennaris, Veterinary Services, Cyprus
12.00 – 13.00	Lunch
13.00 – 13.30	Pilot case II – Estonian dairies, Raivo Vokk, Tallinn University of Technology, Estonia
13.30 – 14.00	Pilot case III – model for ready-made meals, Hanne Løje, National Food Institute, DTU, Denmark
14.00 – 14.30	Pilot case IV – training and education needs, Alan Friis, National Food Institute, DTU, Denmark
14.30 – 15.00	Coffee/tea break
15.00 – 15.30	Pilot case V – Romanian bakeries, Alina Dobre, Institutul de Bioresurse Alimentare, Romania & Satu Salo, VTT
15.30 – 16.00	Pilot case VI – Poultry meat production in Slovenia, Prof. Sonja Smole Možina, Tina Tusar & Vanja Uhan, University of Ljubljana, Slovenia
16.00 – 16.30	Coffee/tea break
16.30 – 16.45	Pilot case VII – Traditional white cheese from Turkey, Dr. Mehlika Borcakli, Tübitak, Turkey
16.45 – 17.00	Summary report on workshop 1 – Detection and identification of harmful microbes, Dr. Ivan Rychlik, VRI
17.00 – 17.15	Summary report on workshop 2 – Microbial risk management in food processes, Hanne Løje, DTU
17.15 – 17.45	Summary of the day, Prof. Raivo Vokk, TUT

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Gun Wirtanen & Satu Salo (eds.)

Title

RISK MANAGEMENT BY HYGIENIC DESIGN AND EFFICIENT SANITATION PROGRAMS

Abstract

Hygiene in food processing factories is highly dependent on hygienic design of the process lines and equipment as well as on cleaning efficiency. Principles of hygiene design are simple but several faults appear in food processing premises. Hygienically designed process lines are supposed to be cleanable but still optimization of cleaning programs is worthwhile. Cleaning of complex equipment is very challenging. Choosing suitable cleaning agents and disinfectants for various food processing equipment and process environment requires knowledge about efficacy of chemicals and properties of surface materials. It is important to apply the knowledge of good cleaning practices in everyday cleaning routines in food factories and improve sanitation programs constantly.

The seminar on *risk management by hygienic design and efficient sanitation programs* was held in Tallinn (Estonia) 4th – 6th of May 2009. This final seminar of SAFOODNET EU-project (Food Safety and Hygiene Networking within New Member States and Associated Candidate Countries; FP6-022808-2006) provided information about hygienic design and cleaning procedures and concluded the pilot case studies performed during this 3-year-project. Participants were challenged to create risk management plans for different imaginary factories using all the knowledge gained in the workshops and seminars of the project. These groupworks interactively summarized the lectures and more importantly built firm network between group participants. The groupworks as well as participant abstracts on risk management are published in this publication.

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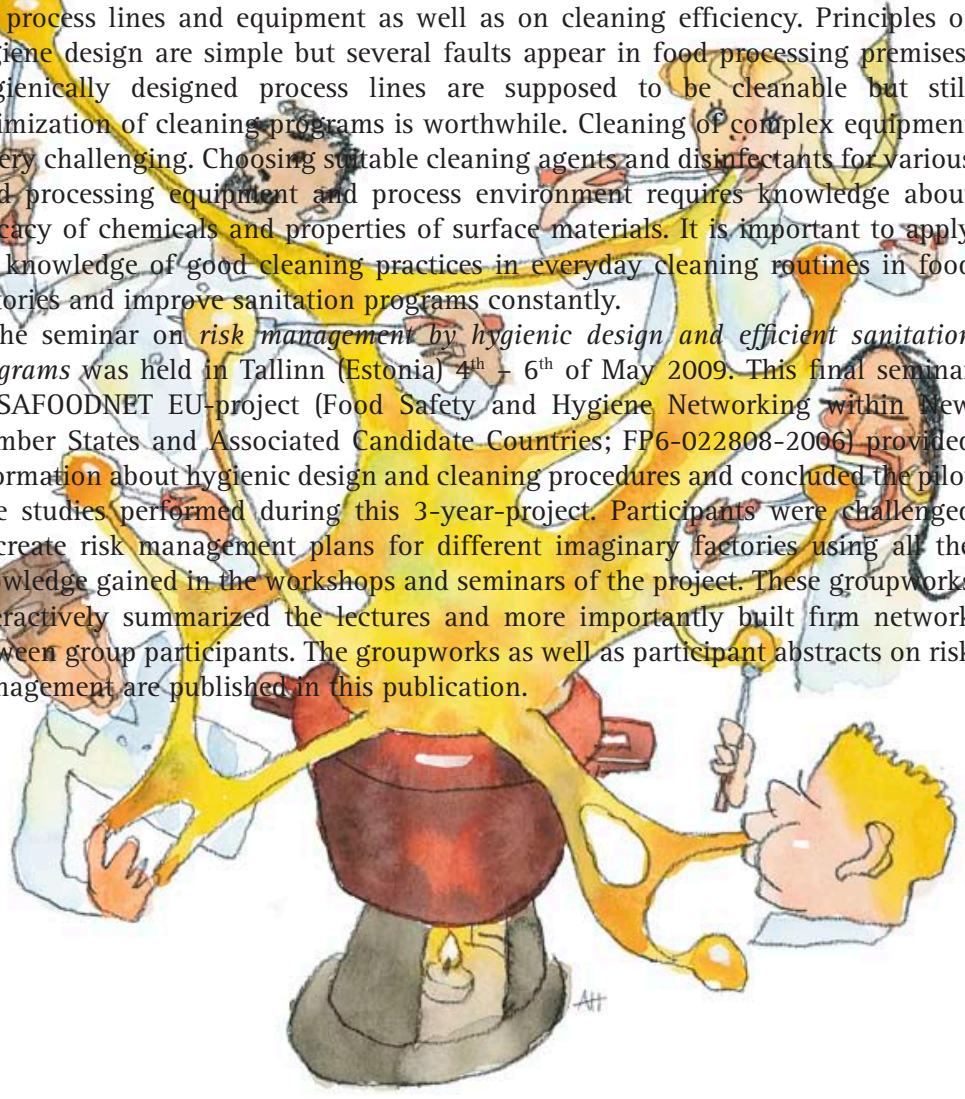
microbial risk management, food processing, preventive activities, cleaning, disinfection, equipment design, surface materials, layout. Good Management Practice, GMP, contamination routes, corrective actions, critical control points, HYGRAM, harmful microbes, pathogens, sampling, monitoring, training, documentation	VTT Technical Research Centre of Finland P.O. Box 1000, FI-02044 VTT, Finland Phone internat. +358 20 722 4520 Fax +358 20 722 4374

RISK MANAGEMENT BY HYGIENIC DESIGN AND EFFICIENT SANITATION PROGRAMS

Hygiene in food processing factories is highly dependent on hygienic design of the process lines and equipment as well as on cleaning efficiency. Principles of hygiene design are simple but several faults appear in food processing premises. Hygienically designed process lines are supposed to be cleanable but still optimization of cleaning programs is worthwhile. Cleaning of complex equipment is very challenging. Choosing suitable cleaning agents and disinfectants for various food processing equipment and process environment requires knowledge about efficacy of chemicals and properties of surface materials. It is important to apply the knowledge of good cleaning practices in everyday cleaning routines in food factories and improve sanitation programs constantly.

The seminar on *risk management by hygienic design and efficient sanitation programs* was held in Tallinn (Estonia) 4th – 6th of May 2009. This final seminar of SAFOODNET EU-project (Food Safety and Hygiene Networking within New Member States and Associated Candidate Countries; FP6-022808-2006) provided information about hygienic design and cleaning procedures and concluded the pilot case studies performed during this 3-year-project. Participants were challenged to create risk management plans for different imaginary factories using all the knowledge gained in the workshops and seminars of the project. These groupworks interactively summarized the lectures and more importantly built firm network between group participants. The groupworks as well as participant abstracts on risk management are published in this publication.



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SIXTH FRAMEWORK PROGRAMME

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