THE FUTURE OF FOOD SAFETY RESEARCH IN THE EUROPEAN UNION:

FOSARE SEMINAR SERIES 4

NOVEL PRESERVATION TECHNOLOGIES IN RELATION TO FOOD SAFETY

THURSDAY & FRIDAY JANUARY 22 –23, 2004

DORINT HOTEL
BOULEVARD CHARLEMAGNE 11-19, BRUSSELS

PROGRAMME, PROCEEDINGS AND PARTICIPANT LIST

SUPPORTED BY THE EUROPEAN COMMISSION QUALITY OF LIFE PROGRAMME UNDER KEY ACTION 1, FOOD AND NUTRITION AND HEALTH, CONTRACT NO. QLK1 2002 30480

INTRODUCTION

On account of a growing consumer demand towards foods that are safe, but retain the characteristics of fresh or freshly-prepared foods, mild preservation technologies are gaining more and more importance. Examples include high-pressure processing, pulsed electric fields treatment, light technologies, cold plasma, and use of biopreservatives. These mild preservation technologies enhance the shelf life of foods, are usually applied at room temperature and have a minor impact on the quality and fresh appearance of food products. They are referred to as mild since they pose little stress on foods. This on the other hand increases the importance of food safety considerations. Extended shelf life and a "fresh-like" product presentation emphasise the need to take full account of food safety risks, alongside possible health benefits to consumers.

The introduction of novel preservation methods stimulated research in microbiology, technology and food processing. The adoption of mild preservation technologies under European legislation is an ongoing process, as shown by the Novel Food Regulation.

Compared to a decade ago, research on mild preservation technologies has made a tremendous step forward. At this moment, novel technologies such as high pressure, pulsed electric fields and the use of biopreservatives are beyond the first development phase. Equipment is available on different scales and several process conditions have been described. Possibilities to use these technologies for preservation of food products are reported and effects on quality of food products become more and more known. Currently, some products are on the market (e.g. high pressure processed) or will be on the market in the near future (e.g. pulsed electric fields treated) (Ohlsson & Bengtsson, 2002).

In the fourth Seminar of the Safe Consortium, representative aspects of current research on mild preservation technologies were presented and discussed. The aim of the seminar was to promote the further integration of microbiology, preservation techniques, processing methods and food safety considerations related to mild preservation technologies. Table 1 shows the oral and poster presentations of the participants of the seminar. In this chapter, we will refer to these contributions to focus on the research topics discussed.

ORGANISED BY:

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SEMINAR PROGRAMME

Thursday January, 22, 2004

14.30 – 17.30	Arrival of participants and speakers: posters to be displayed
17.30 – 18.00	Informal drinks reception
18.00 – 19.30	Dinner
19.45 – 20.30	Developments in high-pressure processing in USA. Dr. Dan Farkas (Oregon State University, USA)
20.30 – 21.15	Hurdle approach for mild preservation. Prof. Chris Michiels (KU Leuven, Belgium)
21.15 -	Informal networking of young scientists and speakers

Friday, January, 23, 2004

Microbiology and mild preservation

Chair:	Dr. Paul Bartels
8.45 – 9.15	Food safety developments relating to mild preservation. Dr. Servé Notermans (TNO Nutrition and Food Research, The Netherlands)
9.15 – 9.45	Microbiology concerns with mild preservation. Prof. Pablo Fernandez (Universidad Politécnica de Cartagena, Spain)
9.45 – 9.55	The combined effects of high pressure and nisin on micro-organisms in milk. Elaine Black (University College Cork, Queens University Belfast, Northern Ireland)
9.55 – 10.05	(Nisin activity in meat) Virginia Stergiou (University of Surrey, UK)
10.05 – 10.15	Assessment of psychrotolerance features of *B. cereus.* Yvonne Thomassen (WCFS, Wageningen UR, The Netherlands)
10.15 – 10.25	Poster presentations 1-5
10.25 – 10.45	Poster presentations / Tea and coffee served

Novel technologies for mild preservation

Chair:	Dr. Dan Farkas
10.45 – 11.15	Overview of novel technologies. Prof. Dietrich Knorr (TU Berlin, Germany)
11.15 –11.45	Recent developments in high pressure relating to food safety. Dr. Michael Gänzle (TU München, Germany)

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11.45 – 12.15	Recent development in pulsed electric field treatment relating to food safety. Dr. Hennie Mastwijk (Wageningen UR, The Netherlands)
12.15 – 12.25	Paula Maria Periago Bayones (CEBAS-CSIC, Spain)
12.25 – 12.35	Optimization of pulsed electric field processing parameters and treatment chambers for liquid food pasteurisation. Stefan Toepfl (Berlin University of Technology, Germany)
12.35 – 13.00	Poster presentations 6-xx
13.00 – 14.15	Lunch/ Poster discussions continued

Processing and product aspects

Chair:	Dr. Servé Notermans
14.15 – 14.45	Hygiene processing in relation to mild preservation. Ing. Tineke Mostert (Unilever, The Netherlands)
14.45 – 15.15	Integrated concepts for mild preservation. Thomas Ohlsen (SIK, Sweden), Paul Bartels (Wageningen UR, The Netherlands)
15.15 - 15.30	Coffee/tea break/ Poster presentations
15.30 – 15.40	High pressure effects on microbial stability in low acid beverages. Roman Bukow (Berlin University of Technology, Germany)
15.40 – 15.50	Novel radio frequency water bath process and the consequences for quality of fish products. Dagbjorn Skipnes (Norconserv AS, Norway)
15.50 – 16.00	Comprehension of the effect of high hydrostatic pressures and sub-zero temperatures on cell-inactivation. Moussa Marwen (Université de Bourgogne, France)
16.00 – 16.10	Application of high pressure to frozen aqueous systems. Cornelius Luscher (Berlin University of Technology, Germany)
16.10 – 17.00	Round-up discussion, including comments from young scientists
17.00 – 18.00	Closure and drinks

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Table 1. Lectures and poster presentations at the 7th SAFE seminar

Name	Affiliation	Title
Key note lectures		
Farkas, D.	Oregon State University, USA	Developments in high-pressure processing in USA.
Fernandez, P.	Universidad Politécnica de Cartagena, Spain	Microbiology concerns with mild preservation.
Gänzle, M.	TU München, Germany	Recent developments in high pressure relating to food safety.
Mastwijk, H.C.	Agrotechnology & Food Innovations, Wageningen, The Netherlands	Recent development in pulsed electric field treatment relating to food safety.
Michiels, C.	KU Leuven, Belgium	Hurdle approach for mild preservation.
Notermans, S.	TNO Nutrition and Food Research, The Netherlands	Food safety developments relating to mild preservation.
Mostert, T.	Unilever, The Netherlands	Hygiene processing in relation to mild preservation.
Ohlsen, T, Bartels, P.V.	SIK, Sweden, Agrotechnology & Food Innovations, Wageningen, The Netherlands	Integrated concepts for mild preservation.
Lectures		
Black, E.	University College Cork, Queens	The combined effects of high pressure and nisin on micro-organisms in milk.

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	University Belfast, Northern Ireland	
Bukow, R.	Berlin University of Technology, Germany	High pressure effects on microbial stability in low acid beverages.
Luscher, C.	Berlin University of Technology, Germany	Application of high pressure to frozen aqueous systems.
Marwen, M.	Université de Bourgogne, France	Comprehension of the effect of high hydrostatic pressures and sub-zero temperatures on cell-inactivation.
Periago Bayones, P.M.	CEBAS-CSIC, Spain	Potential risk of *Yersinia enterocolitica* recontamination in ready-to-eat meals.
Skipnes, D.	Norconserv AS, Norway	Novel radio frequency water bath process and the consequences for quality of fish products.
Stergiou	University of Surrey, UK	Inhibition of nisin activity in meat.
Thomassen, Y.E.	WCFS, A&F, Wageningen, The Netherlands	Assessment of psychrotolerance features of *B. cereus.*
Toepfl, S.	Berlin University of Technology, Germany	Optimization of pulsed electric field processing parameters and treatment.
Poster presentations		
Boonman, M., Matser, A.M., Bartels, P.V., Van den Berg, R.W.	Agrotechnology & Food Innovations, Wageningen, The Netherlands	Improving the quality of fresh meat with mild high pressure technology.
Brindza, P.	Slovak University of Agriculture,	Evaluation of cornelian cherries.

	Nitra, Slovak Republic	
Chrysoula, T.	Institute of Technology of Agricultural Products, Greece	Effect of high pressure on spoilage micro-organisms isolated from traditional salads and ham products.
Cruz-Romero, M., Smiddy, M., Kerry, J.P., Hill, C., Kelly, A.L.	University College Cork, Ireland	Effects of thermal pasteurisation or high pressure processing on physical and biochemical characteristics of oysters.
De Angelis, M., Di Cagno, R., Huet, C., Crecchio, C., Fox, P.F., Gobetti, M.	Institute of Sciences of food production, Bari, Italy	Heat stress resistance in *Lactobacillis plantarum.*
Dimitrov, A.G.	Department MAHVP, Burgaria	High pressure in combination with high temperature treatment for food products.
Halkman, H.B., Kozat, P., Basbayraktar, V., Cetinkaya, N.	TAEA-Ankera Nuclear Research Center in Agriculture and Animal Sciences, Ankara, Turkey	To improve the safety and quality of minimally processed foods.
Jansen, O.	Groupe Armes et Metrologie Sensorielle, INPT-ENSIACET, Toulouse, France	Olfactory evaluation of flavour changes in high-pressure treated black truffles.
Jofre, A.	IRTA, Monells, Spain	Application of high hydrostatic pressure and biopreservatives as a new preservation alternative in cooked meat products.
Lechner, N.	Central Food Research Institute, Budapest, Hungary	Pulsed electric field processing effects on physical and chemical properties of vegetable juices.
Mamone, G.	Institute of Food Science and	Structural characterization of food proteins and their posttranslational

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	Technology, Avellino, Italy	modification by proteomic techniques.
Murchie, L.	Queen's University of Belfast, Northern Ireland	Effect of high pressure on microbiology of shellfish.
Nikolov Iliev, I.	Bulgary	Study on biologically active substances isolated from lactic acid bacteria from Bulgarian dairy products.
Nozkova, J.	Institute of Biodiversity and Biosafety, Nitra, Slovac Republic	Research on pumpkin seeds.
Schuten, H.K., Gulfo-Van Beusekom, K., Pol, I.E., Mastwijk, H.C., Bartels, P.V.	Agrotechnology & Food Innovations, Wageningen, The Netherlands	Mild preservation of fresh orange juice by pulsed electrical field treatment: enzymatic stability.
Tuboly, E., Farkas, L., Simon, A.,	Faculty of Food Science, Budapest, Bulgary	Changes of egg properties induced by high hydrostatic pressure or gamma irradiation.
Van Beek, J.	Agrotechnology & Food Innovations, Wageningen, The Netherlands	The MPN method is indispensable for evaluating spore inactivation by high hydrostatic pressure.
Voigt, D., Ananta, E., Zenker, M., Knorr, D.	Berlin University of Technology, Germany	Applicability of high-intensity ultrasound to inactivate *Escherichia coli* and *Lactobacillus rhamnosus* and to increase sensitivity against nisin.

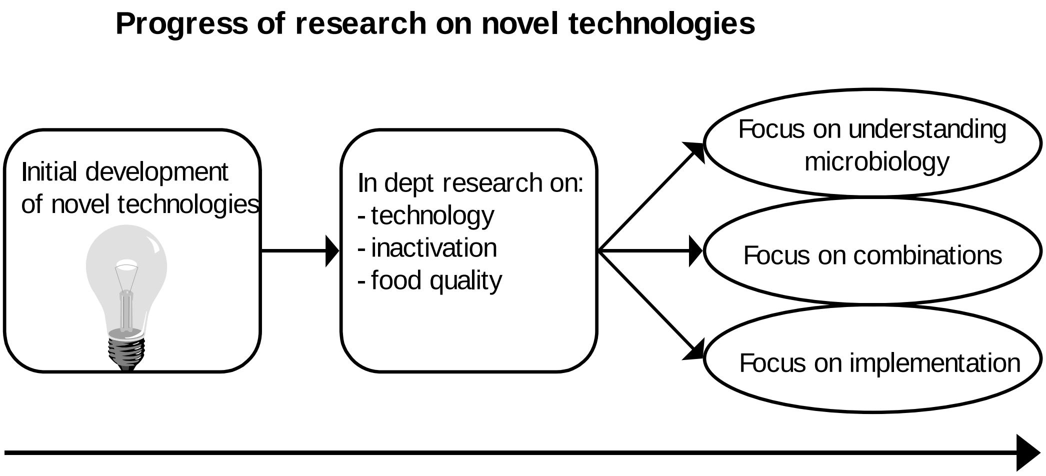

Figure 1. Development of the research on novel technologies.

From the contributions to this Seminar, we conclude that there are three major trends in the research on novel preservation technologies (Figure 1.). The first trend is related to understanding the effects of novel preservation technologies on micro-organisms. As these technologies are currently used or will be used in the near future for producing food products, focus on the mechanisms of inactivation of micro-organisms becomes crucial. Not only the amount of inactivation by a certain technology is important, but also aspects such as sublethal inactivation, differences between strains and between subpopulations, and stress adaptation.
The second trend is related to the use of combinations of technologies. The presentations at the Seminar demonstrated that more and more research is performed on combinations of novel technologies with other novel or existing technologies. Research on high pressure treatment at non-room temperature, such as high-pressure sterilisation (using elevated temperatures) or high-pressure shift freezing (using sub zero temperatures) was discussed. Another example is the use of biopreservatives in combination with high pressure or pulsed electric fields treatment. Using combinations of technologies broadens the range of possible applications of novel mild preservation technologies.
The third focus is on implementation of novel preservation technologies. This involves the growing attention on food safety issues regarding these technologies. Issues are not only related to microbiology but also aspects such as toxicology, for example corrosion of electrodes that are used during pulse electric fields treatment, are considered. Besides this, processing conditions of novel technologies are accordingly chosen, so safe foods are produced without the use of extreme conditions that could result in unwanted negative effects regarding quality. A final topic is the need for hygienic processing since the use of mild preservation technologies mostly results in pasteurised products which have to be stored at low temperatures untill consumption.
In this chapter, after a brief introduction to novel technologies, these three trends will be discussed in more detail demonstrating results of current research as presented by the various participants of the Seminar.

Introduction to novel technologies

High pressure processing

By subjecting foods to high pressures in the range of 400-800 MPa, micro-organisms and enzymes can be inactivated without degradation of flavour and nutrients, which is normally found with traditional thermal processing. High pressure processing is a relatively old technique. Hite (1899) already described that milk can be pasteurised by high pressure. The process is applied in the chemical industry for the production of polyethylene. Pressure

systems used for this application consists of pressure vessels of several hundreds litres and are loaded till 300 –400 MPa. Application of high pressure to food is reintroduced in Japan in the early 1990s.
At present, the technique is successfully used in Japan, United States and Europe for pasteurisation of food products such as citrus juices, guacamole, ham, oysters, rice and fruit desserts. Pressures up to 1000 MPa can be applied in liquids for food conservation and preparation. At these high pressures, macromolecules may be altered. Examples of processes taking place at these pressures are protein denaturation, lipid crystallisation and starch gelatinisation. Small molecules associated with flavours, colours and vitamins are not influenced (see figure 2).

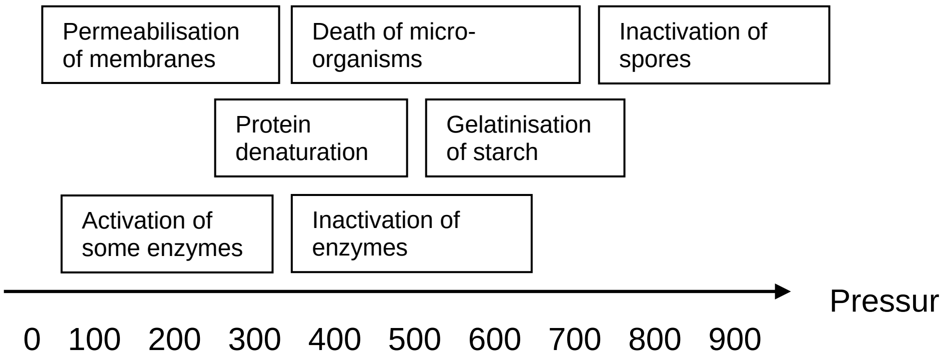

Figure 2. Indicative effects of high pressure on food components (high pressure treatment at roomtemperature).

High pressure processing is performed in a pressure vessel. The packed food is placed in a pressure fluid that is usually water. Additional fluid is pumped into the vessel, resulting in an increase in pressure till the desired end pressure has been reached. After a treatment time of 1 to 10 minutes, pressure is decreased and the product has been treated.
The pressure is applied isostatically. Therefore, pressure is uniform in the product and thus the entire product undergoes the same treatment. High pressure is non-thermal in principle, even though the pressure increase in itself causes a small adiabatic rise in temperature (Ohlsson & Bengtsson, 2002).
The main applications of high pressure processing are preservation and preparation. For preservation applications, enzymes and micro-organisms are inactivated under high pressure. This results in a preserved product while remaining fresh taste, flavour and colour. Examples are pasteurisation of fruit juices, fruit desserts and guacamole. For preparation applications, high pressure can be used to modify product characteristics. In Japan, for example, several rice-based foods are available with novel textures induced by high-pressure treatment. Other examples are gelatinisation of starches and proteins, preparation of pectin gels (jams) and tenderisation of meat.

Pulsed electric field treatment

Preservation of bulk products by pulsed electric field (PEF) is an emerging technology that opens new perspectives for the food and pharmaceutical industry. This process can be applied homogeneously through the product and is applicable for the pasteurisation of pumpable products at reduced temperatures. At field strengths in the order of 15-30 kV/cm, microbial

cells are inactivated by electroporation of their membranes without any considerable temperature rise and without changes in the chemical or physical composition of the food material. For adequate processing, a number of short pulses (1-100 µs) is used. Recently, the interest in this potential food preservation method has increased significantly and resulting in many international research cooperation (Ohlsson & Bengtson, 2002).
PEF treatment relies on the microbial action of intense electrical impulses on a microsecond time scale. When an electrical impulse is applied, the osmotic balance of the micro-organisms present is disturbed. If processing conditions are chosen correctly, micro-organisms are inactivated. A single pulse can establish reductions in plate counts exceeding 5 orders of magnitude.
The feasibility of PEF technology has been demonstrated. Preservation conditions have been established at temperatures less than 30^{o}C while the initial product quality was retained. Some examples include the PEF treatment of skim milk, resulting in an extended shelf-life. Other examples can be found in treatment of fruit juices. Treatment of fresh squeezed orange juice increased refrigerated shelf life to more that six weeks while loss of flavour compounds and vitamin C were decreased compared to traditional heat processing. For apple juice a 3-4 weeks extended shelf-life (at +4°C) was reached with PEF treatment (Ohlsson & Bengtson, 2002).

Other non-thermal novel technologies

High pressure processing and pulsed electric field treatment are both volumetric treatments that inactivate micro-organisms at any spot in the product. Beside these processes, there are some interesting novel surface treatment technologies for decontamination at room temperature. The first group of technologies is related to light technology and includes ultraviolet light and pulsed white light. Ultraviolet light processing involves the use of radiation from the ultraviolet region, ranging from 100-400 nm. The germicidal range, which inactivates bacteria and viruses, is located between 100-280 nm. Pulsed white light treatment involves the use of intense and short duration pulses of broad-spectrum "white light". The spectrum for pulsed light treatments includes UV and near infrared (80 % of the electromagnetic energy is within the range of 170-260 nm) (Ohlsson & Bengtsson, 2002). Both light treatments result in considerably inactivation of micro-organisms on surfaces without effects on the product quality. Since light technologies have the disadvantage of casting shadows, applications for rough surfaces can be difficult. Equipment for these technologies is now available.
A second surface technology is cold plasma. Plasma is a high-energy gas that is created when an electrical current is passed through a gas. A well-known application of this principle is fluorescent lighting. Until recently, plasmas could only be created at relatively high temperatures in a vacuum and the use of plasma in the agro-sector and the packaging industry, was therefore impractical. In the last few years technological breakthroughs have made it possible to produce cold plasma under atmospheric conditions. Thus, it is now possible to apply the plasma technology in the food industry.
Although plasma is a surface treatment it can reach shadow places since it is a gas and in this way, it differs from light treatments. Several gases can be used for creation of plasma. Noble gases are used when radical formation is unwanted while oxygen is used for the production of ozone in the plasma. Depending on the used gases, temperature, activity and stability of the plasma can be varied. No commercial equipment is available at the moment.

Focus on Microbiology

Modelling

Classic log-linear survival model

Behavior of microbial cells can be modeled in order to describe and/or predict microbial survival or growth. In the beginning of the 20th century methods for calculating thermal death times were developed and so modeling of microbial inactivation started. Traditionally it was assumed that inactivation of microbial cells and spores exposed to heat or another harsh environment were governed by first-order reaction kinetics (in analog with chemical reactions) (Figure 3A.). The now classic log-linear method of D and *z*-values (Stumbo, 1973) is based on this theory and is widely accepted and practiced. In this mechanistic model it is assumed that all cells have a similar (heat-) resistance and cell death occurs as a single critical event. The microbial inactivation is expressed by the following equation:

$$\log(N_t) = \log(N_0) - (1/D)t \tag{1}$$

where N_t is the number of cells at time t, N_0 is the initial cell concentration, and D is the decimal reduction time or D-value.

The decimal reduction time or D-value is the time needed for a 1-log (or tenfold) reduction of viable cells, thus independent of the population numbers. This D-value is dependent on temperature and can be coupled this parameter by introducing a second parameter: the z-value. The z-value is the temperature (T) change corresponding with a 10-fold change in D-value.

$$\log(D_1/D_2) = (T_2/T_1)/z \tag{2}$$

The D/z method has proven its value in food preservation for over 80 years, mainly in sterilization of canned products, but should we still use it? Upon consumers requests for higher quality foods preservation methods have become milder. Analysis of microbial inactivation resulting from these preservation methods revealed that many deviations from log-linearity occur. These deviations can lead to over- or underprocessing when linearity is assumed and may result in a decrease in product quality or food spoilage respectively. Simply assuming first-order kinetics of survival curves can therefore not be accepted anymore.

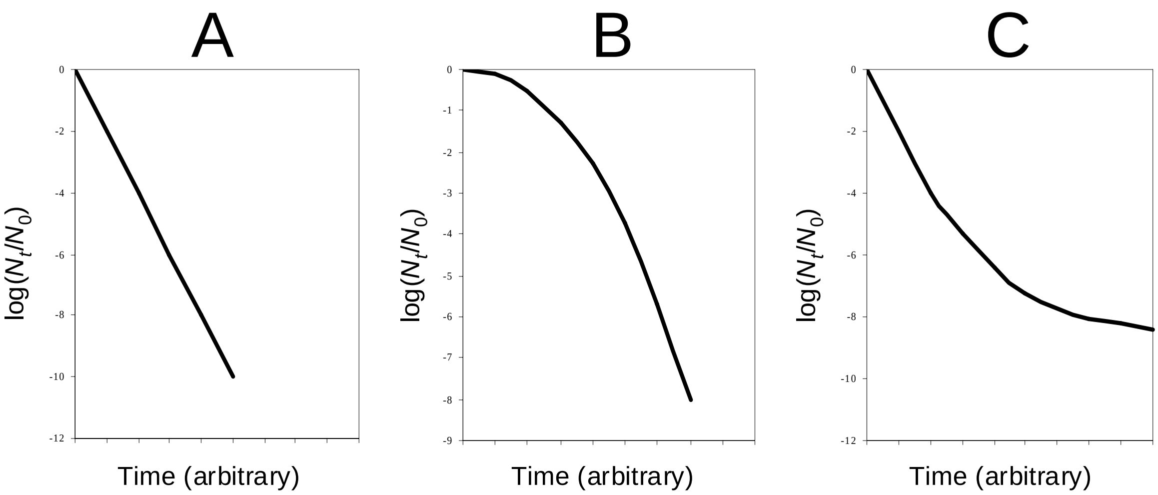

Figure 3. Semilogarithmic survival curves. A) Log-linear reduction; B) Lag or shoulder; C) Tailing

Modelling non log-linear survival curves

Activation of dormant spores and presence of subpopulations of differing resistance give rise to the so-called shoulders (Figure 3B.) and tails (Figure 3C.) appearing as deviations from log-linearity in survival curves (Heldman and Newsome, 2003). In the last decade several models have been developed to describe non-linear survival curves. An overview of these models can be found in reviews covering this subject (Geeraerd et al., 2000; Smelt et al., 2002; Whiting and Buchanan, 2001). Here we will discuss a probabilistic approach to model non-linear survival curves.

In contrast to mechanistic models, probabilistic models are not based on presumed molecular or physical mechanisms but on variability. This can be justified since biological systems are very complex and it is not likely that all cells behave in the same way or that cell death is the cause of a single event. Consequently a lethal event is considered a probabilistic event. Peleg and colleagues (Peleg and Cole, 1998) developed a probabilistic model using the Weibull distribution to cope with heterogenity.

$$\left(N_t / N_0\right) = e^{-\left(t/a\right)^n} \tag{3}$$

Where a is the scale and n is the shape. The parameters a and n can be estimated when fitting Eq. (3) to survival data.

The use of the Weibull model has been thoroughly investigated using many available data sets on heat inactivation (Peleg and Cole, 1998; van Boekel, 2002) and it performs much better than the classic log-linear model. It a flexible yet simple model that can be used to describe microbial heat inactivation (Fernandez et al., 1999; Peleg and Cole, 1998), but also pulse electric field (Peleg, 1995) and high pressure inactivation (Heinz and Knorr, 1996).

Arguments against the Weibull model are on one hand that the parameters a and n (Eq. 3) have to be estimated for each specific process and on the other hand the fact that the Weibull model is an empirical model. This implies that it is based on statistical distribution and that the specific mechanisms underlying cell physiology are not taken into account.

Predictive modelling

Predictive modeling is regarded as the solution for microbial safety. Several papers describe predictive models for food preservation (see for instance (Fernandez et al., 2001)). These models can provide useful information on microbial behavior without experimental effort. Although they can be used within the limits of the model, extrapolations or true predictions are not always allowed or correct.

The complexity of food and the range of microorganisms create many variables and formulas to incorporate in a model. The present models are not valid for all foods because they do not take all the variables into account. It is even doubtful that it is feasible to obtain models for all conditions. Besides this, models might be based on actual data that are not accurate enough. For example surviving cells have been enumerated using plate counts instead of a most probable number (MPN) method in which microorganisms with a delayed outgrowth are also enumerated. This may lead to an underestimation of the number of survivors.

In the future new tools should be developed to describe the potential hazards with a huge variety of products and formulations. In these models a precise description of the behaviour of the mircoorganisms should be accounted for. However, the development of a precise or complete mechanistic model requires more information on the physiology of cell populations as well as single cell behavior.

Microbial behaviour in preservation

Survival of microorganisms that have been exposed to the stress of preservation technology can be the result of three main systems. Firstly, the resistance is induced and the survival is a cause of intrinsic properties. Secondly, the resistance is genetically acquired through mutation and selection. And thirdly, survival of microorganisms is the result of interactions between the microorganisms and the environment. This section will discuss these survival systems in relation to food preservation techniques.

Build-in resistance mechanisms

Bacterial cells have many mechanisms at their disposal that allow them to adapt to a change in environment. These mechanisms range from the drastic sporulation which is found in *Bacilli* to the more transient production of proteins that repair damage, maintain the cell or eliminate the stress agent. Here we will not provide a comprehensive molecular overview on bacterial stress responses and the reader is referred to the books of (Storz and Hegge-Aronis, 2000) and (Yousef and Juneja, 2003), but we will discuss the meaning of bacterial stress responses for preservation technologies.
Throughout the food chain bacteria encounter various stresses, ranging from temperature changes in the fields to the cooking during food preparation at home. Preservation technologies themselves are stresses and thus invoke a stress response (e.g. De Angelis, Table 1). In Table 2 a listing of several stresses in the food chain and their corresponding stress responses is given. Although for most stress types a specific stress response mechanism is present in cells, nearly all stresses invoke activation of the general stress response and many stress mechanisms can contribute to protection against other stress types. For instance mild heat shock prior to high-pressure treatment results in more survivors (Aertsen et al, 2004). On the other hand, the different stresses can have a synergistic effect. Cells can be killed by a combination of heat and high pressure while the heat or pressure treatment alone would not suffice (Patterson and Kilpatrick, 1998).
When designing preservation conditions using a hurdle approach, it is important to keep in mind the molecular basis of stress responses to gain optimal benefit of each mild stress. Moreover the molecular basis of new technologies such as pulse electric field should be investigated in order to predict the occurrence of resistance.

Table 2. Stresses in the environment, foods, food preparation and cooking and responses to such stresses (adapted from (Yousef and Juneja, 2003))

Stress	Relevance to food	Damage	Stress responses, cross responses	Induced gene products
Acidity	Occurs in aquatic environment* and in food production	DNA, outer membrane	Acid tolerance[1]; alkali sensitivity, UV resistance, salt tolerance, thermotolerance, H_2O_2 tolerance, resistance to polymixin B	Hyd genes[2] RpoS PhoP Heat shock proteins Lysine[3] or arginine decarboxylase
Acid pH + weak acids	In acidic or acidulated foods	DNA, outer membane, cytoplasmic membrane	Acid tolerance[1]	Fur AhpC Heat shock proteins
Weak acids at pH 7.0	In certain foods	Outer membrane, cytoplasmic membranes	Acid tolerance[1]	

Alkalinity	In egg-white	DNA, outer membane, cytoplasmic membrane	Alkali tolerance, Thermotolerance Resistance to UV Acid sensitivity Alkylhydroperoxide tolerance	AhpC Heat shock proteins NhaA
Heat	During food production, preparation and cooking	DNA, outer membranes, ribosomes	Thermotolerance Acid tolerance[1] Alkali tolerance UV tolerance HHP tolerance	Heat shock proteins
Cold	Refrigeration	RNA	HHP tolerance	Cold shock proteins
Osmotic stress (low a_w)	In many foods with high level of salt or sugars	Outer membrane, transport processes	Osmo-tolerance Tolerance to oxidative components Thermotolerance HHP tolerance	Heat shock proteins ProP, ProU etc
Salt shock	Food containing high [NaCl]	Effects on enzyme activity and on protein synthesis	Acid sensitization	NhaA PhoE
Starvation[4]	In contaminated waters, if used for food processing	Proteins[5]	Thermotolerance Acid tolerance Alkali tolerance Salt tolerance H_2O_2 tolerance Osmotolerance HHP tolerance	Proteases Heat shock proteins
Exposure to Cu^{2+}	In contaminated waters	DNA, proteins	Cu^{2+}-tolerance Thermotolerance Acid tolerance Alkali tolerance Cd^{2+}-tolerance	
Irradiation	Preservation	DNA	UV-tolerance Thermotolerance Acid tolerance[6] Alkali tolerance	Heat shock proteins UvrA,B,C RecA LexA
High hydrostatic pressure	Preservation	Outer membrane, cytosolic membrane, ribosomes, proteins	Thermotolerance Acid sensitization Sensitation to biopreservatives	Heat shock proteins Cold shock proteins proteases
Pulse electric field	Preservation	Cytoplasmic membrane	Not known	

[*] Relevant to food in contaminated water is used in production or preparation.
[1] Tolerance to inorganic acid
[2] Anaerobiosis and formate also needed
[3] Lysine and anaerobiosis needed
[4] For carbon

[5] A major effect of starvation is degradation of many proteins
[6] Irradiation induces acid tolerance and UV can activate and acidity ESC

Genetically acquired resistance-Differences between strains and in strains, tailing, subpopulations

Different microbial species demonstrate a different survival when exposed to preservation technologies, as it was long known for treatments such as temperature. Not unexpectedly, treatments such as high hydrostatic pressure result in differences in survival as well. Generally, Gram-positive bacteria are more resistant towards heat and pressure than Gram-negative bacteria (Smelt, 1998). And different strains from a single species can demonstrate a widely varying pressure resistance (Alpas et al., 1999). However, it is more important that cells of the same population can exhibit differences in, for instance, pressure resistance (Hauben et al., 1997; Karatzas and Bennik, 2002). These differences are either physiological as discussed above or ar based on genetic differentiations, resulting in more resistant subpopulations causing for instance tailing in the survival curves.
DNA-mutations that allow enhanced bacterial survival can be distinguished in two types: spontaneous and adaptive (or directed) mutations. Spontaneous mutations occur mostly in exponential growing cells, when all intracellular activities, and thus also the replication of the genome, are at high speed. Often these mutations allow survival to the encountered stress but are not beneficial to the bacterial populations in the end.
On the other hand, adaptive mutations occur in stressed, starving, non-growing, or slowly growing cells. Some of these mutations allow resistance towards one of more stresses and so permit growth. A key role in adaptive mutation is played by the general stress response transcription factor RpoS (σ^S), DNA polymerase IV and the SOS response (a DNA damage response) (Lombardo et al., 2004; McKenzie et al., 2000; McKenzie et al., 2001).
One of the known adaptive strategies of pathogenic bacteria is the generation of genetic variation at individual loci, also known as the "contingency loci". These loci are controlled by simple repetitive DNA sequences that are mutated with high frequencies (Bayliss et al., 2001). The *ctsR* gene of *L. monocytogenes* contains such a repetative DNA sequence, and natural mutation causes deletion of a 3 bp region resulting in a barotolerant mutant (Karatzas et al., 2003).
Occurrence of mutations is natural, but using mild stresses to preserve food allows induction and selection of more resistant microorganisms. Besides it allows different types of organisms to become a problem in food spoilage or poisoning. An example is the appearance of cold-tolerant strains in dairy products and ready-to-eat meals that are stored at low temperature after mild treatments.
In relation to genetic diversity the question of which species or strain to use when validating a new preservation technology arises as well. Till now a 12-log reduction of *Clostridium botulinum* spores was standard for claiming sterilization and a 6-log reduction corresponds with pasteurization. This was based on heat treatment but other preservation technologies might need a different standard for validation.

Environmental influences on resistance

Chemical agents or biopreservatives are being used widely in food preservation. Bacteriocins such as nisin are broadly studied to investigate their way of action, possibilities, and limitations. But, food components can have an antibacterial effect as well. Louis Pasteur already described the antibacterial effect of garlic in 1858. Unfortunately, food components can also have an opposite effect on preservation techniques such as temperature and pressure.
The relation between the food matrix and/or the food ingredients on resistance of microorganisms towards temperature or pressure treatment is very complex. Studies both on foods and model media have been performed to assess the mechanisms underlying this

resistance. Milk for example protects microorganisms against pressure treatment (Patterson et al., 1995). But whether this is due to the protective effect of calcium (Hauben et al., 1998), low water activity (a_w) (Oxen and Knorr, 1993) or proteins in general (Simpson and Gilmour, 1997) is difficult to determine. Most likely it is a combination of all the above.

Several studies demonstrated that low water activity increases resistance of microorganisms towards stress. Proteins, carbohydrates, oil and salts can offer some protection against pressure treatment (Oxen and Knorr, 1993; Simpson and Gilmour, 1997; Van Opstal et al., 2003). However, not all solutes offer equal protective effects. Salts result in less protection than carbohydrates and some carbohydrates are especially protective, i.e. trehalose. The subsequent recovery after a low water activity stress in 2% salt was lower than without salt (Patterson et al., 1995) while recovery in 40% sucrose was not inhibited (Van Opstal et al., 2003).

Acidic pH can be used to inhibit microbial growth, but acidity also plays a role during heat or pressure treatment. Both temperature and pressure treatment result in a decrease of the pH during treatment while the buffer or food system influences the extent of the pH change. Since the cells suffer a similar treatment, their internal pH is influenced following similar rules. Additional effects are observed due to the damage caused by the treatment. Molina-Gutierrez *et al* (Molina-Gutierrez et al., 2002) have demonstrated that upon sublethal pressure treatment the internal pH decreases to the level of the external pH. This effect may be assigned to increased membrane permeability or inactivation of proteins involved in pH homeostasis. Acidic food products, such as fruit juices, not only have pH benefits during pressure treatment; also outgrowth of survivors is inhibited due to the low pH (Garcia-Graells et al., 1998).

The compounds that can play a role in increased resistance towards preservation stresses are for instance lysozyme and calcium. Although lysozyme has an antibacterial effect against *E. coli*, recovery of *C. botulinum* spores is enhanced (Peck and Fernandez, 1995). Calcium and also other metal ions (Mg^{2+}, Mn^{2+}, Fe^{2+}) can have an protective effect against pressure but other metal ions (Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}) stimulate pressure-killing (Hauben et al., 1998).

Analysis of the influence of the environment, or food matrix, on surival is complex, yet the knowledge is needed when milder preservations are wanted.

Outlook on microbiology in relation to food preservation

Understanding microbial behavior at a molecular level allows assessment of microbial responses to environmental conditions, i.e. preservation conditions, beyond the level of live or dead. This triggers an impulse to the development of predictive models that can be trusted beyond the measured values. The genome of many of the food pathogens has been sequenced (Table 3), and recently the first genomics papers in relation to food preservation technologies have been published (Fernandes et al., 2004; Iwahashi et al., 2003; Quillardet et al., 2003). The use of these new "-omics" techniques allows a better understanding of microbial physiology. The next challenge will be integration of this knowledge into a predictive model.

Table 3. Sequenced pathogenic microorganisms

Organism	Reference
Bacillus cereus	(Ivanova et al., 2003)
Brucella spp.	(DelVecchio et al., 2002)
Campylobacter jejuni	(Parkhill et al., 2000)
Clostridium botulinum	Sanger institute
Escherichia coli O157:H7	(Hayashi et al., 2001)
Listeria monocytogenes	(Glaser et al., 2001)
Salmonella spp.	(McClelland et al., 2001)
Shigella spp.	University of Wisconsin
Staphylococcus aureus	(Kuroda et al., 2001)

Streptococcus, Group A	(Ferretti et al., 2001)
Vibrio spp.	(Heidelberg et al., 2000)
Yersinia enterocolitica	Sanger institute

For a complete and up-to-date list of sequenced genomes see
http://www.ncbi.nlm.nih.gov/genomes/MICROBES/Complete.html

Focus on combinations

High pressure, pulsed electric fields, biopreservatives and radio frequency heating are examples of novel technologies, which are currently used or will be used in the near future for preservation of food products. In general these technologies are effective in inactivating vegetative cells of bacteria, yeasts, and fungi. However, inactivation of bacterial spores and for some technologies also enzyme inactivation remains difficult using these technologies alone. To extend the use of non-thermal processing technologies in the food industry, combinations of these technologies with traditional or emerging food preservation techniques are interesting (Raso & Barbosa-Cánovas, 2003).
An often-made combination used is cold storage after a (non-thermal) pasteurisation treatment. Also other consecutive or simultaneous combinations are interesting to broaden the application range of these technologies. Examples include combinations of novel technologies with elevated temperature, lowered temperature and/or biopreservatives. In general, these combinations may result in an enhanced shelf life combined with a good preservation of product quality. However, as discussed above, it is important to keep in mind the molecular basis of microbial stress responses to gain optimal benefit of each mild stress. This section will discuss some successful combinations of novel technologies with high or low temperatures and with biopreservatives.

Combinations with heat

High pressure processing at room temperature is commercially applied for pasteurisation of food products. However, these conditions do not inactivate bacterial spores and some enzymes are only partially inactivated. So, most of the current commercially available high-pressure treated products have to be stored at low temperature. Using elevated temperatures in combination with high pressure processing can overcome these limitations, resulting in a sterilised product (Dimitrov, Table 1). In general, sterilisation by high pressure is possible by starting high-pressure treatment at temperatures of 60-90°C, the subsequent adiabatic compression causes rapid heating to higher temperatures. High-pressure sterilisation is a combined process in which both pressure and temperature contribute to sterilisation by the inactivation of spores and enzymes. The result is a shelf stable product. In many cases a higher general quality is obtained compared to products sterilised via conventional heat sterilisation (De Heij et al, 2003, Matser et al, 2004).
Introduction of high-pressure sterilised food products to the market requires validation of the safety of the process. For heat sterilisation, 12D inactivation of *Clostridium botulinum* is used as the target process. However, it is unknown if this is also a suitable validation method for high-pressure sterilisation. The first question regards the target micro-organisms that should to be used to validate high-pressure sterilisation processes. Various contributors demonstrated that there can be a large strain to strain variation in pressure resistance. Moreover, a direct correlation between heat and pressure resistance of spores is not always found.
Another topic to be dealt with is the method that should be used for determination of the inactivation rates of high pressure treatments. Depending on the conditions chosen, high pressure can result in sublethal inactivation of micro-organisms. When using plate count methods, spores with long lag times for germination are not detected and the number of inactivated spores is therefore overestimated. Other methods, such as the most probable number MPN method, are therefore necessary (Van Beek, Table 1).

High temperature can also be used in combination with pulsed electric field treatment. Pulsed electric field treatment at room temperature is not entirely a non-thermal process, as some heat will also be generated from electric currents, depending on e-fields and the electric conductivity of the food. The treatment time is usually only a matter of seconds and heat generation will have limited influence on the killing effects of the treatment. PEF treatment can also be done at higher temperatures. This enhances the inactivation rate of micro-organisms, and especially the inactivation rate of enzymes (Toepfl, Mastwijk, Table 1; Heinz et al, 2003). To lower processing costs part of the heat can be recovered to reduce the energy input of the process.

Combination with low temperatures

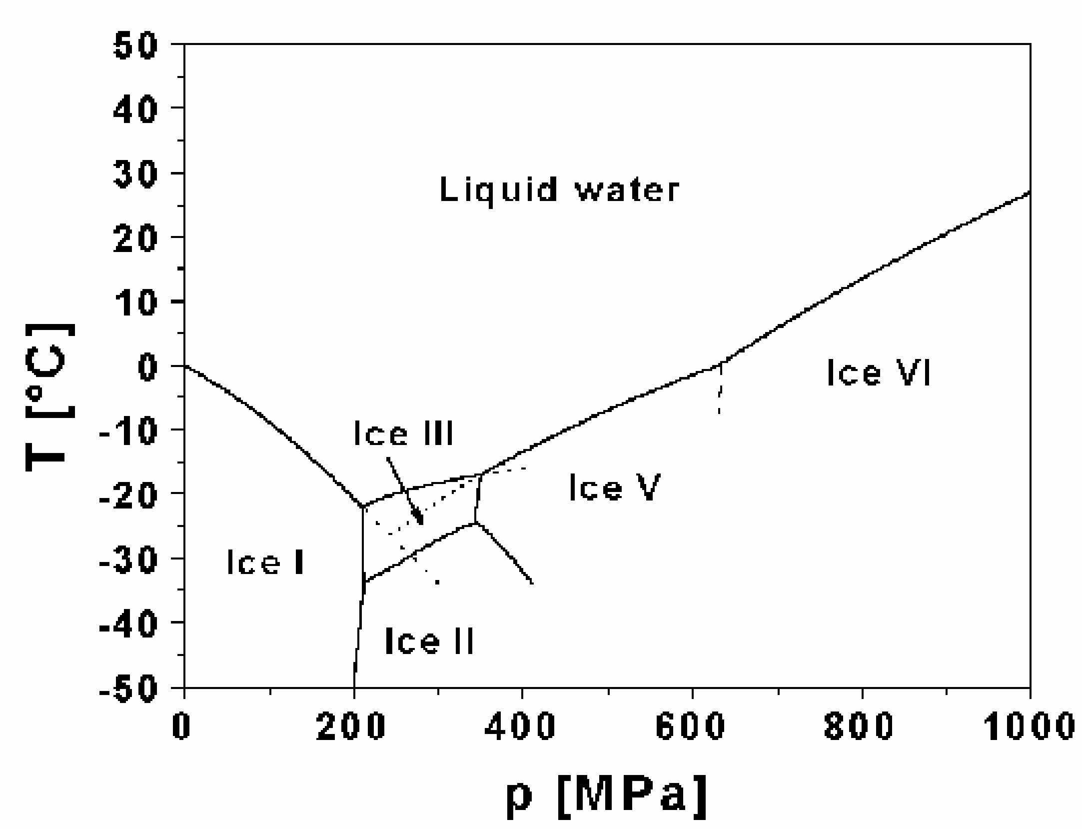

Figure 4 Phase diagram of water (adapted from Bridgman, 1911)

Just as high temperatures enhance the efficiency of mild preservation methods, the use of sub zero temperatures can also be an option. Especially considering the combination with high pressure is interesting as pressure influences the phase diagram of water (Figure 4.). Increased pressure results in a lower freezing point, allowing fluid water at temperatures below zero. Decreased temperatures or increased pressures result in formation of various ice phases and temperature or pressures changes can result in ice phase transitions.

There are several interesting applications of these effects. A well-described application is pressure shift freezing. With these pressure-temperature combination it is possible to super cool the product resulting in an instantaneous ice formation when pressure is released. This leads to formation of many small ice crystals, which has a positive effect on the texture of a product. Several contributors evaluated the effect of the super cooling domain on the

inactivation rate of micro-organisms, demonstrating an enhanced inactivation (Marwen, Table 1; Denys et al, 2002).
Other possibilities are the use of different ice phases as preservation or processing method (Luscher, Table 1). But not much is known on this topic and questions such as "What will happen with food and micro-organisms in the different ice phases?" or "Is inactivation possible in these domains?" remain to be answered. Ice phase transitions will result in density changes, which can probably result in inactivation of micro-organisms and so it can have a positive effect on shelf life. However, effects on product quality and texture in particular have to be evaluated, just as the technical and economical feasibility of the process.
A specific application of high pressure at low temperature, is the use of relatively low pressures (150-200 MPa) to enhance the shelf life of fresh meat. By processing at low temperatures (in this application in the region of 0-5°C), a high pressure treatment result in a preservation of the fresh red colour of meat combined with a elongation of the shelf life due to microbial growth (Boonman, Table 1).

Combinations with biopreservatives

Combinations with biopreservatives can enhance the efficiency of mild preservation technologies. Examples are the use of essential oils (e.g. carvacrol or thymol) and bacteriocins (e.g. nisin) (Jofre, Nikolov Iliev, Voigt, Bukow, Table 1). Nisin is the only bacteriocin that has been approved by the WHO to be used as food preservative. It has been successfully used to suppress the growth of pathogenic and spoilage micro-organisms. However, combining it with other preservation technologies can enhance its practical application. Research demonstrated that pulsed electric field treatment acts synergistically with nisin in reducing the viable count of vegetative cells of *Bacillus cereus* (Pol et al, 2000). Moreover, high pressure and nisin act synergistically to effectively inactivate both Gram-positive and Gram-negative bacteria in milk. In some food matrices however, the activity of nisin is limited due to interactions with food components. For instance the application of nisin in meat is limited, probably due to reaction of nisin with glutathione (Black, Stergiou, Table 1; Rose et al, 2002).

Focus on implementation

Novel food technologies such as high pressure and pulsed electric field treatments are used or will be used in the near feature for commercial applications. So, research is needed to evaluate the effects on food quality of high pressure processing (e.g. Crysoula, Cruz-Romero, Jansen, Murcie, Tuboly, Table 1) or pulsed electric field processing (e.g. Lechner, Schuten, Table 1). This implies also that issues such as safety and legislation have to be considered. In the production of acceptable, safe foods for human consumption, a generic framework for setting food safety criteria has been gradually established (Figure 5.). This framework is based on an internationally agreed food safety policy that comprises certain general rules. An important principle is the ALARA principle, which means that levels ‘as low as reasonably achievable’ are required. For most chemicals, concentrations below the no-effect level are considered to provide an appropriate safety level . The subsequent risk assessment and management procedure, which follows the requirements of the Codex Alimentarius Commission, results in the final food safety objectives. These objectives must be incorporated in the relevant food production process using Good Manufacturing Practices (GMP) and the Hazard Analysis Critical Control Point concept (HACCP). Important is the food safety at the point of consumption, which implies the importance of the whole chain of the food process, including the role of the consumer. (Notermans, 2003).

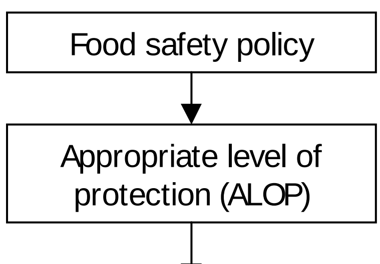

Figure 5. Generic framework of current food safety systems (based on Notermans, 2003)

The legislation of novel food technologies is incorporated in the Novel Food Regulation (Anon., 1997) and includes new production processes. These processes are not used on a significant scale before introduction to the Novel Food Regulation and result in significant differences in composition or structure of the food products. Central in this regulation is the concept of substantial equivalence. If a new process results in products, which are not substantial equivalent with common products, the Novel Food Regulation has to evaluate the process on safety issues such as microbiology, toxicology and allergy. In 2001, the EU authorized the Groupe Danone to launch high pressure pasteurised fruit-based preparation (Anon., 2001).
It is expected that the first pulsed electric field treated products will be on the market in one to two years. A special issue for the safety of pulsed electric field treatment is the possible toxicity due to metal release as a result of electrode degradation. However, presentations of Mastwijk and Toepfl on the Seminar (Table 1) showed that well designed and engineered pulsed electrical field systems result in metal release of less than 0.5 µg/kg product.
Mild preservation technologies have in common that they are often limited in the type and number of micro-organisms killed. For example, pulsed electrical field treatment does not inactivate bacterial spores and high pressure inactivates spores only if applied at elevated temperatures. The implementation of mild preservation technologies requires special attention to the quality of raw materials, optimisation of process conditions, hygiene of process lines, high quality packaging (pack integrity, packing under modified atmosphere) and specific storage conditions (e.g. Periago Bayones, Table 1). Good hygienic design is essential to prevent the contamination of minimal processed products with substances that would adversely affect shelf life or the health of the consumer. This contamination might be microbial (e.g. pathogens), chemical (e.g. lubricating fluids, cleaning chemicals) and physical (e.g. glass). Processing skills are essential and especially hygienic engineering of the equipment is essential. Basis hygienic design requirements include the construction material, surface finish, joints, fasteners, drainage, internal angles and corners, dead spaces, bearings and shaft seals, instrumentation, doors, covers and panels, and controls (Mostert, Table 1; Lelieveld et al, 2003).

Conclusions

Novel preservation technologies are an interesting option to produce high quality food products with an extended shelf life. For introduction of technologies such as high pressure processing or pulsed electric field treatment it is essential that the quality is better than the quality of conventional processed food products. The starting point of an evaluation of the possibilities of novel technologies will therefore be the effect on quality combined with the safety of the product after processing. Research on the effect on microorganisms of mild preservation technologies resulted in an increased knowledge of microbiology related to preservation in general. This knowledge is essential for mild preservation technologies because these technologies are often performed with a smaller safety margin compared to for example conventional heat sterilisation. This Seminar demonstrated that several research groups are working on topics related to the safety and implementation of novel technologies including extended microbial research, hygiene and safety issues.
Using simultaneous or consecutive combinations of technologies can broaden the application of novel technologies. Especially using higher or lower temperatures than room temperature is an interesting option to increase the effectiveness of novel technologies. The Seminar included also an increased interested in combinations with biopreservatives that enhances the effect of novel technologies.

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References

Anon. Novel Food Regulation, Regulation N° 258/97, 1997.

Anon. Commission Decision N° 2001/424/EC, 23 may 2001.

Aertsen, A., Vanoirbeek, K., De Spiegeleer, P., Sermon, J., Hauben, K., Farewell, A., Nystrom, T., Michiels, C.W. (2004) Heat Shock Protein-Mediated Resistance to High Hydrostatic Pressure in Escherichia coli, Appl. Environ. Microbiol., 70, 2660-2666.

Alpas, H., Kalchayanand, N., Bozoglu, F., Sikes, A., Dunne, C.P. and Ray, B. (1999) Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. Appl Environ Microbiol, 65, 4248-4251.

Bayliss, C.D., Field, D. and Moxon, E.R. (2001) The simple sequence contingency loci of Haemophilus influenzae and Neisseria meningitidis. J Clin Invest, 107, 657-662.

Bridgman, 1911

De Heij, W.B.C., Van Schepdael, L.J.M., Moezelaar, R., Hoogland, H., Matser, A.M., Van den Berg, R.W. (2003) High-pressure sterilization: maximizing the benefits of adiabtic heating. Food Technology, 3, 37-41.

DelVecchio, V.G., Kapatral, V., Redkar, R.J., Patra, G., Mujer, C., Los, T., Ivanova, N., Anderson, I., Bhattacharyya, A., Lykidis, A., Reznik, G., Jablonski, L., Larsen, N., D'Souza, M., Bernal, A., Mazur, M., Goltsman, E., Selkov, E., Elzer, P.H., Hagius, S., O'Callaghan, D., Letesson, J.J., Haselkorn, R., Kyrpides, N. and Overbeek, R. (2002) The genome sequence of the facultative intracellular pathogen Brucella melitensis. Proc Natl Acad Sci U S A, 99, 443-448.

Denys, S., Schluter, O., Hendrickx, M.E.G., Knorr, D. Effects of high pressure on water-ice transitions in foods. In Ultra high pressure treatments of foods, eds. M.E.G. Hendrickx, D. Knorr. Kluwer Academic, New York, 2002.

Fernandes, P.M., Domitrovic, T., Kao, C.M. and Kurtenbach, E. (2004) Genomic expression pattern in Saccharomyces cerevisiae cells in response to high hydrostatic pressure. FEBS Lett, 556, 153-160.
Fernandez, A., Ocio, M.J., Fernandez, P.S. and Martinez, A. (2001) Effect of heat activation and inactivation conditions on germination and thermal resistance parameters of Bacillus cereus spores. Int J Food Microbiol, 63, 257-264.

Fernandez, A., Salmeron, C., Fernandez, P.S. and Martinez, A. (1999) Application of a frequency distribution model to describe the thermal inactivation of two strains of Bacillus cereus. Trends in Food Science & Technology, 10, 158-162.

Ferretti, J.J., McShan, W.M., Ajdic, D., Savic, D.J., Savic, G., Lyon, K., Primeaux, C., Sezate, S., Suvorov, A.N., Kenton, S., Lai, H.S., Lin, S.P., Qian, Y., Jia, H.G., Najar, F.Z., Ren, Q., Zhu, H., Song, L., White, J., Yuan, X., Clifton, S.W., Roe, B.A. and McLaughlin, R. (2001) Complete genome sequence of an M1 strain of Streptococcus pyogenes. Proc Natl Acad Sci U S A, 98, 4658-4663.
Garcia-Graells, C., Hauben, K.J. and Michiels, C.W. (1998) High-pressure inactivation and sublethal injury of pressure-resistant Escherichia coli mutants in fruit juices. Appl Environ Microbiol, 64, 1566-1568.

Geeraerd, A.H., Herremans, C.H. and Van Impe, J.F. (2000) Structural model requirements to describe microbial inactivation during a mild heat treatment. Int J Food Microbiol, 59, 185-209.
Glaser, P., Frangeul, L., Buchrieser, C., Rusniok, C., Amend, A., Baquero, F., Berche, P., Bloecker, H., Brandt, P., Chakraborty, T., Charbit, A., Chetouani, F., Couve, E., de Daruvar, A., Dehoux, P., Domann, E., Dominguez-Bernal, G., Duchaud, E., Durant, L., Dussurget, O., Entian, K.D., Fsihi, H., Garcia-del Portillo, F., Garrido, P., Gautier, L., Goebel, W., Gomez-Lopez, N., Hain, T., Hauf, J., Jackson, D., Jones, L.M., Kaerst, U., Kreft, J., Kuhn, M., Kunst, F., Kurapkat, G., Madueno, E., Maitournam, A., Vicente, J.M., Ng, E., Nedjari, H., Nordsiek, G., Novella, S., de Pablos, B., Perez-Diaz, J.C., Purcell, R., Remmel, B., Rose, M., Schlueter, T., Simoes, N., Tierrez, A., Vazquez-Boland, J.A., Voss, H., Wehland, J. and Cossart, P. (2001) Comparative genomics of Listeria species. Science, 294, 849-852.

Hauben, K.J., Bartlett, D.H., Soontjens, C.C., Cornelis, K., Wuytack, E.Y. and Michiels, C.W. (1997) Escherichia coli mutants resistant to inactivation by high hydrostatic pressure. Appl Environ Microbiol, 63, 945-950.

Hauben, K.J., Bernaerts, K. and Michiels, C.W. (1998) Protective effect of calcium on inactivation of Escherichia coli by high hydrostatic pressure. J Appl Microbiol, 85, 678-684.

Hayashi, T., Makino, K., Ohnishi, M., Kurokawa, K., Ishii, K., Yokoyama, K., Han, C.G., Ohtsubo, E., Nakayama, K., Murata, T., Tanaka, M., Tobe, T., Iida, T., Takami, H., Honda, T., Sasakawa, C., Ogasawara, N., Yasunaga, T., Kuhara, S., Shiba, T., Hattori, M. and Shinagawa, H. (2001) Complete genome sequence of enterohemorrhagic Escherichia coli O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res, 8, 11-22.

Heidelberg, J.F., Eisen, J.A., Nelson, W.C., Clayton, R.A., Gwinn, M.L., Dodson, R.J., Haft, D.H., Hickey, E.K., Peterson, J.D., Umayam, L., Gill, S.R., Nelson, K.E., Read, T.D., Tettelin, H., Richardson, D., Ermolaeva, M.D., Vamathevan, J., Bass, S., Qin, H., Dragoi, I., Sellers, P., McDonald, L., Utterback, T., Fleishmann, R.D., Nierman, W.C. and White, O. (2000) DNA sequence of both chromosomes of the cholera pathogen Vibrio cholerae. Nature, 406, 477-483.

Heinz, V. and Knorr, D. (1996) High pressure inactivation kinetics of Bacillus subtilis by a three-state model considering distrubuted resistance mechanisms. Food Biotechnol., 10, 149-161.

Heinz, V., Toepfl, D., Knorr, D. Impact of temperature on lethality and energy efficiency of apple juice pasteurisation by pulsed electric field treatment. Innovative Food Science & Technologies, 4, (2003) 167-175.

Heldman, D.R. and Newsome, R.L. (2003) Kinetic models for microbial survival during processing. *Food Technology*, 57, 40-46

Ivanova, N., Sorokin, A., Anderson, I., Galleron, N., Candelon, B., Kapatral, V., Bhattacharyya, A., Reznik, G., Mikhailova, N., Lapidus, A., Chu, L., Mazur, M., Goltsman, E., Larsen, N., D'Souza, M., Walunas, T., Grechkin, Y., Pusch, G., Haselkorn, R., Fonstein, M., Ehrlich, S.D., Overbeek, R. and Kyrpides, N. (2003) Genome sequence of Bacillus cereus and comparative analysis with Bacillus anthracis. Nature, 423, 87-91.

Iwahashi, H., Shimizu, H., Odani, M. and Komatsu, Y. (2003) Piezophysiology of genome wide gene expression levels in the yeast Saccharomyces cerevisiae. Extremophiles, 7, 291-298.

Karatzas, K.A. and Bennik, M.H. (2002) Characterization of a Listeria monocytogenes Scott A isolate with high tolerance towards high hydrostatic pressure. Appl Environ Microbiol, 68, 3183-3189.

Karatzas, K.A., Wouters, J.A., Gahan, C.G., Hill, C., Abee, T. and Bennik, M.H. (2003) The CtsR regulator of Listeria monocytogenes contains a variant glycine repeat region that affects piezotolerance, stress resistance, motility and virulence. Mol Microbiol, 49, 1227-1238.

Kuroda, M., Ohta, T., Uchiyama, I., Baba, T., Yuzawa, H., Kobayashi, I., Cui, L., Oguchi, A., Aoki, K., Nagai, Y., Lian, J., Ito, T., Kanamori, M., Matsumaru, H., Maruyama, A., Murakami, H., Hosoyama, A., Mizutani-Ui, Y., Takahashi, N.K., Sawano, T., Inoue, R., Kaito, C., Sekimizu, K., Hirakawa, H., Kuhara, S., Goto, S., Yabuzaki, J., Kanehisa, M., Yamashita, A., Oshima, K., Furuya, K., Yoshino, C., Shiba, T., Hattori, M., Ogasawara, N., Hayashi, H. and Hiramatsu, K. (2001) Whole genome sequencing of meticillin-resistant Staphylococcus aureus. Lancet, 357, 1225-1240.

Lelieveld, H.L.M., Mosters, M.A., Curiel, G.J. Hygienic equipment design. In Hygiene in food processing. Eds. Lelieveld, H.L.M., Mostert, M.A., Holah, J., White, B. Woodhead Publishing Ltd., Cambridge UK, 2003.

Lombardo, M.J., Aponyi, I. and Rosenberg, S.M. (2004) General Stress Response Regulator RpoS in Adaptive Mutation and Amplification in Escherichia coli. Genetics, 166, 669-680.

Matser, A.M., Krebbers, B., Van den Berg, R.W., Bartels, P.V. Advantages of high pressure sterilisation on quality of food products. Trends in Food Science and Technology, 15 (2004) 79-85.

McClelland, M., Sanderson, K.E., Spieth, J., Clifton, S.W., Latreille, P., Courtney, L., Porwollik, S., Ali, J., Dante, M., Du, F., Hou, S., Layman, D., Leonard, S., Nguyen, C., Scott, K., Holmes, A., Grewal, N., Mulvaney, E., Ryan, E., Sun, H., Florea, L., Miller, W., Stoneking, T., Nhan, M., Waterston, R. and Wilson, R.K. (2001) Complete genome sequence of Salmonella enterica serovar Typhimurium LT2. Nature, 413, 852-856.

McKenzie, G.J., Harris, R.S., Lee, P.L. and Rosenberg, S.M. (2000) The SOS response regulates adaptive mutation. Proc Natl Acad Sci U S A, 97, 6646-6651.

McKenzie, G.J., Lee, P.L., Lombardo, M.J., Hastings, P.J. and Rosenberg, S.M. (2001) SOS mutator DNA polymerase IV functions in adaptive mutation and not adaptive amplification. Mol Cell, 7, 571-579.

Molina-Gutierrez, A., Stippl, V., Delgado, A., Ganzle, M.G. and Vogel, R.F. (2002) In situ determination of the intracellular pH of Lactococcus lactis and Lactobacillus plantarum during pressure treatment. Appl Environ Microbiol, 68, 4399-4406.

Notermans, S. Food authenticity and traceability. Ensuring the safety of animal feed. In Food authenticity and traceability. Eds. Lees, M., Woodhead Publishing Ltd, Cambridge, UK, 2003.

Ohlsson, T., Bengtsson, N. (2002) Minimal processing technologies in the food industry. Woodhead Publishing Limited, Cambridge.

Oxen, P. and Knorr, D. (1993) Baroprotective effects of high solute concentrations against inactivation of Rhodotorula rubra. Lebens.-Wiss. Technol., 26, 220-223.

Parkhill, J., Wren, B.W., Mungall, K., Ketley, J.M., Churcher, C., Basham, D., Chillingworth, T., Davies, R.M., Feltwell, T., Holroyd, S., Jagels, K., Karlyshev, A.V., Moule, S., Pallen, M.J., Penn, C.W., Quail, M.A., Rajandream, M.A., Rutherford, K.M., van Vliet, A.H., Whitehead, S. and Barrell, B.G. (2000) The genome sequence of the food-borne pathogen Campylobacter jejuni reveals hypervariable sequences. Nature, 403, 665-668.

Patterson, M.F. and Kilpatrick, D.J. (1998) The combined effect of high hydrostatic pressure and mild heat on inactivation of pathogens in milk and poultry. J Food Prot, 61, 432-436.

Patterson, M.F., Quinn, M., Simpson, R.K. and Gilmour, A. (1995) Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. Journal of Food Protection, 58, 524-529.

Peck, M.W. and Fernandez, P.S. (1995) Effect of lysozyme concentration, heating at 90 degrees C, and then incubation at chilled temperatures on growth from spores of non-proteolytic Clostridium botulinum. Lett Appl Microbiol, 21, 50-54.

Peleg, M. (1995) A model of microbial survival after exposure to pulsed electric field. Journal of the Science of Food and Agriculture, 67, 93-99.

Peleg, M. and Cole, M.B. (1998) Reinterpretation of microbial survival curves. Crit Rev Food Sci Nutr, 38, 353-380.

Pol, I.E., Mastwijk, H.C., Bartels, P.V., Smid, E.J. Pulsed electric field treatment enhances the bactericidal action of nisin against *Bacillus cereus*. Applied and Environmental Microbiology, 66 (2000) 428-430.

Quillardet, P., Rouffaud, M.A. and Bouige, P. (2003) DNA array analysis of gene expression in response to UV irradiation in Escherichia coli. Res Microbiol, 154, 559-572.

Raso, J., Barbosa-Cánovas, G.V., Nonthermal preservation of foods using combined processing techniques. Critical Reviews in Food Science and Nutrition, 43 (2003) 265-285.

Rose, N.L., Palcic, M.M., Sporns, P., McMullen, L.M. Nisin: a novel substrate for glutathione S-transferase isolated from fresh beef. Journal of Food Science, 67 (2002) 2288-2293.

Simpson, R.K. and Gilmour, A. (1997) The effect of high hydrostatic pressure on Listeria monocytogenes in phosphate-buffered saline and model food systems. J Appl Microbiol, 83, 181-188.

Smelt, J.P.P.M. (1998) Recent advances in the microbiology of high pressure processing. Trends in Food Science & Technology, 9, 152-158.

Smelt, J.P.P.M., Hellemons, J.C., Wouters, P.C. and van Gerwen, S.J.C. (2002) Physiological and mathematical aspects in setting criteria for decontamination of foods by physical means. International Journal of Food Microbiology, 78, 57-77.

Storz, G. and Hegge-Aronis, R. (2000) Bacterial stress responses. ASM Press, Washington.
van Boekel, M.A. (2002) On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. Int J Food Microbiol, 74, 139-159.

Stumbo, C.R. (1973) *Thermobacteriology in Food Processing*. Academic Press, New York.

Van Opstal, I., Vanmuysen, S.C. and Michiels, C.W. (2003) High sucrose concentration protects E. coli against high pressure inactivation but not against high pressure sensitization to the lactoperoxidase system. Int J Food Microbiol, 88, 1-9.

Whiting, R.C. and Buchanan, R.L. (2001) Predictive modeling and risk assessment. In Doyle, M.P. and Beuchat, L.R. (eds.), Food microbiology: fundamentals and frontiers. ASM press, Washington, pp. 813-831.

Yousef, A.E. and Juneja, V.K. (2003) Microbial stress adaptation and food safety. CRC Press, Boca Raton, FL [etc.].

ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

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Misc Details
Research interests: preservation techniques for food (i.e. potatoes and especially apples) of lactic acid bacteria by using carbohydrtaes (i.e. trehalose); preservation techniques forlactic acid bacteria by using polysaccharides (alginates); treatment of high perishable foods by gamma rays: evaluation of protein, peptide and aminoacidic pattern, to optimise the dose rate of gamma rays to apply to food such as truffles, fresh sausage and fishes, also a genotoxic study to develop substances eventually forming due to the treatment.

Gianluca Picariello
National Council of Research Institute of Food Science, Avellino, Italy
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Misc Details
Research interests: Modifications indeced by thermic and technological treatments on protein foods. Molecular charaterizatin of markers of treatments by mass spectrometry and on-line HPLC/mass spectrometry.

Functional foods.

Abstract or summary of benefits: Technological treatments on foods induce a modifications in their chemical and/or physical properties. The aim of my research activity is to identify markers of the treatment undergone, to assure the preservation of all the originary qualities of food.

I'd like to gain information's about new methodologies of food preservation, to be able in future to study their possible effects on protein fractions and, in special way, the probable formation of allergenic amino-acidic sequences.

Gianfranco Mamone
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Misc Details
Research interests: Structural characterization of food proteins and their posttranslational modifications by proteomic tecniques.

Research interests is focuse mainly on proteins from wheat, milk.

Abstract or summary of benefits: Transglutaminase-catalyzed post-translational modification of proteins plays a key hole in celiac disease generating a variety of haptens responsible, among others, for an autoimmune response. By using a peptidomic approach, the glidian peptic-tryptic digest has been evaluated in terms of possible modification by Transglutaminase. Mono-dansylcadaverine was used as a fluorescent probe to tag Transglutaminase – susceptible glutamine (Q) residues. Six a/b-, g- gliadin, and LMW glutenin peptides including then Q epitopes were identifies by nanoES-MS/MS. Transglutaminase deadmidated, in the absence of an acyl acceptor, synthetic peptides Q belonging to the sequence consensus Q-X-P at the Q site susceptible to transamidation. The formation of a covalent Transglutaminase-gliadin peptide complex was also demostrated through immonoblotting experiments. The benefit of this procedure lies in its vast applicability for defining Q susceptibilit pathways of any dietary protein to Transglutaminase
Rossella Rotondi
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ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

Misc Details
Research Interests: Food Processing, Agricultural mechanics, Agricultural Components design

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Misc Details
Research interests: High-pressure analysis of the ethereal extracts from tomato.
- Microbiology and mild preservation.
- Food safety developments relating to mild preservation.
- High-pressure processing in relation to food safety.

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Misc Details
Research Interests: Food Microbiology

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Misc Details
Research Interests: Food Microbiology

Yvonne E. Thomassen
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Misc Details
Research Interests: Molecular microbiology especially in the field of foodpathogens in relation to preservation techniques.

Abstract or summary of benefits:
Assessment of psychrotolerance features of B.cereus. Yvonne E. Thomassen, Floris Bosveld, Marjon J.H. Bennik.

There is an increased consumer demand for ready-to-eat, cooked-chilled and fresh foods. These products are often stored in refrigerators to lower the possible microbial growth. This however creates a niche for psychotolerant bacteria, which are able to survive the mild preservation techniques used.
Bacillus weihenstephanensis is a psychrotolerant spore-forming food pathogen. Due to its spore-forming capacities it can survive mild preservation techniques and subsequently grow at temperatures as low as 4 to 7°C. B. weihenstephanensis was described as the psychrotolerant variant of the closely related food pathogen Bacillus cereus1. However, not all cold tolerant B. cereus can be classified as B. weihenstephanensis2.
To identify genetic determants allowing B. weihenstephanensis to grow at low temperatures a PCR based subtractive hybridization was performed between psychrotolerant B. weihenstephanensis DSM 11821 and

mesophilic B. cereus ATCC 14579. Thus allowing the construction of a library enriched for B. weihenstephanensis genes.
To identify which of these genes play a role in cold-tolerance, hybridization experiments were performed to assess their presence in mesophilic and/or psychrotolerant B. cereus and B. weihenstephanensis strains. Several genes were found to be only present in cold-tolerant B. cereus strains, and these are currently studied to determine if and what their role in psychrotolerance is.

1. Lechner, S. Int J Syst Bacteriol (1998)
2. Stenfors, L. P Fems Microbiology Letters (2001)

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Research Interests: Pulsed Electric Field Pasteurization of liquid food. Investigation of Membrane Permeabilization Mechanism and Kinetics. Improvement of Mass Transfer Processes by pulsed Electric Fields. Impact of Process Parameters on Microbial Inactivation of liquid food by Pulsed Electric Fields. Energy efficiency of PEF treatment of liquid food and process optimization. Modeling and optimization of electric field distribution, Treatment Chamber Design and Impulse Generating Systems.

Abstract or summary of benefits:
Optimization of Pulsed Electric Field Processing Parameters and Treatment Chambers for Liquid Food Pasteurization.

Preservation of liquid food by high intensity pulsed electric fields (PEF) is an interesting, mild alternative to traditional techniques like thermal pasteurization, but high costs of operation inhibited an industrial application so far. This work is focussed on improving treatment efficiency by a combination of PEF and synergetic effects by mild heat as well as optimization of treatment chamber geometry and selection of electrode material to enhance PEF lethality and ensure product safety.

By flow cytometric analysis it was shown that an exposure of biological cells or liposomes to an external electrical field induces an electroporation of phospholipid bilayers. Based on the underlying mechanism of action and making use of highly synergetic effects of mild heat in the range of 35-55°C a gentle processing concept for pasteurization of liquid media by PEF was developed. The need to preheat the media before treatment provides a possibility to recover the dissipated electrical energy, leading to a drastic reduction in costs of operation. The inactivation of three bacteria (E. coli, Bacillus megaterium, Listeria innocua) and a yeast (Saccharomyces cerevisiae), different in size and geometry has been investigated, proving theoretical considerations that cell shape influences a microbes susceptibility against PEF.

By selection of an appropriate treatment chamber configuration and optimization of its geometry treatment homogeneity, a decisive factor for product safety was improved. Choice of suitable electrode material may help to reduce electrochemical reactions and gas bubble formation at the electrode-media interface, which leads to electric field perturbation. The erosion of stainless steel as well as carbon electrodes and the impact of electrode erosion on treatment efficacy was investigated. Carbon electrodes show the potential to improve treatment homogeneity by redution of bubble formation due to electrolysis, enhancing microbial inactivation and a higher electrode lifetime.

ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

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Misc Details
Research Interests: High pressure impact on frozen systems – microstructure and micro-organisms. High pressure impact of aqueous systems and lipd systems – phase transitions, thermodynamics. Impedance analysis of plant tissue material.

Abstract or summary of benefits:
Application of high pressure to frozen aqueous systems.

Little is known so far about the impact of high pressure on micro-organisms in frozen systems. Treatments were carried out with E. coli and Listeria innocua in frozen model systems and foods in the range of –45 °C to 0 °C and up to 400 Mpa. Inactivation kinetics with considerable differences to inactivation kinetics in liquid aqueous systems were obtained. In general the inactivation rates were lower in the frozen state, possible due to the low water activity. However, the phase transition of the commonly known ice I to other ice polymorps (ice II, ice III) lead to an instant inactivation effect due to the high density changes at pressures above 210 Mpa. Flow cytometric analyses showed signs for complete cell rupture, which is obtained after high pressure applications in the liquid state. Potential applications, restrictions and future research directions will be discussed.

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Misc Details
Research Interests: Supercritical fluids

Satu Vuorela
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Misc Details
Research Interests: The topic of my PhD studies is "Functional properties of rapeseed phenolics". It concerns analysis techniques, antioxidant, antibacterial and anti-inflammatory tests as well as food applications.

Abstract or summary of benefits:
I would like to promote my knowledge concerning food processing and preservation in safety aspect. Safety aspect is important in my research work with rapeseed phenolics and their food applications.

Hanna Salminen
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Misc Details
Research Interests: Interaction reactions of functional food components: lipids, proteins and phenolic antioxidants. Oxidation of proteins n meat products and improvement of the quality of meat products by phenolic antioxidants.

Abstract or summary of benefits:
I hope to provide new information relating to food safety and technology. This seminar would also give new aspects of improving novel food applications regarding to my research interests.

ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

Paula Maria Periago Bayonas
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Misc Details
Research Interests: I am interested in new mild technologies used to produce ready to eat foods which are nutritive and natural tasted and have a long shelf-life (Safety Microbiology) in refrigerated conditions.

Abstract or summary of benefits: Abstract of the presentation.
Demografic, social and cultural changes since the Second World War have established a sustained demand for high quality ready to eat meals which can be rapidly and easily prepared for consumption in the home (Conner et al., 1989). One such process is vacuum packaging, holding the product for extended periods under refrigeration, prior to a further reheating step immediately before consumption.

Yersinia enterocolitica is considered to be the most significant genus member with respect to foodborne disease (Varnum and Evans, 1991). Y. enterocolitica occupies a broad range of ecosystems including the intestinal tract, birds, flies, fish and a variety of terrestrial and aquatic ecosystems. Isolation of Y. enterocolitica has been reported from a variety of foods including raw vegetables (Barton et al., 1997; Delmas and Vidon, 1985; Tassinari et al., 1994). Y. enterocolitica can grow at refrigeration temperatures and under aerobic and anaerobic conditions (Barton et al., 1997).

To evaluate the potential risk of this foodborne pathogen recontamination in ready to eat meals, growth of a cocktail of five strains of Y. enterocolitica in Nutrient Broth at different pHs and refrigeration temperatures has been carried out. Results have been analysed to evaluate its ability to grow using a frequency distribution approach.

ÌOlla gitanaî (a complex Spanish dish based on vegetables) was onoculated with the same Y. enterocolitica cocktail and incubated under aerobid and anaeobic conditions at different temperatures. Results were compared with data in laboratory media and recommendations of preservation conditions and shelf-life have been proposed.

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Misc Details
Research Interests: Enzymatic hydrolysis of hemicelluloses and use of formed oligosaccharides in food products.

Laura Murchie

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Misc Details
Research Interests: High pressure Microbiology Shellfish

Abstract or summary of benefits:
Although the implementation of sanitary controls for the production of bivalve shellfish has reduced the incidence of diseases, such as typhoid, the number of reported outbreaks of shellfish-associated illnesses continues to rise. Recent decades have seen a change in the aetiology of shellfish-borne disease, and viruses are now implicated in

the majority of cases. This may be explained, in part, by the limitations of current regulations and purification methods, for example, the lack of parity between bacterial indicators and viruses, and the efficacy of purification methods for these pathogens. Therefore further processing of shellfish is advisable to ensure food safety. Shellfish such as oysters and clams are often eaten raw or lightly cooked. This preference not only has implications for disease transmission but also limits the processing methods suitable for these products. High pressure processing allows inactivation of micro-organisms in foods, while maintaining sensory and nutritional properties of the product. Currently, high pressure processing has several commercial food applications, including the processing of oysters. However, relatively little is know of the efficacy of high pressure in reducing pathogens in shellfish. The present study was initiated to investigate the inactivation of viruses in shellfish. Results of the inactivation of a bovine enterovirus in culture medium, seawater and shellfish are presented. Preliminary results suggest that while treatment of 450 Mpa for 5 minutes at 20ºC is effective at reducing the enterovirus to undetectable levels in culture medium and seawater, the virus may still be detectable, at reduced levels in shellfish following this treatment.

Fandi Ibrahim
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Misc Details
Research Interests: Food microbiology, probiotics

Abstract or summary of benefits:
It is a fact that I got my BSc and MSc in the area of Food Science and Technology and now I am continuing my PhD in the same field. I also was working as an assistant lecturer for six years in the department of Food Science and Technology, Assiut University, University, Egypt (November, 1995 – December, 2001). Therefore, this course is strongly relevant to my career now and in the Future. I came to Finland to complete my PhD and I will come back to continue working in the same place where I was. That means simply that I will be involved in the Food Science and Technology in the future too. So I hope I have this chance to attend this course which is pertinent to my studies.

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Misc Details
Research Interests: Thermal processing of foods (fish)

Abstract or summary of benefits:
Novel radio frequency water bath process and the consequences for quality of fish products.

Heating of vacuum packed foods by a novel radio-frequency (RF) water bath process bears the potential of significant quality improvements for minimal processing of convenience cook/chill fish products compared to conventional methods such as water baths, convection ovens and autoclaves. The rapid volume heating of the process is expected to reduce overcooking of the heat sensitive fish flesh and to result in a better product quality while maintaining product safety and shelf life.

An experimental RF-heater has been constructed which offers great flexibility in investigating and optimising the novel heating process. Several RF-heating experiments have been carried out and demonstrated the feasibility of the process. It seems possible to reduce the heating time of the products by one order of magnitude compared to the heating time needed for conventional heating. Results of the tests and possible quality improvements by rapid heating will be presented together with the presentation of some obstacles and limitations of the method.

ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

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Misc Details
Research Interests: Food Microbiology and Preservation Technologies

Abstract or summary of benefits:
Food preservation in the tropics is a difficult post harvest problem with the humidity, insects and heat hampering some food preservation systems thus denying longer shelf life. Yet Improved post harvest management depends on the quality and efficiency of handling, processing and preservation techniques used. Processing of food to a form which has a longer shelf life (food preservation) and adding value to the original food form helps not only to overcome the spoilage and losses, but provides a form of assurance in the food security error.

Therefore, as a preparation for the start of a PhD, the seminar will be gainful to enable me learn with and from fellow young scientists, to evaluate the food preservation technologies, their impact on the diversity of micro-organisms responsible for both food spoilage and food borne illness. To get an insight into the challenges faced as a result of 1) the successful survival and growth of food micro-organisms, 2) the different global conditions (temperate and tropics), 3) very little knowledge about alternative food preservation methods in the tropical regions. Knowledge and techniques from ongoing research by fellow young scientists will be enriching for the kind of direction to take in tackling these and other identified challenges.

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Misc Details
Research Interests: New methods for preservation and mainly High Pressure and Electric Field

Abstract or summary of benefits:
High-Pressure in combination with high-temperature treatment for food products.

Extremely prospective method for agro-food industry is treatment under high pressure. By this operation are producing foods, with different degree of residium contamination. The food, which is produced by this method, must have the same requirements like the food treated by conventional methods. The food produced (treated) by novel methods of preservation must be with better quality and equal price. The novel technology must ensure energy effectiveness, better quality taste and safety. We use combination of two factors. The factors are high-pressure as first and high temperature as second factor. Both of them are applied for short time. The combination of these factors for short time (pressure and temperature) there are in high temperature extrusion. This treatment is very complicated because the factors like pressure in different zones, residence time distribution (RTD) and the temperature of flow of the product are not easy for measure. We create new method for measurement of RTD and pressure in different zones in extruder Brabender 20 DN. These two factors and the temperature of flow are the main factors for analyse of samples. For analyse of samples we have to know the conditions (the exact values of factors) of the samples into machine and then we search the correlation between temperature, pressure and RTD. Тhis poster exposes our pattern, results and conclusions which we determined.

Rauni Karenlampi
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Misc Details

ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

Research Interests: Campylobacter jejuni epidemiology, transmision and control.

Abstract or summary of benefits:
I hope to get knowledge of new preservation technologies possibly applicable for C. jejuni elimination especially in poultry products.

Virginia Stergiou
School of Molecular and Biomedical Sciences, University of Surrey, UK
virgako@lycos.com

Misc Details
Research Interests: It has been shown that nisin recovery in meat is unsatisfactory. A number of factors have been proposed for this. However, few researchers have actually attempted to study some of them in depth. The current research focusing on the availability of nisin in meat and meat products, aims to determine those factors and investigate to what extent each of them contributes to reduced nisin activity. It will also be attempted to diminish the undesirable effect of those attributes found to be significant for the poor nisin activity.

Abstract or summary of benefits: Nisin is a small antimicrobial peptide produced by strains of Lactococcus lactis. It has a wide spectrum of activity against Gram-positive bacteria, particularly spore-formers. It has been approved as a preservative in over 50 countries for use in numerous food systems. However, the use of nisin in meat is limited as some of the meat components are believed to interfere with its activity. It has been proposed that its reduced activity may be due to nisin binding on meat proteins, enzymic inactivation and adherence to fat.
Glutathione (GSH) is a small molecular weight thiol compound found in substantial amounts in animal tissues. We have confirmed earlier observations that nisin reacts with glutathione and have found that the formation of the glutathioneñnisin adduct is a non-enzymic process. The product of the reaction is inactive and results in reduced antimicrobial activity. Glutathione is heat-stable under pasteurisation conditions. The retention of nisin activity in heat-treated meat appears to be the result of the loss of free glutathione during cooking rather than enzyme inactivation.

Tassou Chrysoula
National Agricultural Research Foundation, Institute of Technology of Agricultural Products , Greece
microlab.itap@nagref.gr

Misc Details:
Research interests: Food safety, physiology of pathogens, new emerging technologies, natural antimicrobials.

Abstract or summary of benefits: effect of High Pressure on spoilage micro-organisms isolated from traditional salads and ham products.

Moussa Marwen
University de Bourgogne (Burgundy), Dijon, France
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Misc Details:
Research interests: Comprehension of the effect of high hydrostatic pressures and sub-zero temperatures on cell-inactivation: application to the pasteurisation of foods

Abstract or summary of benefits: Coupling effect of sub-zero temperature and water activity on high pressure inactivation of Escherichia coli:

ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

The purpose of this work is to study pressure inactivation of a Gram-negative bacteria at low temperatures and different water activity levels. Escherichia coli K12 TG1 was submitted 10 minutes at different pressure levels (from 0,1 to 450 MPa), temperatures (25 and -20°C without freezing) and water activity (from 0,85 to 1). Results confirm the synergistic effect of sub-zero temperature and pressure with regard to the microbial destruction for pressure levels up to about 300 MPa. In this range, a temperature of -20°C allows to reduce pressure level of about 100 MPa to achieve the same microbial inactivation obtained for 25 °C. This effect decreases for pressure levels between 300 and 400 MPa, and seems to be completely cancelled for 450 MPa. It was strongly amplified after an hypo-osmotic shock and reduced after an hyper-osmotic shock.

In order to approach target mechanisms of pressure-temperature synergistic effect, it was proposed a thermodynamical way. Microbial destruction seems to be related to the quantum of energy received by microbial cell during pressurisation witch depends on water compressibility.
Key Words : High Pressure, Sub-zero Temperature, Water Activity, Synergistic effect, E. coli K12 TG1.

Hilal B. Halkman

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Misc Details:
Research interests: Food microbiology, predictive microbiology, risk assesment, new food preservation techniques.

Abstract or summary of benefits:
To improve the safety and quality of minimally processed
H.B. Halkman, P.Kozat, V. Basbayraktar, N.Cetinkaya

Fresh pre-cut produce and other minimally processed food of plant origin are often consumed as such, without cooking or after undergoing a microbial inactivation process. Occurrence of some of the pathogenic organisms in raw vegetables and their implication in causing ill health resulting in diarrhea/dysentery or serious diseases like yersiniosis and listeriosis have been document in recent years.

In recent years, because of health interests and renewed diet trends, leafy vegetables and salads are gaining increasing importance in the human diet. For weight conscious persons also, due to their high vitamins and fiber contents, salads are very beneficial. In response to consumer demands minimal processing of food is gaining importance.

Minimally processed vegetables, shredded carrots may have high levels of micro-organisms. In commercial shredded vegetables, mesophilic counts of about 106 to 107 have been reported. Commercial processes for preparing fresh-cut vegetables usually use chlorine in the wash water to control the microbiological population. However chlorine cannot be relied on to eliminate pathogenic micro-organisms such as Listeria monocytogenes. Irradiation and other mild preservation techniques have been shown to be effective in reducing the microbial population of shredded vegetables.

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Misc Details:
Research interests: Food microbiology, food preservation techniques.

ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

Misc Details:
Research interests: Food microbiology, predictive microbiology, risk assesment, new food preservation techniques.

Abstract or summary of benefits:
To improve the safety and quality of minimally processed
H.B. Halkman, P.Kozat, V. Basbayraktar, N.Cetinkaya

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In recent years, because of health interests and renewed diet trends, leafy vegetables and salads are gaining increasing importance in the human diet. For weight conscious persons also, due to their high vitamins and fiber contents, salads are very beneficial. In response to consumer demands minimal processing of food is gaining importance.

Minimally processed vegetables, shredded carrots may have high levels of micro-organisms. In commercial shredded vegetables, mesophilic counts of about 106 to 107 have been reported. Commercial processes for preparing fresh-cut vegetables usually use chlorine in the wash water to control the microbiological population. However chlorine cannot be relied on to eliminate pathogenic micro-organisms such as Listeria monocytogenes. Irradiation and other mild preservation techniques have been shown to be effective in reducing the microbial population of shredded vegetables.

Mbara Kamaso
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Misc Details:
Research interests: Agric. Food Management

Abstract or summary of benefits: Basic ways of food management and preservation.

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Misc Details:
Research interests: Food enzymology, Biosythesis of differen types of oligosaccharides as prebiotics-glucooligosaccharides and fructooligosaccharides; bacteriocins from LAB.

Abstract or summary of benefits: Study on biologically active substances isolated from lactic acid bacteria from Bulgarian dairy products.
Functional foods hold a great promise for future trends in human nutrition. Lactic Acid Bacteria produce a variety of homopolysaccharides and heteropolysaccharides wich account for increasing interest in the nutritional application. Extracellular glucansucrases from LAB belonging to the genera: Leuconostoc, Streptococcus and Lactobacillus catalyze the transfer of glucose units from sucrose onto acceptor molecules, mainly sugars, resulting in the synthesis of glucooligosaccharides. Some of glucooligosaccharides have foung new applications in the food industry as dietary compounds stimulating the growth of beneficial bacteria of the intestinal microflora. From the other point of view LAB are important producers of biologically active peptides (bacteriocins) important for food

safety. The proteolytic system of lactic acid bacteria consists a cell wall bound proteinase and several intracellular peptidases, and can contribute to the liberation of bioactive peptides.
In our study we focused our attention on 3 aims:
A/ Biosynthesis of the EPS by strains with proved anticancer effect Lactobacillus bulgaricus LB3 and Lactobacillus bulgaricus LB5 on chemically-defined medium and whey. A study on the action of different carbohydrate sources (glucose, fructose and sucrose) on the productivity of EPS and the rheological properties of medium was done. When chemically-defined medium was used the highest production of EPS was determined at 4% glucose - 150 mg/g-1 . The carbohydrate substrate influenced the EPS composition.
Not only the concentration but also the structure of the EPS is important for its thickening effect. The functional properties of the EPS, produced from LAB could be modified by influencing the growth conditions. The influence of the different content of glucose, fructose and sucrose (3-6%) on the fermentation process and the viscosity of the skimmed milk and whey were performed. The carbohydrate substrate influenced the viscosity of the fermented milk.
B/ Biosynthesis of an extracellular dextransucrase by new starins Leuconostoc mesenteroides.
The strains were isolated from different bulgarian dairy products. Some of the studied strains produced large amounts of extracellular dextransucrase activity when grown on sucrose as carbon source – strain No 22 – 9,6 U/ml and strain No. 28 – 8,85 U/ml. The effects of different carbohydrates as substrates on bacterial growth and dextransucrase synthesis were studied for the isolated strains. No enzyme production occurs on a glucose and fructose medium or during growth on glucose, fructose, maltose, raffinose and lactose. In addition, the effect of C/N ratio on the production of extracellular dextransucrase was studied. SDS-PAGE analysis showed that the molecular masses of the dextransucrases from the studying strains were approximately 180 kDa.
In the presence of sucrose and maltose (acceptor), DS from strains No. 22 and 28 synthesized the same mixture of products as GTS from strain NRRL – 152 F. The products observed were glucooligosaccharides formed of only a-1,6 linkages with a maltosyl residue at the reducing end.
C/ Study of b - lactoglobulin and a - lactalbumin in early stages of yogurt fermentation of traditional Bulgarian products. Biochemical techniques were used to measure the concentration of these two whey proteins during fermentation. At a result of the done study alteration in the concentration of b - lactoglobulin and a - lactalbumin were detected. The studied proteolytic activity of the strains, used in the fermentation process confirmed the received results.

Olaf Jansen
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Misc Details:
Research interests: High-Pressure Preservation Mushroom aroma, fresh and processed, flavour Analysis, sensory evaluation, GC-MS, GC-O

Abstract or summary of benefits: Olfactory evaluation of flavour changes in high-pressure treated black truffles.

Black truffles (Tuber melanosporum Vitt.) are subterranean mushrooms growing in symbiosis and certain trees. As the collecting season is limited to the winter months, part of the harvest is traditionally preserved by heat sterilisation. These tinned truffles are of intense and typical flavour, but lack some subtle notes of the fresh merchandise. High pressure treatment was applied instead, aiming at the conservation of a flavour profile closer to the fresh reference product. Evaluation was carried out by GC-Olfactometry, thus allowing an odourant-by-odourant follow-up of flavour change. Odourant intensity was compared using the detection frequency approach on fresh, pressure-treated and heat-treated truffles, respectively. While no differences in low-boiling-point aroma constituents were observed, some "heavier" notes were perceived only in pressurised samples. These possible artefacts are however not found in heat-treated samples.

ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

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Misc Details:
Research Interests: High Pressure and Bacteriocins in Food.

Abstract or summary of benefits:
The combined effects of high pressure and nisin on micro-organisms in milk
Black1, 2, E., A. L. Kelly2, M. F, Patterson3 and G. F. Fitzgerald1,2
Departments of 1Microbiology and 2Food and Nutritional Sciences, University College Cork, Ireland and 3Food Science Department, The Queen’s University of Belfast, Northern Ireland
High pressure has been found to kill micro-organisms in food over a wide range of pressures (250 – 800 MPa). The commercially-available bacteriocin nisin has been safely used in a range of foods as a biopreservative for over 40 years. Nisin is active against Gram-positive bacteria such as Listeria, and is used to prevent the outgrowth of spores in dairy products. Applying high pressure or the addition of nisin to a food are thus effective preservative treatments; however they also have disadvantages. Nisin is unable to act against Gram-negative bacteria such as E. coli and high pressure needs to be extreme (> 600 MPa) to kill certain organisms or spores. Using both treatments as part of the hurdle process can eliminate these disadvantages. This study demonstrates that, when applied together at ambient temperatures, high pressure and nisin act in a synergistic manner and a log reduction of 8 log units of both Gram-positive (e.g. L. innocua) and Gram-negative bacteria (e.g. E. coli) can be achieved. Using a pressure treatment of 500 MPa for 5 min cycled twice in combination with nisin and mild heat (40oC) has been found reduce the numbers of Bacillus subtilis spores in milk by 5.9 log units. These results demonstrate the potential for the use of such combined treatments for the preservation of milk and milk products.

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Misc Details:
Research Interests: High pressure processing, pulsed electric fields, process engineering

Abstract or summary of benefits: Improving the quality of fresh meat with mild high pressure technology

A major issue in the shelf life of fresh meat is discoloration. The bright red colour of fresh meat is caused by oxymyoglobin, which is formed from myoglobin (deep purple) due to the presence of oxygen. During storage this red colour will turn into a grey brownish colour. The brown colour comes from the layer of metmyoglobin in the meat, this metmyoglobin is formed by an oxidation of myoglobin and a reduction of oxymyoglobin. To what extend the formation of metmyoglobin will occur depends on many factors; what kind of animal is used, what part of the animal is used, gender, age, pre-slaughter stress, hygiene during slaughter, packaging and storage conditions, etc.
In this project the influence of mild high pressure treatment on the colour and micro-biological contamination of fresh meat is examined. The pressures used vary from 50 MPa (500 bar) up to 200 MPa (2000 bar) and a holding time of approximately 5 to 20 minutes. The initial temperatures were between 0 and 10°C. In these ranges of pressures, temperatures and times it is possible to improve the quality and shelf life of many meat types without changing the fresh appearance of the meat.

Our research shows an improvement in the colour stability and a decrease in the micro-biological contamination and growth in fresh beef.

Ledward (University of Reading) has done initial research in this area, his articles have formed the basis on which we started our research.

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Misc Details:
Research Interests: Novel preservation technologies especially PEF and Plasma, drying and Microwave technology, al technologies in relation to food properties.

Abstract or summary of benefits: Mild preservation of fresh orange juice by pulsed electrical field treatment: enzymatic stability.

H.J. Schuten, K. Gulfo-Beusekom, I.E. Pol, H.C. Mastwijk, P.V. Bartels

In this contribution the shelf live of orange juice is determined with respect to microbial and enzymatic spoilage during storage. Fresh juice with pulp obtained from oranges has been treated by pulsed electrical field (PEF) and is compared to untreated and heat-pasteurised samples. The PEF treatment under consideration is a well-defined combination of mild heat and electrical pulses at moderate field strength.
The enzymatic stability of the juice is evaluated by measurement of the activity of pectinmethylesterase (PME), by determination of the juice cloud stability and by the appearance of the product after sedimentation during storage.
PEF treatment did not show any significant reduction in PME activity due to non-thermal action. The activity after treatment is still 79 +/- 3 % when compared to the activity of untreated samples. The observed level of inactivation could be attributed to the heating inextricable connected to this preservation process.
In contrast, cloud stability shows an improvement of enzymatic quality by PEF treatment. An extension of the shelf live towards 21 days has been demonstrated. This extension in shelf live should be compared to untreated juice, which is subject to a combination of microbial and enzymatic spoilage within 5 days.
References
H.W. Yeom et al. Journal of Agricultural and Food Chemistry, 48 (2000) 4597–4605.
G. V. Barbosa-Canovas, Q.H. Zhang, Pulsed Electrical Fields in Food Processing, Fundamantel Aspects and Applications,Pennsylvania, (2001).
A. Van Loey et al. Trends in Food Science & Technology 12 (2002) 94–102

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Misc Details:
Research Interests: High hydrostatic pressure treatment.

Abstract or summary of benefits: CHANGES OF EGG PROPERTIES INDUCED BY HIGH HYDROSTATIC PRESSURE OR GAMMA IRRADIATION
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The future implication of non-thermal food processing techniques, as high hydrostatic pressure and irradiation, develop rapidly in the food industrial area. In order to gain experience on feasibility of non-thermal pasteurization of egg, the effects of high hydrostatic pressure (HHP) treatment or gamma irradiation levels inactivating several log cycles of vegetative pathogenic bacteria were investigated. In the present study fresh liquid egg yolk and

albumen were subjected to high hydrostatic pressure of 300-500 MPa for 15 min or gamma irradiation of 0.5-3 kGy doses. Shell eggs were also irradiated with the same radiation dose levels. Mainly rapid instrumental examinations were performed in order to determine the major quality changes caused by the applied treatments. One of the aims was to study the thermal stability of liquid albumen and yolk by determining DSC thermograms, where the protein denaturation resulted by the non-thermal processes could have been followed. Color changes in egg yolk and albumen were also determined, spectra of egg yolk were performed. Functional properties, such as whipping power and foam stability were also investigated. Surface areas of untreated and irradiated broken whole eggs were compared as well. DSC studies revealed changes related to protein denaturation/aggregation in HHP treated egg albumen. Conalbumin seems to be the most pressure sensitive main egg white protein. Slight loss of color intensity of egg yolk and thinning of the thick egg white by gamma irradiation were noted. HHP treated egg white started to partially gelify after 400 MPa level. Decreased viscosity of irradiated egg albumen became statistically significant already at 0.5 kGy radiation dose, whereas foam stability were significantly reduced from 2 kGy onward. The applied methods proved to be suitable for detecting quality changes resulted by high hydrostatic pressure and irradiation o fresh liquid egg. Due to these changes of properties non-thermal pasteurization treatment levels of high hydrostatic pressure or gamma irradiation would require careful optimization. Further studies are in progress to investigate changes in proteins of egg by gel-electrophoresis and eventual changes in their immuno-reactivity.

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Misc Details:
Research Interests: Pulsed Electric Field Technology.

Abstract or summary of benefits: PULSED ELECTRIC FIELD (PEF) PROCESSING EFFECTS ON PHYSICAL AND CHEMICAL PROPERTIES OF VEGETABLE JUICES
Pulsed Electric Field (PEF) processing is an emerging, non-thermal technology involves the application of pulses of high voltage for food preservation. As a consequence of the PEF treatment the shelf life of food products can be significantly extended - without considerable physical and chemical changes as compared to the traditional thermal pasteurisation - by inactivation of micro-organisms and by decreasing the enzyme activity.

The objective of this research was to study the effect of PEF treatment on some physical and chemical properties of vegetable juices. The °Brix, pH, conductivity, non-enzymatic browning index and hydroxymethylfurfurol content of samples were investigated.

The vegetable juices (carrot, pumpkin, red beet) were treated by OSU 4-B laboratory equipment. The PEF system, that has six treatment chambers connected in series, containing stainless stell electrodes with a gap of 0.29 cm, worked with square electric field pulse waves with 2 microsiemens pulse duration. During the treatment 28 kV/cm electric field strength and 8.3 pulses/treatment chamber were applied. The treatment temperature was under 40 °C. The °Brix and non-enzymatic browning index did not change practically. The pH slightly decreased while the conductivity and HMF content slightly increased. According to the absorbances measured in total visible light range and to the absorbance values at 520 nm the treatment did not cause change in the colour of juices. The total colour difference of vegetable juices determined by tristimulus colour measurement showed slightly noticeable difference between the treated and untreated samples.

Summarizing our results, it was established, that the PEF treatment didn't affect the studied properties of samples significantly.

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ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

Misc Details:
Research Interests: Food Research (Food Quality, Food Processing, Food safety, Novel food technologies etc.).

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Misc Details:
Research Interests: Novel technologies, High-pressure treatment of shellfish.

Abstract or summary of benefits: Effects of thermal pasteurisation or high pressure processing on physical and biochemical characteristics of oysters (Crassostrea gigas)
Treatments currently in use for post-harvest treatment of oysters include cryogenic individual quick freezing, a mild thermal process referred to as cool pasteurisation (CP) and high pressure (HP) processing. CP and HP processing are used for both raw half shell and shucked oysters. Preservation of food with HP is of particular interest as it does not affect the natural appearance, taste and flavour of food. The objective of this study was to determine the effects of commercial pasteurisation (CP, 50°C x 10 min) or HP (260 MPa x 3 min) treatments on some quality parameters of depurated oysters. HP-treated oysters had higher pH than untreated or CP oysters, but differences were not significant ($P > 0.05$). HP and CP both modified the gross composition of oysters; however, the moisture content of HP-treated whole oyster tissue was higher than that of pasteurised or untreated oysters. Neither HP nor CP treatment affected the salt content or water activity of oysters. HP treatment and CP both increased Hunter L and b values and decreased a values of oysters; overall, however HP treatment had less negative effects on the colour of oyster tissue than CP. HP-treated and CP oysters both had higher shucked meat yields (15.5 and 12.5 % respectively) than untreated oysters. One significant advantage of HP treatment over CP was that the former process opened the oyster and separated the muscle of the oyster from the shell.

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Misc Details:
Research Interests: Preservation and Hygiene (Mild preservation technologies)

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Misc Details:
Research Interests: Food Microbiology and Proteomics

Abstract or summary of benefits: Abstract or summary of benefits: Heat stress resistance in Lactobacillus plantarum
Maria De Angelis,1 Raffaella Di Cagno,2 Claude Huet,3 Carmine Crecchio,4 Patrick. F. Fox,5 and Marco Gobbetti2*
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ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

Heat stress resistance and response were studied in strains of Lactobacillus plantarum. Stationary phase cells of L. plantarum DPC2739 showed a D-value in sterile milk of 32.9, 14.7 and 7.14 sec at 60, 72 and 75°C, respectively. When mid-exponential phase cells were used, the D-values decreased. The Z-values ranged from 9 to 20°C, depending on the strain. A part of the cell population treated at 72°C for 90 sec recovered viability during incubation at 7°C in sterile milk for 20 days. When mid-exponential or stationary phase cells of L. plantarum DPC2739 were adapted to 42°C for 1 h, the heat resistance at 72°C for 90 sec increased ca. 3 and 2 log cycles, respectively. Heat-adapted cells also showed an increased growth at pH 5 and in the presence of 6% NaCl. Two-dimensional gel electrophoresis of protein expressed by control and heat-adapted cells showed changes in the level of expression of 31 and 18 proteins in mid-exponential and stationary phase cells, respectively. Twelve proteins were commonly induced. Nine proteins induced in the heat-adapted mid-exponential and/or stationary phase cells of L. plantarum DPC2739 were subjected to N-terminal sequencing. Proteins were identified as DnaK, GroEL, trigger factor, ribosomal proteins L1, L11, L31 and S6, DNA-binding protein II HlbA and CspC. All proteins have been found to play a role in the mechanisms of stress adaptation in other bacteria. Antibodies against GroES detected a protein which was induced moderately, while antibodies against DnaJ and GrpE reacted with proteins which did not vary the level of expression after heat-adaptation. This study showed that the heat resistance of L. plantarum is a complex process involving proteins with various roles in cell physiology: chaperone activity, ribosome stability, stringent response mediator, sensing temperature and control of ribosomal function. The physiological mechanisms of response to pasteurization in L. plantarum are fundamental for survival in cheese during manufacture.

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Misc Details:
Research Interests: High pressure processing for pasteurization & sterilization use, inactivation of microorganism, inactivation and stabilization of enzymes, inactivation of viruses, PCR methodology

Abstract or summary of benefits: High hydrostatic pressure effects on microbial stability in low acid beverages

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High pressure processing is rarely considered as a tool for industrial food preservation. This is related to high investment costs, the long processing times and consequently the difficulty to process large quantities in a continuous way at pressure levels higher than 500 MPa. In order to make the HP technology more applicable for an industrial and commercial use, a new concept for high pressure processing was developed which is characterized by very short processing times. The inactivation kinetics of Lactobacillus rhamnosus GG (LGG) in fruit juices as well as Lactobacillus brevis in lager beer have been tested in a range of 300 to 600 MPa and processing temperatures from 30 to 60°C. A new system for high pressure generation, with extremely fast compression and decompression rates was used to perform inactivation experiments in a range of treatment times from 0.5 to 60 seconds. Making use of a modified MPN (Most Probable Number) method, it was possible to rule out the ìtailingî and ìshoulderî phenomena which have been found in some experiments. A first order kinetic was found to accurately describe most inactivation kinetics. Higher treatment temperatures accelerated the inactivation. A pressure treatment of 600 MPa for less than 20 seconds was found to meet the safety demands of the consumers and to come to sufficient short processing times which allow high throughputs. In order to predict the pressure-temperature impact on the inactivation of LGG in different fruit juices and L. brevis in lager beer, mathematical models were performed for a wide range of processing conditions.

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Abstracts of Poster Presentations by Young Scientists

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Misc Details: Food microbiology in relation to mild
preservation techniques e.g. High-pressure

Abstract or summary of benefits:
I am a microbiologist.
By attending this seminar I hope to gain new practical insights in food microbiology in relation to mild preservation. I am interested in meeting scientists with the same interests and expertise and discuss with them about the subject in general.

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Misc Details: Food-born pathogens, High hidrostatic pressure, bacteriocins, Rapid dectection methods

Abstract or summary of benefits: Application of high hidrostatic pressure and biopreservatives (bacteriocins and lactate) as a new preservation alternative in cooked meat products.

Food safety in meat and meat products is under scrutiny for the consumers and one of the standards of the European Union is to implement high standards of food quality and safety. Cooked, packed and ready to eat products are of major concern as low levels of contamination during post-processing allow a quick development of food-born pathogens due to the absence of competitive endogenous microbiota and favourable physico-chemical parameters. High hydrostatic pressure (HHP) and biopreservatives are potential alternatives to reduce the effects of cross contamination. HHP allows treatment of the products at room temperature and allows preservation of the original quality parameters of the products. Among biopreservatives, lactate has been widely used in the meat industry for increasing flavour and safety and biopreservative potential and inhibitory effect of nisin and enterocin, alone and in combination with HHP has been shown. In this study, we have combined HHP and the biopreservatives nisin, enterocin and lactate to control the growth of L. monocytogenes and Salmonella in sliced and vacuum packed cooked ham during 84 days. HHP treatment at 400 MPa for 10 min at 17ºC produced an immediate decrease to L. monocytogenes and Salmonella counts. Salmonella maintained at low levels during 84 days of storage at both 1 or 6ºC. L. monocytogenes was inhibited in all kinds of cooked ham after HHP treatment for 42 days. However, after 84 storage days it could not be fully retained, especially if stored at 6ºC, where Listeria reached 106 ufc/g. At 1ºC storage, the pathogen was more retained in nisin and lactate treatments than in control ham.

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Misc Details: oil pumpkin and flax genotypes biodiversity; preservation, maintenance of collected genotypes; new utilization of rare used crops in the conditions of Slovakia; restoration of land races and old cultivars as rich material for production of new food products and for maintenance of narural and cultural heritage of our country

Abstract or summary of benefits: Some pumpkin (C. pepo) cultivars produce what is sometimes called ìnaked seedî (C. pepo convar. Citrullinina var. Styriaca).

The result of this inherited variant characteristic is very thin nearly transparent seed coat in place of the normally thick coat. The spontaneous hull less seeded mutant was segregated in Styria from the common species C. pepo (Rubatzky, Yamaguchi 1983; Teppner 2000; Winkler et al. 2000). According to science research the styrian pumpkin seed oil has a positive effect on health and effective influence to relieve prostate problems. Unsaturated oil made a stronger immunity system. Nutrition content of hull less seeds may be regarded as a health food of no or minimal processing well-suited for a properly balanced diet.
The aim of our research is detection of genotypes with seeds appropriate for another use and food processing technologies by the morphometrical analyze. In addition, we have also oriented our research for monitoring of health state of pumpkins plants during a vegetation, but also health state of fresh, dry and frozen hull less seeds. Besides of morphometrical analyzes we will deal with chemical and nutrition analyzes of seeds and seed oil of chosen genotypes, and so we will detect high quality seed genotypes of oil pumpkin. At the same time experimentally are checked conditions of storage and also appropriate conditions for providing safety food products.

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Misc Details: : Food microbiology
Food safety
Novel food preservation technologies (high-pressure, cold plasma)

Abstract or summary of benefits: poster abstract:
The MPN method is indispensable for evaluating spore inactivation by high-pressure
In microbiological kinetic studies, bacterial endospores are generally enumerated by plate count.

Incubation times are short, so spores with long lag times for germination are not detected. This may result in an overestimation of the number of inactivated spores.
In this study, a modified MPN method was developed for evaluating high-pressure spore inactivation. In this modified method, spores were diluted before treatment rather than after treatment. Additional advantages were that post-process contamination could not occur and treated spores were not exposed to oxygen. Results were compared with standard plate counts.
Clostridium sporogenes PA3679 spores were high-pressure treated under adiabatic-isobaric conditions (initial temperature 70ºC, applied pressure 800 MPa, single pulse) in a batch system.
The stabilization of the MPN during incubation took longer than standard incubation times for plate counts (up to 50 days, compared to 5 days for plate counts). The MPN score after incubation was considerably higher than the plate count immediately after the treatment.
These results indicate that some surviving spores that were detected by the modified MPN method were not detected by plate count. This may be caused by the longer lag times that sub-lethally damaged spores need for germination.
It is our opinion that the (modified) MPN method is indispensable for evaluating spore inactivation by high-pressure.

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Misc Details: : Flow cytometry,non thermal preservation technologies

Abstract or summary of benefits: Applicability of high-intensity ultrasound to inactivate Escherichia coli and Lactobacillus rhamnosus and to increase sensitivity against nisin

ABSTRACTS OF POSTER PRESENTATIONS BY YOUNG SCIENTISTS

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D. Knorr

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The inactivation behaviour of Escherichia coli (Gram negative bacteria) and Lactobacillus rhamnosus (Gram positive bacteria) in response to a high-intensity ultrasound treatment was examined. Classical plate count technique was applied to evaluate the loss of culturability on optimal growth media, whereas flow cytometric technique was performed to acknowledge the types of injury on cellular organelles of treated bacteria, which possibly contributed to the deficiency of recovery. According to plate count results E. coli (D-value 8 min) was far more sensitive than L. rhamnosus (D-value 18 min) in their response to the applied ultrasound. Significant differences were observed on the fluorescence pattern of ultrasound treated Gram positive and Gram negative bacteria. Ultrasound was highly effective in inducing damage on the lipopolysaccharide layer of the outer membrane of Gram negative bacteria. This rupture was visualized under utilization of fluorescence-based flow cytometric analysis. However, only a small population exposed to ultrasound (20 kHz, 17.6 W) up to 20 min showed indication of breakdown of cytoplasmic membrane. For Gram positive bacteria L. rhamnosus cFDA could freely diffuse across the cytoplasmic membrane, resulting in cells emitting green fluorescence. Similarly, the application of high-intensity ultrasound within the experimental conditions did not considerably affect cytoplasmic membrane either. It could be speculated that in absence of thermal effect ultrasound induced non-membrane related cell death. Moreover, the possibility to combine ultrasound processing with antimicrobials e.g. Nisin, whose bactericidal efficacy was reduced by the presence of an intact outer membrane was also explored.