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Edited by V. Gaukel
and W.E.L. Spieß

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A. Karl
T. Storck

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Preface

This supplement volume contains those manuscripts of papers presented as oral presentations or posters at the EU-Congress 'European Research towards Safer and Better Food' which had not been available at the time of printing of the congress proceedings volumes 1 and 2. With this supplement, all contributions - except for a few - are available in printed form, allowing to evaluate results achieved in the scientific segments Food, Nutrition and Well Being - Food Safety and Monitoring of Safety Aspects - Meat - Technological Methods to Improve Food Quality - Consumer Perception and Transfer Strategies supported by the EU-Framework Programmes. To facilitate access to the contributions, an author and a subject index were added. The present volume also contains some information about the congress as well as list of participants.

This supplement is also intended to reflect the organizers' satisfaction with, and gratitude for the congress which brought together so many European colleagues in a friendly atmosphere. The organizers wish to thank also many other European based scientific actions as, for example, COST Action '93 which contributed significantly to the success of the event. The presence of the congress participants at the reception and housewarming party in the new building of the Federal Research Centre for Nutrition has been regarded as a promising start of this institution into the future.

W.E.L. Spieß   V. Gaukel

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Keywords: food science, nutrition, Japan

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Session 1:

Food Safety and
Monitoring of Safety Aspects

Rapid detection methods for pathogens

D. Blivet, C. Soumet, G. Ermel, P. Colin

National Veterinary and Food Research Centre (CNEVA), B.P. 53, Zoopole-Beaucemaine
F-22440 PLOUFRAGAN, France

Abstract

The conventional methods for the detection of potentially pathogen bacteria in food products have been standardized. These methods are often very sensitive and specific and fit very well in official assessment ; but these conventional cultural techniques are labour intensive and time consuming. This is the reason why, in the food industry, some alternative and rapid methods have been developed. The aim of the development of these methods is to have a very quick answer, to be able to treat samples as soon as possible, without fastidious and heavy work, while keeping specificity and sensibility. Of course, these new methods should also be, if possible, cheaper !

In the last three decades, we observed a fantastic increase in the number of new rapid methods. Some of them are based on the change or adaptation of the conventional procedures to reduce the time of analysis. Also the measurement of the metabolic activity of microorganisms (impedance) can be used to detect pathogens by using appropriate selective media. Finally, immunological methods and, more recently DNA-based methods, including gene amplification procedure (P.C.R.), are probably the most popular. Many ELISA kits give very good results, comparable for specificity and sensitivity to conventional methods. The P.C.R. is a highly sensitive and specific procedure, which can be used to detect very small number of bacteria cells, but some compounds, present in samples may inhibit the reaction.

Among these procedures, it is now very easy, for the microbiologist, to choose the best one, or to combine different steps (immunocapture and P.C.R. for example) to increase the specificity and the sensitivity together with a quicker answer. Nevertheless the microbiologist has, before using a new rapid method, to compare with the standard one. In the same way, validations should be done before marketing.

Introduction

The detection of pathogen bacteria in food is always very important to guarantee the safety of the product.

Traditionally the microbial quality was evaluated by counting bacteria after decimal dilutions, or by using different procedures of specific enrichment and isolating media on selective agar to detect different microbial populations. These procedures take generally more than two or three days and consequently are not really adapted to a modern quality assurance policy. Some of them have been standardised by national or international organisms (A.F.N.O.R., C.E.N., F.I.L., I.S.O. ...) and can be considered as references because they are very sensitive and specific. Unfortunately, they need media, labor and time to have an answer, and also for the analysis ; they are rather expensive, and for this reason, not adapted to the analysis of a large number of samples.

In the last three decades, rapid methods were introduced in food microbiology to replace conventional procedures. Whatever the principle on which these methods are based, the following

criteria should be considered before choosing a given test : precision, rapidity, reproducibility, cost acceptability, simplicity of use, quality and stability of the reagents, training of the staff, reliability of the sale services, quality of the technical assistance, usefulness and the space required. Some of them are based on the improvement on sampling and sample preparation, or in automation, saving time and labour (Fung, 1992). For the detection of pathogens, especially *Salmonella*, these alternative methods can be classified by the principles on which they are based (van Der Zee and Huis In't Veld, 1997).

1 Modified conventional methods

Some procedures try to decrease the enrichment step. For example, a method for *Salmonella* detection has been described by Entis *et al.* (1982), using the HGMF (Hydrophobic Grid Membrane Filter) technique: after a pre-enrichment and a very short enrichment step (6 hours), samples are filtered through a membrane and incubated onto a selective agar medium. Using this procedure, they showed that *Salmonella* detection is better and faster (24 or 48 hours) in the presence of a great number of competitive bacteria.

De Smedt *et al.* (1991) used one specific character, the motility, combined with selective agar medium, to detect *Salmonella*, after a pre-enrichment and a short enrichment step (6 hours). This procedure (M.S.R.V.), based on the migration of mobile *Salmonella*, allow to save time (24 hours).

In the same way, different combinations of media for biochemical test can be used together in order to have more results in the same time ; for example, miniaturization (Fung, 1971), or Oxoïd *Salmonella* Rapid Test (O.S.R.T.) (Holbrook *et al.*, 1989) can give good results to detect *Salmonella*.

2 Bacterial metabolism measurement

In its initial status, a cultural medium is composed of uncharged or weakly charged substrates. If a bacterial population converts these substrates, the medium nature goes to the production of highly charged end products. The impediometry measures the conductance changes of the cultural medium, involved by bacterial metabolism. The conductance curve as a function of time is similar to a growth curve, and the start of exponential phase of the curve coincides with the detection time (DT). The DT is inversely proportional to the initial bacterial population which is present in the cultural medium, thus allowing a quantitative evaluation of the inoculum.

The conductance method can be applied to various analysis, in microbiology as well as in biochemistry. Up to date, the more significant developments have been observed in food analysis which requires the detection and/or the enumeration of micro-organisms. The most prevalent uses are the replacement of the enumeration by colony counting on agar surface for the analysis of raw materials or finished products, the estimation of food spoilage micro-organisms and the detection of groups of micro-organisms indicators of sanitary status, of both equipments and products. On another side, the attention for the detection of food pathogens such as *Salmonella* is increasing (table 1).

Table 1: Detection of pathogens in foods by using impedance microbiology

BACTERIA	PRODUCTS	REFERENCES
Coliforms	Meat	Martins and Selby, 1980
	Cheese	Khayat *et al.*, 1988
	Milk products	Madden and Gilmour, 1995
Campylobacter spp.	Poultry	Bolton, 1991
Listeria spp.	Milk products	Philipps and Griffiths, 1989
	Cheese	Hancock *et al.*, 1993 - Rodrigues *et al.*, 1995
Salmonella spp.	Meat	Di Falco *et al.*, 1993
	Poultry	Saco, 1993 - Blivet, 1998

Conductance technique offers both main advantages of rapidity and enumeration. In that way, many researchers have developed specific media for the detection of different food pathogens, as described above. However, we must bear in mind that the specificity of the method is limited to the only selectivity of the medium developed, and that is the reason why most impedance protocols have shown inadequate efficiency. Nowadays, impedance techniques are largely restricted to the enumeration of general flora such as total coliforms, faecal coliforms, *Enterobacteriaceae* or Yeasts and Moulds.

3 Immunological methods

The use of antibodies that will react with antigens on the surface of the organism to be detected, and with no other, has formed the basis of many detection systems. Antibodies may be poly- or monoclonal.

The most common kind of assay is called the Enzyme Linked ImmunoSorbent Assay (ELISA) ; an enzyme is linked to the antibody that targets the micro-organism to be detected. Following the antibody/antigen reaction, a chromogenic substrate is added. The change in colour in presence of the enzyme reveals the presence of the organism. Among the different assays developed, most are directed towards *Salmonella* detection. Many kits are available (D'Aoust, 1992): Salmonella-Tek, BacTrace, Tecra, but false positive reactions due to *C.freundii*, *E.coli*, *Pr.vulgaris*... are encountered (D'Aoust and Sewell 1988a, D'Aoust *et al.* 1991).

Immunological techniques can be separated into three groups, according to their objective: concentration, detection or confirmation (table 2).

An automated ELISA system has been developed, the Vitek Immuno Diagnostic Assay System (VIDAS). All ready-to-use reagents and the sample to be tested are on a special stick (Bourouina *et al.*, 1998). The solid receptacle is a pipette tip on which anti-*Salmonella* antibodies (four flagellar and two somatic) are adsorbed and this tip also serves as pipettor during the process. The different reagents are successively sucked up and released in a way to reach the transformation of a substrate (4-methyl-ombelliferin) in a fluorescent product (4-methyl-ombelliferone). Same systems have been developed for *E.coli* O157, *Listeria*, *Campylobacter* and staphylococcal enterotoxin detection.

Table 2: Commercial kits of immunological methods, dedicated to concentration, detection or confirmation of food pathogens.

BACTERIA	CONCENTRATION	DETECTION	CONFIRMATION
Salmonella spp.	DYNABEADS VIDAS-ICS	TECRA TRANSIA LOCATE VIDAS MASTAZYME	*Salmonella* Rapid Test *Salmonella* 1-2 Test SPECTATE
Listeria spp.	DYNABEADS LISTERSCREEN	TRANSIA VIDAS	*Listeria* RAPID Test
L. monocytogenes		VIDAS	
E. coli 0157	DYNABEADS		
Campylobacter spp.	*VIDAS-ICC**	VIDAS	

* in study

The 1-2 Test system for the *Salmonella* detection is based on immunodiffusion (D'Aoust and Sewell, 1988b). The pre-enrichment is introduced in the inoculation chamber which contains a tetrathionate-brilliant green broth, allowing the bacterial migration through the chamber which contains a non selective semi-solid medium. *Salmonella* are then immobilised in the second chamber by polyvalent flagellar anti-*Salmonella* antibodies, producing a immunodiffusion band after a 14 hours incubation.

The ImmunoMagnetic Separation (IMS) is a new concept based on the coating of magnetic beads by specific antibodies. This assay is usually associated to another technique later applied, such as microscopy, culture and enrichment, immunological assays, nucleid probes hybridisation, PCR or impediometry. Main disadvantages of this technique are a partial loose of cells and a lack of specificity due to cross reactions with « not so specific » antibodies.

The agglutination technique consists in the attachment of the antibodies on small latex beads which, in the presence of the micro-organism, clump together to form visible aggregates due to the reaction between the antibody and the antigen on the surface of the bacterial cell. Some kits for *Salmonella* detection are available: Bactigen, Microscreen, Spectate.

If all of these immunological methods can be considered as rapid (some minutes...), we must bear in mind that large numbers of cells are required for the antibody/antigen reaction to proceed, and this involves pre-enrichment of the food sample for several hours to obtain the desired number of cells. Moreover, problems of cross-reactions may be encountered.

4 Nucleic acid technology

The DNA probes provide specific microbial identifications and are used to replace biochemical and serological assays. Numerous DNA probes have been developed and commercial kits are now available (table 3). Gene-Trak first developed radioactive labelled probes, then systems composed of sticks (« cold probes ») which confer simplicity and safety to the method. Detection is based on the hybridisation of a polydeoxadenylic (poly-dA) tailed « capture » probe and a fluorescein labelled detector probe to the *Salmonella* ribosomal RNA. The 2 probes hybridise to adjacent regions on the

same target rRNA molecule. This target complex is captured onto a polystyrene « dipstick » coated with poly-dT and detected colorimetrically using an antifluorescein antibody conjugate of peroxidase. The system can detect the main pathogen bacteria in foods : *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, *E.coli*, *Yersinia enterocolitica*, *Campylobacter*.

Table 3: Commercial kits for food pathogen detection, based on nucleic acid technology

BACTERIA	DNA PROBES	P.C.R.
Salmonella spp.	Gene-Trak	Probelia
		BAX
Listeria spp.	Gene-Trak	
L. monocytogenes	Gene-Trak	Probelia
	Accuprobe	BAX
E.coli O157		BAX
Campylobacter spp.	*Gene-Trak**	*Probelia**
	Accuprobe	

* in study

Another procedure consists to amplify a specific region of the micro-organism genome by Polymerase Chain Reaction (P.C.R.). That method generates many thousands of copies of the original sequence in a matter of 1-4 hours. However, many foods contain substances that may interfere with the reaction and some pretreatment of the food sample is generally required before the assay can be performed reliably.

At the CNEVA Ploufragan, we developed a multiplex P.C.R. with three primers couples (Soumet *et al.*, 1996). For *Salmonella* detection, we have a specific and sensitive method to visualise the presence of *Salmonella* genus, *Salmonella* Typhimurium and *Salmonella* Enteritidis in the same assay. With the same approach, we are now able to specifically detect *Campylobacter* genus, *Campylobacter* jejuni and *Campylobacter* coli in poultry products.

The recent development of that technology lead to the commercialisation of kits : Probelia (Sanofi D.P.) for *Salmonella* detection and the BAX system (Qualicon) for *Salmonella*, *E.coli* 0157:H7 and *Listeria monocytogenes* detection. That is also known that some routine laboratories of food industry, or veterinary laboratories begin to use PCR based methods for the detection of specified pathogens. These PCR based methods are up-and-coming methods.

Validation

Even if rapid methods are sensitive and/or specific, they must be approved by an official department to certify their equivalence (or superiority) compared to standard methods. This guaranties a conviction in analysis results, in addition to the time saved. In France, 18 rapid methods have been validated by the AFNOR (French association of standardisation) (table 4).

Table 4: Rapid methods for pathogens detection validated by AFNOR (French association of standardisation) until June 1998

PATHOGEN	TYPE OF DETECTION	NAME OF COMMERCIAL KIT	SUPPLIER
Salmonella spp.	immunoenzymatic	TECRA	Bioentreprises / Australia
	immunoenzymatic	TRANSIA	Diffchamb / France
	immunological	*Salmonella* Rapid Test	Oxoid / U.K.
	immunoenzymatic	LOCATE	RPD / U.K.
	P.C.R.	PROBELIA	SANOFI D.P. / France
	immunological	*Salmonella* 1-2 Test	Biocontrol / U.S.A.
	immunoenzymatic	VIDAS	bioMérieux / France
	immunological	DYNABEADS	DYNAL / Norway.
	immunoenzymatic	MASTAZYME	Mast Diagnostic / U.K.
Listeria spp.	immunoenzymatic	TRANSIA	Diffchamb / France
	immunological	*Listeria* Rapid Test	Oxoid / U.K.
	immunological	LISTERSCREEN	VICAM / U.S.A.
	immunoenzymatic	VIDAS	bioMérieux / France
	molecular hybrid	GENE-TRAK	GENE-TRAK / U.S.A.
L. monocytogenes	immunoenzymatic	VIDAS	bioMérieux / France
	molecular hybrid.	GENE-TRAK	GENE-TRAK / U.S.A.
	molecular hybrid	PROBELIA (milk products)	SANOFI D.P. / France
E.coli 0157	immunological	DYNABEADS	DYNAL / Norway

Conclusion

The range of tests for the rapid detection of pathogen contamination in foods is vast and new technologies are emerging daily. Even if improvements may be achieved, and if the operator can combine some rapid methods together such as immunoconcentration with P.C.R., the following questions must stay in mind:

> what specificity level do I need?
>
> what sensitivity level do I need?
>
> what is the nature of my food products?
>
> is the cost of that method in relation with the number of samples I treat?
>
> do I have enough place in my lab to acquire a large apparatus?
>
> if my staff competent for very new technologies?

As all rapid methods are quite equivalent in efficacy, the user -by answering those questions- may be able to select the best method for his own needs.

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Quality management of food

St. Ditchev

Higher Institute of Food and Flavour Industries, Plovdiv, BG

Abstract

In this paper a special attention has been paid to the definition of "quality", its main characteristics, and the chain of responsibilities for food products quality.

The total quality management (TQM) has been presented as an integrated approach and as the main business strategy of the company that require a revolution of thought in the field of management. The series of standards ISO 9000, conception HACCP and GMP has been presented as one of the powerful levers for quality assurance in food industries.

Introduction

Food quality cannot be easily scientifically defined, because it has many qualitative criteria. On other hand the evaluation criteria for food quality are controversial to a great extent among the different groups of interest - producers, processors, tradesmen and consumers.

A certain perplexion has arisen in the understanding of the terms quality control, quality assurance, quality management and total quality management, as well. We shall try to clear up these concepts by means of the International standard ISO 8402 and publications in this field (Leitzmann, 1993; Paulus ,1993).

For several years now, the Commission to the European Union has been developing a global approach strategy for stimulation of industrial enterprises that have to take the main responsibility for production quality. In this sense, the series of standards ISO 9000 acquire greater and greater significance for quality guarantee, irrespective of the separate branches of industry. However, we should bare in mind the necessity of adaptation of these general standards to specificity of each branch, particularly as regards food industry. The series of standards ISO 9000, conception HACCP and GMP can be used as an integrated approach for quality assurance.

The total quality management (TQM) should be treated as an integrated approach and as the main business strategy of the companies that require a revolution of thought in the field of management.

Development of definition for food products quality

The shortest definition for quality is a pursuit of "Suitability for use".

According to the International standard ISO 8402 (1994), the term "quality" in its generally accessible use has been defined as the totality of features and characteristics of the product that bear on its ability to satisfy stated or implied needs, i.e. the product should correspond to the requirements of those who will use it.

(Leitzmann , 1993) describes in details the definition for "food products quality" and gives a generalized evaluation for foods quality by means of a great number of criteria.

As we have already noted, it is difficult to make a strict scientific definition of "food quality" because in their qualitative evaluation are included many qualitative criteria, i.e., quality can be determined by a great number of (objectively or subjectively) available criteria. Quality can be discussed from a different point of view according to the specific interests of the user and consumer.

For example, the main criterion for evaluation of a product quality for the producers is the yield and crop, for the processors - the technological characteristics, for the traders - the shelf-life of the product, and for the consumers - the flavour characteristics and safety.

The complex and in a way a controversial interconnection between food quality and user, (producer, processor, trader) on one hand and consumer on the other, have been presented schematically on Fig.1 as a complex functioning system with a direct connection and feedback.

In this system there is a direct connection and a feedback between product quality and user and consumer. The feedback as a requirement towards quality have a positive, regulating effect to food products quality.

Having in mind the above-mentioned circumstances, we can note that defining food products quality has its own evolution (Fig. 2.)
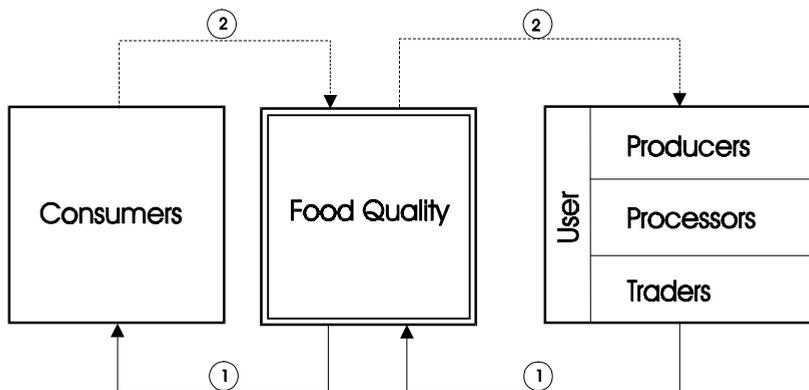

Fig. 1: A diagram of interconnection between product quality, consumer and producer: 1 - direct connection; 2 - feedback

Qualitative criteria for food

The food quality has been determined at the beginning mainly by means of their energetic content. Today this index continues to be used as a qualitative criterion but having a different value: for the developing countries it is higher, while for the developed countries it is lower. At present, food quality is determined by a great number of criteria which have been united in different value groups (groups of qualitative criteria) - transitional (main) and additional. The traditional qualitative criteria have been united in three value groups: suitability, sensory and health; and the additional qualitative criteria - in the following four value groups: psychological, social, political and ecological.

The qualitative criteria for food products which have been described (Leitzmann , 1993) can be presented in the process of development on three levels (Fig. 2) of their qualitative evaluation.

The total set of quality attributes of food products united in value groups and groups of interest have been presented in Table 1.
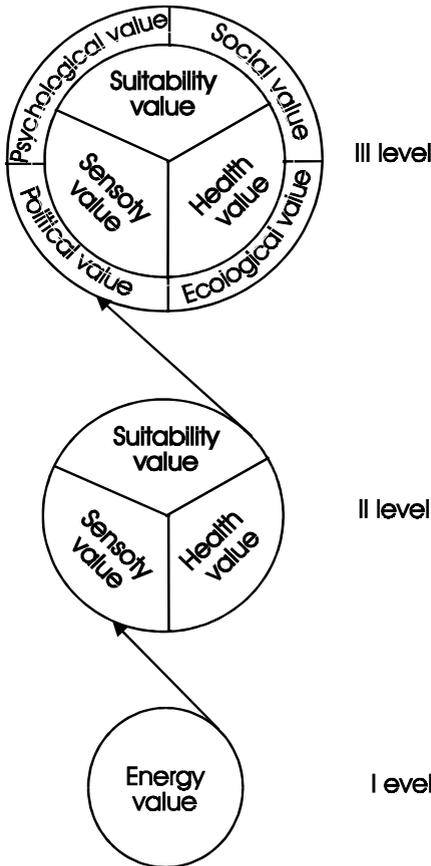
Fig. 2: Development spiral of the qualitative evaluation of food

MANAGEMENT AND QUALITY ASSURANCE

The quality management can definitely be defined as a science for quality that includes methods and techniques for quality management.

The quality liability should be by everybody and for everything. The liability of product quality lies upon the total organization and company's activity, namely: planning, marketing, and sales of production; development of production; processing; purchasing; management of production activity; control and testing; packing and market delivery; service of consumers; advertisement activity; legislative activity.

A guarantee for product quality stability are quality assurance systems. These systems can be built on the basis of the standard series ISO 9000, in combination with the system "Good Manufacturing Practice" (GMP) and/or the conception "Hazard Analysis and Control of Critical Points" (HACCP). The standards of the series ISO 9000 represent in total the best achievements and experience in the field of quality management and assist companies when utilizing a complex approach in product quality assurance.

The standards of the series ISO 9000 represent an integrated concept of the world for quality systems which endure a guaranteed quality of production, and confidence in the consumer.

The main elements of quality system in the standard series ISO 9000 have been presented in Table 2.

The HACCP system, which is science based and systematic, identifies specific hazards and measures for their control to ensure the safety of food. HACCP is a tool to assess hazards and establish control systems that focus on prevention rather than relying mainly on end-product testing. Any HACCP system is capable of accommodating change, such as advances in equipment design, processing procedures or technological developments.

HACCP can be applied throughout the food chain from the primary producer to final consumer and its implementation should be guided by scientific evidence of risks to human health.

The successful application of HACCP requires the full commitment and involvement of management and the workforce. It also requires a multidisciplinary approach. HACCP is a management technique which primarily used to address microbiological risks in the food supply, but it is one advantageous technique for assuring that the foods we eat are free of microbiological, chemical and physical hazards. The application of HACCP is compatible with the implementation of quality management systems, such as the ISO 9000 series, and is the system of choice in the management of food safety within such systems.

Table 1: Quality criteria of food

FOOD QUALITY							
Basal quality criteria			**Additional quality criteria**				
I group	II group	III group	I group	II group	II group	IV group	

Basal quality criteria:

I group (Group of interest)

- Producers: - yield; - harvest attributes; - stability; - disposability; - market value
- Processors: - properties for processing; - technological characteristics
- Traders: - transport quality; - stability; - disposability; - surface condition
- Consumers: - degree of processing; - stability; - suitability for certain usage

II group: - appearance (form, color); - small; - taste; - consistency; - temperature; - ripeness; - cleanness; - freshness

III group: - essential nutrients; - density of essential nutrients; - macro nutrient content; - energy content; - energy density; - satiety effect; - digestibility; - compatibility; - microorganism content; - toxicity; - freshness

Additional quality criteria:

I group: - concepts; - opinions; - expectations; - supposed properties; - sham satisfaction; - attraction; - advertizing

II group: - prestige of food; - compensation; - taboos; - entertainment; - experience; - emblence; - example for others

II group: - Import; - export; - food surplus; - food aid; - food as a weapon

IV group: - effects of food production on the enviroment; - effects of the processing of food by the food industry; - effects of food marketing (energy and material demands for transport, storage, advertizing)

Table 2: A list of quality system elements in the series standards ISO 9000

• Management responsibility	• Control of measuring and test equipment (inspection, measuring and test equipment)
• Quality system principles	
• Auditing the quality system (internal)	• Nonconformity (Control of nonconforming product)
• Economics - Quality-related cost considerations	
• Quality in marketing (Contract review)	• Corrective action
• Quality in specification and design (Design control)	• Handing and post-production functions (Handing, storage packaging and delivery)
• Quality in procurement (Purchasing)	• After-sales servicing
• Quality in production (Process control)	• Quality documentation and records (Document control)
• Control of production	
• Material control and traceability (Product identification and traceability)	• Quality records
	• Personnel (Training)
• Control of verification status (inspection and test status)	• Product safety and liability
	• Use of statistical methods (Statistical techniques)
• Product verification (inspection and testing)	• Purchaser supplied product

The successful application of HACCP requires the full commitment and involvement of management and the workforce. It also requires a multidisciplinary approach. HACCP is a management technique which primarily used to address microbiological risks in the food supply, but it is one advantageous technique for assuring that the foods we eat are free of microbiological, chemical and physical hazards. The application of HACCP is compatible with the implementation of quality management

systems, such as the ISO 9000 series, and is the system of choice in the management of food safety within such systems.

The HACCP system consist of seven principles (Diagram 1). The application of HACCP principles consist of the following tasks as identified in the Logic Sequence for Application of HACCP (Diagram 2). Example of a decision tree to identify Critical Control Points (CCPS) and answer questions in sequence was showed at Diagram 3.

The concept "Good Manufacturing Practice is designed to achieve food quality control".

Good Manufacturing Practice may in turn be viewed as having two complementary components, namely effective manufacturing operations, and the effective exercise of quality control.

Important requirements of the GMP system for quality assurance:

1. BUILDINGS AND EQUIPMENT
   - factory ground (location and environment)
   - existing construction
   - equipment
   - hygienic measures and pests control
   - sanitary equipment
   - water-supply
   - waste and environmental protection

2. MANUFACTURING EQUIPMENT
- design
- reliability
- cleaning and disinfecting
- process control and regulation

3. PRODUCT MANUFACTURE
   - raw and supplemental materials
   - methods and processes

4. STORAGE AND DISTRIBUTION

5. DEFFECTS

6. LABORATORY TESTS

7. DEFFECT ANALYSIS

8. SYSTEM FOR PRODUCT RECALL

9. PERSONNEL AND TRAINING

10. RECORDS AND DOCUMENTATION

Good Manufacturing Practice is not a static concept, but an evolutionary mechanism by which overall improvements can be made and maintained.

Diagram 1. Principels of HACCP system

1. Conduct a hazard analysis

2. Determine the Critical Control Points (CCPs)

3. Establish critical limit (s)

4. Establish a system to monitor control of the CCP

5. Establish the corrective action

6. Establish procedures for verification to confirm that the HACCP system is working effectively

7. Establish documentation concerning all procedures appropriate to these principles
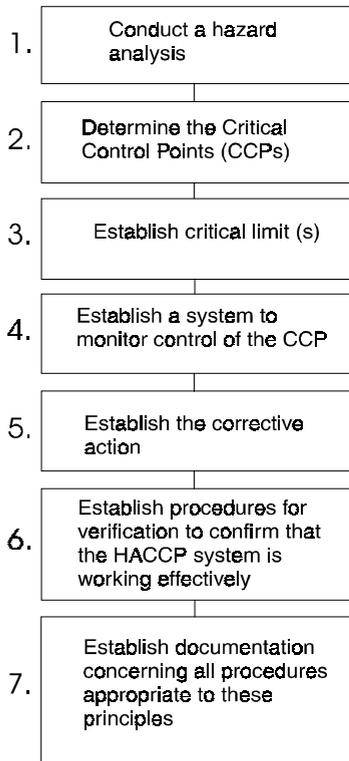
Diagram 2. Tasks sequence for application of HACCP

1. Assemble the HACCP team

2. Describe the product

3. Identify intended use

4. Construct Flow Diagram

5. On-site confirmation of Flow Diagram

6. List all potential hazards Conduct a hazard analysis Determine control measures

7. Determine CCPs

8. Establish Critical Limits for each CCP

9. Establish a monitoring system for each CCP

10. Establish corrective action for deviations that may occur

11. Establish verification procedures

12. Establish documentation and record keeping

For quality assurance greater emphasis should be played as well on statistical process control (SPC) and on statistical quality control (SQC).

To interpret all these quality systems methods and techniques it is most important to develop quality circle programmes or quality improvement teams to encourage employee participation in improving food quality and safety.

In the current food industry we are somewhere between the old-fashioned system of quality control and the described system of quality assurance. But there is another step to be taken- total quality management (TQM), as it can be seen from other areas of industrial production. TQM is an approach which involves managing all phases in a business for quality (Fulks, 1991)

The total quality management is the most advanced field of management which requires educational activity on all levels - from the manager to the worker at the department or production line.

Diagram 3. Example of a Decision Tree to Identify CCPs
(answer questions in sequence)

Q_1 Do control measure(s) exist?

Modify step,
process or
product

Yes   No

Is control at this step
necessaty for safety?

Yes

No   Not a CCP   Stop(*)

Q_2 Is the step specifically designed
to eliminate or reduce the likely
occurrence of a hazard to an
acceptable level?

Yes

No

Q_3 Could contamination with
identified hazard(s) occur in excess
of acceptable level(s) ot could
these increase to unacceptable levels?

Yes   No   Not a CCP   Stop(*)

Q_4 Will a subsequent step eliminate
identified hazard(s) or reduce likely
occurrence to an acceptable level?

Yes   No

Critical Control
Point

Not a CCP   Stop(*)

On Fig. 3 the managing task has been presented by us as the cross of quality management that should be carried equally by ass - from the manager of the company to the worker.

The top in quality management is total quality management (TQM). TQM is an integrated approach for managing a company (organization), oriented towards quality, based on the participation of all its members directed towards achieving a long-lasting success for satisfying the customer and benefit for the members of the company and the society.

Fig. 3: Cross of quality management

Conclusion

Quality assurance is not the end. Quality assurance is quality management and most probably the term quality assurance will be replaced by the term quality management in the future to underline that it is of utmost importance that management is involved.

If TQM is something like a vision in the food field today, all our efforts should be devoted to realizing this vision as soon as possible (Paulus, 1993).

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Hygienic aspects of ion exchange resins used to reduce the nitrate content in spinach blanching water

I. Kristanti, E. Mayer-Miebach, W.E.L. Spieß

Institute of Process Engineering, Federal Research Centre for Nutrition, Haid-und-Neu-Strasse 9, D-76131 Karlsruhe, Germany , Phone/Fax: +49 721 6625-65 /111
ivt@bfe.uni-karlsruhe.de http://www.dainet.de/bfe

Abstract

Ion exchange resins are suitable to reduce the nitrate content of spinach blanching water selectively during production of deep-frozen spinach. Recycling of the nitrate-free blanching water minimizes leaching of nutrients from spinach and saves cost of water and energy. This new application to food processing was investigated in a pilot plant, where ion exchange resins were used at 60°C. Influences of the process on the hygiene of the products in case of a strong base anion-exchange resin, Imac HP 555, are discussed.

For hygienic studies a thermophilic bacterial strain, isolated from blanched spinach, was used in a model system. Bacterial cells were incubated in a growth medium with and without the resin. Bacterial growth in the resin containing system was inhibited after an hour of contact. However, when inert materials with large surfaces, for example glass beads, had been added together with the resin, the bacteria were growing again. After 24 hours, the number of cells suspended in the growth medium was even higher than in a control sample with neither glass beads nor resins. Bacterial growth was promoted by the inert material providing surfaces to which the cells could attach. This 'protective effect' may also occur in practice as spinach blanching water usually contains plant particles, providing surfaces for bacterial growth.

Introduction

Ion exchange resins are suitable to reduce the nitrate content of spinach blanching water selectively during production of deep-frozen spinach. Bacteria with a net negative surface charge strongly attach to positively charged sites of anion exchangers [1]. However, resins even disinfect the treated fluid if the counter ions used to exchange nitrate ions are bactericidal substances such as iodine [2]. In the present study a strong base macroporous anion-exchange resin, Imac HP 555, was used to investigate the hygienic status of the nitrate removal process.

Materials and methods

For hygienic studies a thermophilic *Bacillus stearothermophilus* strain, isolated from blanched spinach, was used in a model system. The bacteria were cultivated in nutrient broth supplemented with 10 mg $MnSO_4 \cdot H_2O$ per litre of distilled water at pH 7.0 and 25° C. Each 40 ml of the bacterial culture from the exponential phase of growth with counts of about 10^6 cfu/ml were mixed with: (a) 20 ml of resin, (b) 20 ml of glass beads with a diameter of 3 mm, (c) 20 ml of resin and glass beads each. These mixtures and a control culture without resin and glass beads were incubated by shaking at 60° C. Viable counts of suspended bacteria were determined directly and after one, four and 24 hours of incubation. Sterile glass beads were used as a positive control of adsorbability. Electron micrographs of the resin and the glass beads were taken with a Stereoscan 200.

Results

After one hour of contact, the bacteria in the resin containing sample had not only been inactivated but partially eliminated. If inert materials such as glass beads were incubated together with the resin, the viable counts of suspended bacteria determined after four and 24 hours of contact were comparable to the control samples. Viable counts of bacteria suspended in the resin containing sample decreased further (fig. 1).
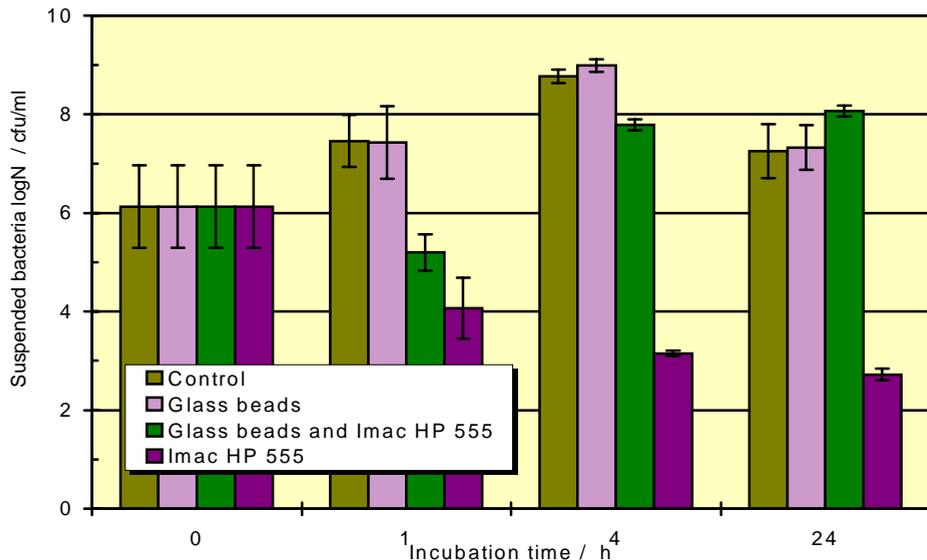

Fig. 1: Bacterial growth during contact with resin and glass beads.

As shown by electron micrographs (fig. 2) bacteria grew preferably on the glass beads, if glass beads and resin were present. This 'protective effect' of inert materials may occur also in practice because spinach blanching water usually contains crude plant particles, providing surfaces for microbial growth.

(a)   (b)   (c)
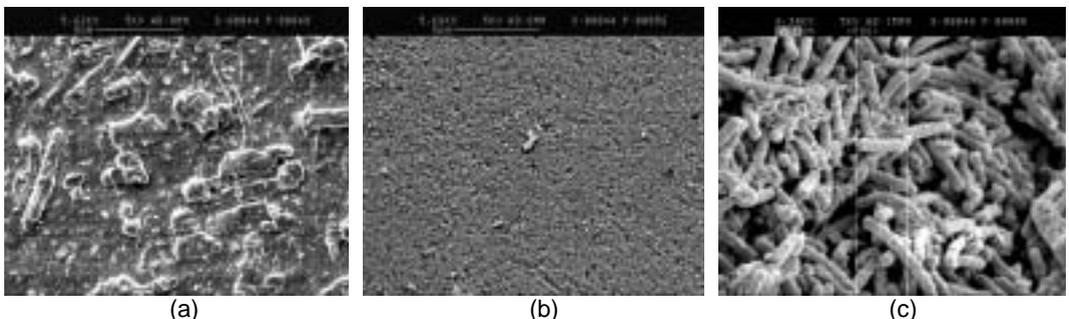
Fig. 2: Electron micrographs of (a) bacterial cells (control), surfaces of (b) resin and (c) glass beads after 24 hours of contact

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Thermal inactivation of microorganisms during blanching of spinach in an industrial blancher

E. Mayer-Miebach, B. Zanoni*, W.E.L. Spiess

Federal Research Centre for Nutrition, Institute of Process Engineering
Haid-und-Neu-Strasse 9, D-76131 Karlsruhe, Germany
Phone/Fax: +49 721 6625-345 /111, ivt@bfe.uni-karlsruhe.de, http://www.dainet.de/bfe

*diSTAM, Università degli Studi di Milano, Phone/Fax +39 (0)2-706-02063/38625
bruno.zanoni@unimi.it, www.distam.unimi.it/distam

Abstract

In previous experiments, thermal inactivation of microorganisms which are associated with spinach had been studied. Using the data obtained, a predictive model was set up which calculates total counts of about 4,000 colony forming units per gram of material (cfu/g) at time/temperature conditions typical of industrial processes. The predicted surviving rate was expected to correspond also to the counts of endospore forming microorganisms which are detectable in the raw material after sublethal thermal treatment to activate spore germination. To transfer this model based on laboratory data to the industrial practice, it was verified by real processing data. Under industrial blanching conditions, the mean counts of endospore forming bacteria on the raw material of 7.820 cfu/g were reduced by 1 - 2 decimal units. This result is not in accordance with the predicted values. The model had been exclusively based on the thermal inactivation data of the thermoresistant *Bacillus stearothermophilus*. Industrial blanching processes fail to inactivate the endospores of this organism, which even survive sterilization conditions. When thermal inactivation data of both mesophilic and thermophilic endospore forming Bacillus sp. are taken into account, experimental and predicted data correspond.

Introduction

Thermal inactivation of microorganisms which are associated with spinach had previously been studied on a laboratory scale. A predictive model was set up (1) which calculated

- a surviving rate of 4,000 cfu/g

- corresponding to endospore counts.

A verification of this model by processing data from an industrial waterbath blancher is presented.

Material and methods

The washed raw produce is transported, on a conveyor belt, through a blanching waterbath to which water vapour is continuously added, cooled down, cut and stored for a short period until packaging and freezing. Within a period of 3.5 hours about 50 samples of the raw produce and produce leaving the blancher were withdrawn and homogenated directly in chilled saline. Total mesophilic counts were analysed according to the agarspot assay or after sterile filtration. Sample homogenates were incubated on Plate Count Agar (Merck, Germany) at 30°C under aerobic conditions. To determine endospore counts, homogenates were subsequently heated to 70°C for 10 minutes before plating and incubation. The water temperatures of the blanching bath were routinely checked by fixed sensors while the time/temperature profiles of the produce were measured by inserting a probe carried along with the produce.

Results and conclusion

Raw spinach was uniformly contaminated by 1.3×10^7 cfu/g mesophilic microorganisms on the average. Endospore counts of 7,820 cfu/g with a standard deviation of ca. 70 % fluctuated strongly. Thermal treatment during blanching reduced total counts by 4 - 5 and endospore counts by ca. 2 decimal units. There was no significant difference calculated between total and endospore counts in the blanched produce(fig. 1).
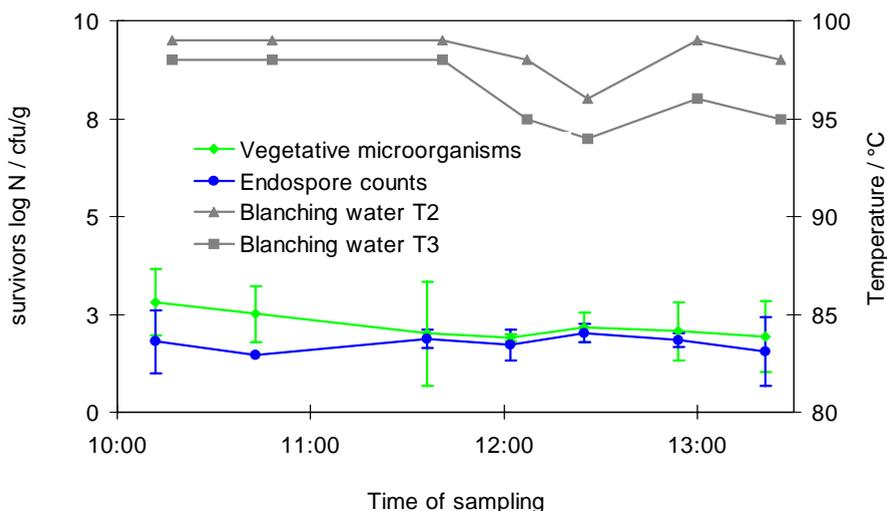
Figure 1: Total counts and endospore counts in the blanched produce (at the end of the conveyer belt) as a function of temperature (T2, T3 are the measuring sites, 95 % confidence of mean range indicated)

This is in accordance with the first model based exclusively on thermal inactivation data of *Pseudomonas fluorescens* and *Bacillus stearothermophilus* ($D_{55°C} = 6$ min, $z = 5°C$; $D_{121°C} = 5$ min, $z = 10°C$) for vegetative and endospore forming organisms. But the reduction of endospore counts in the industrial plant had not been predicted correctly.

Industrial blanching processes fail to inactivate the thermoresistant *B. stearothermophilus* which even survives sterilization conditions. If the model parameters are combined with thermal inactivation data of the mesophilic endospore forming bacteria *B. macerans* ($D_{100°C} = 0.1$ min.; $z = 9°C$) experimental and predicted data correspond (table 1).

Neither inactivation of vegetative organisms nor endospore inactivation depended directly on the water temperature of the blancher (fig. 1). Temperatures within the produce during blanching were on average 3-5°C lower.

Table 1: Comarison of experimental and predicted microbial data

blanching profiles*	predicted values / cfu/g		
	total survivors	*B. stearo-thermophilus*	*B. macerans*
80°C / 60 s **	4,149	241	3,908
87.6°C / 350 s	2,596	242	2,354
90.2°C / 330 s	1,420	243	1,177
91.6°C / 340 s	1,566	242	1,324
94.6 / 660 s	243	242	1
96.2 / 455 s	243	242	1
mean profile			
92°C / 280 s	249	241	8
	experimental values / cfu/g		
92°C / 280 s	270	--	--

*   measured within the blanched produce

** parameter in the first model

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Rapid sol particle immunoassay for the detection of aflatoxin in food products

M.J.F. Niessen[1], J.H. Wichers[1], H.A. Lee [2], M. Alcocer [2], M.R.A. Morgan [2], A. van Amerongen[1]*

[1] Agrotechnological Research Institute (ATO-DLO), P.O. Box 17, 6700 AA Wageningen, The Netherlands; *corresponding author
[2] Institute of Food Research (IFR), Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7 UA, UK

Abstract

A lateral flow one-step Sol Particle ImmunoAssay is described for the detection of aflatoxin B1 in peanuts and maize. This assay is relatively rapid and simple. Signals are generated by colloidal carbon particles onto which a monoclonal antibody specific for aflatoxins has been coupled.

A detection limit of 0.1 ppb (ng/ml) aflatoxin B1 could be achieved in buffer. In extracts of blank sample spiked with aflatoxin B1 the detection limit was 10 ppb (ng/ml).

Samples extracted with either acetonitrile or methanol had to be diluted at least 50 times to eliminate the disturbing effects on assay performance. Because of the dilution step, further research on the present aflatoxin B1 SPIA will be required in order to meet legal regulations.

Introduction

Aflatoxins are a group of toxic and highly carcinogenic secondary metabolites produced and excreted by the moulds *Aspergillus flavus* and *A. parasiticus* under conditions of high temperature and humidity. There are 4 known types of aflatoxin: B1, B2, G1 and G2 (see figure 1). Aflatoxin B1 is the most toxic and in mammals, the liver is the primary target organ for acute injury.

A major metabolic product of aflatoxin B1 is aflatoxin M1 that is usually excreted in the milk and urine of mammals. The primary route of potential human exposure to aflatoxin is ingestion of contaminated food, such as cereals, nuts (e.g. pistachio) and dairy products.

In recent years, much attention has been paid towards the safety and quality of food and feed. Important issues in this respect are pathogenic micro-organisms, their (toxic) secondary metabolic products, natural toxins, contamination with pesticides and additives. For many of these compounds, the legal maximum residue levels (MRLs) are stated by both national and international agencies.

The UK Ministry of Agriculture, Fisheries and Food (MAFF) has proposed that nuts and nut products destined for human consumption should not contain more than 4 ppb (ng/g) of total aflatoxin. Within the European Community, the aflatoxin B1 level is regulated at the 2 ppb level.

Accordingly, there is a need for internationally acceptable, rapid, reliable, sensitive and cost-effective assays for the assessment of these compounds in food(products). Classical biochemical techniques involve the use of paper and thin-layer chromatography, high performance liquid chromatography, gas chromatography and mass spectrometry, coupled with a variety of detection systems. However, the cost of equipment and consumables and the level of experience and technical skills preclude their use in on-line routine monitoring of food quality. Immunoassay techniques provide complementary and/or alternative approaches in reducing the use of sophisticated equipment and analysis time.
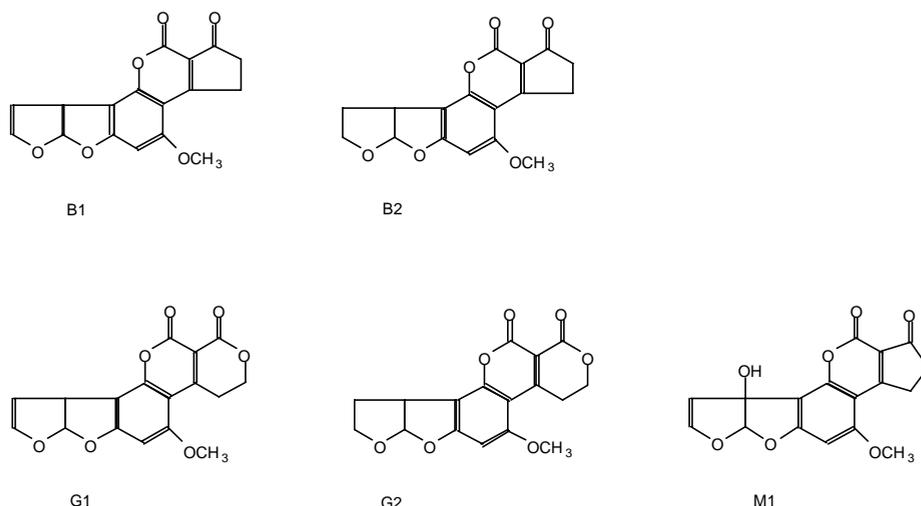
Figure 1: Structures of different types of aflatoxin

In this article, the development of a lateral flow one-step Sol Particle ImmunoAssay (SPIA) is described for the detection of aflatoxin B1 in e.g. nuts, peanuts and maize. Based on monoclonal antibody technology, the aflatoxin B1 SPIA is characterised by its high specificity, rapidity and ease of assay performance.

Materials and methods

Materials

0.2%($^w/_v$) colloidal carbon particles in 5 mM borate buffer pH 8.5

Monoclonal antibody specific for aflatoxins (primarily G1 and B1), developed in rat[6]

BSA-aflatoxin B1	(Sigma, cat# A-6655)
Aflatoxin B1	(Sigma, cat# A-6636)
Goat-anti-Rat IgG	(Sigma, cat# A-5005)
Nitrocellulose membrane	(AE 99, ϕ=8 μm, Schleicher & Schuell)
Plastic backing	(Mylar AD Back 79373, Schleicher & Schuell)
Washing buffer (conjugate):	5 mM Tris-HCl pH 8.5 + 0.02 %($^w/_v$) NaN$_3$ + 1 %($^w/_v$) BSA (ICN, cat#105033)
Washing buffer (membranes):	5 mM Na$_2$HPO$_4$ pH 7.5 + 0.01%($^w/_v$) SDS
Spotting buffer:	0.1 M borate buffer pH 8.8 + 0.02 %($^w/_v$) NaN$_3$
Blocking buffer:	0.1 M borate buffer pH 8.8 + 0.02 %($^w/_v$) NaN$_3$ + 0.05 %($^v/_v$)Tween-20 + 5 %($^w/_v$) sucrose + blocking agent
Running buffer:	0.1 M borate buffer pH 8.8 + 0.02 %($^w/_v$) NaN$_3$ + 0.05 %($^v/_v$)Tween-20

Preparation of detection conjugate

A suspension of colloidal carbon particles (C-sol) was used for the preparation of the detection conjugate. The C-sol was sonified for 1 minute with a Branson Model 250 Sonifier with an output of 27 Watt, 20 kHz.

A rat monoclonal antibody, specific for aflatoxins in 5 mM borate buffer pH 8.5 was added in a ratio of 350 μg of antibodies to 1 ml of C-sol. This mixture was stirred for at least 3 hours at room temperature in the dark.

After incubation, the conjugate was washed with washing buffer for conjugate and centrifuged for 15 minutes at 13,636 x *g*. This washing step was repeated 3 times. After the last washing step the pellet was resuspended with 5 mM borate buffer pH 8.5 in the original volume of C-sol.

Preparation of lateral flow strips

A nitrocellulose membrane was supplied with a plastic backing to prevent breakage.

A quantity of 10 ng of BSA-aflatoxin B1 (capture ligand) per strip in spotting buffer was spotted at a height of 27 mm from the bottom of the membrane with a Camag Linomat IV spotter.

In the same way, a quantity of 20 ng of BSA-aflatoxin B1 in spotting buffer + 10 %($^v/_v$) methanol was spotted on a different piece of membrane.

In both cases, a control line was sprayed 3 mm above the test line with 250 ng of Goat-anti-Rat IgG per strip, in the same spotting buffer as BSA-aflatoxin B1.

After spotting the proteins on the nitrocellulose, the membranes were dried for 16 hours at 37° C.

Additionally, some of the membranes were blocked for 10 minutes at room temperature with blocking buffer + 1 %($^w/_v$) BSA. The membranes for which methanol had been used in the spotting buffer, were blocked with blocking buffer + 1 %($^w/_v$) polyvinyl-pyrrolidone (PVP) 40 kD. After blocking, these membranes were washed with washing buffer for 5 minutes at room temperature. Some of the membranes for which methanol had been used in the spotting buffer, were kept unblocked.

Membranes were dried overnight at 37° C. After drying, the individual strips (size 45 x 7 mm) were sealed in an aluminium package with a desiccant pellet. After incubation for 2 days at 37° C the packages were stored at room temperature.

Principle of SPIA

In SPIA, the capture ligand is spotted onto nitrocellulose strips in a line-format. The strips (1) are mounted in a vertical position on a perspex bridge (2). On the top end of the strip a piece of filter paper (3) is assembled to serve as a fluid drain. The other end of the strip is positioned against a droplet (4) pipetted onto a piece of parafilm (5) (see figure 2). This droplet contains a detection ligand coupled to colloidal carbon particles. The fluid in the droplet is run chromatographically through the membrane by capillary force. Upon passing the immobilised capture ligand the detection ligand on the colloidal conjugate can specifically bind, resulting in the formation of a visual signal, i.e., a black line.

In a competitive format (as used in this study) the droplet of fluid also contains the target molecule of interest (in a pure form or as part of a sample). During the run, the detection ligand will bind the target molecule. This will result in binding of less colloidal particle / detection ligand conjugate to the capture ligand and, as a consequence, in loss of signal intensity.

1. Nitrocellulose strip
2. Perspex bridge
3. Filter paper
4. Droplet (carbon particle / detection ligand conjugate ± target molecule)
5. Parafilm
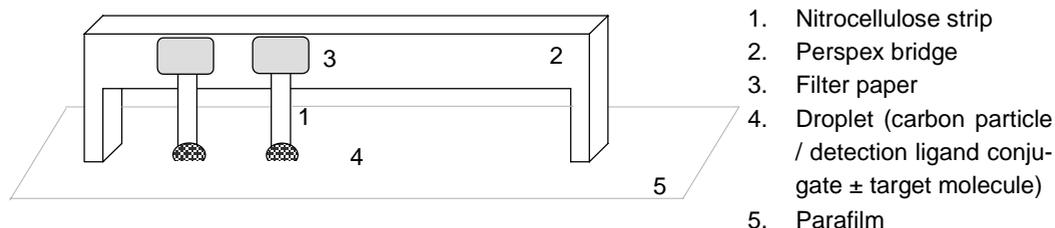
Figure 2: Scheme for experimental SPIA set-up

Sample preparation

A reference blank sample of nutpowder was from the Food Analysis Performance Assessment Scheme (FAPAS), CSL Food Science Laboratory (Norwich Research Park, Colney, Norwich, UK).

In order to extract the aflatoxins from the samples, 5 g of sample was homogenized with 25 ml of acetonitrile/H_2O (1:1) for 1 minute in an Ultra-Turrax at 13,500 rpm. The suspension was filtered through Whatman No. 1 paper under vacuum. The filtrate was diluted 2 times with 0.2 M borate buffer pH 8.8 + 0.02 %($^w/_v$) NaN_3 and stored at 4° C.

As an alternative, extraction with methanol was used according to the AOAC Official Method 990.34. Briefly, 5 g of sample was homogenized with 10 ml of methanol/H_2O (4:1) for 1 minute in an Ultra-Turrax at 13,500 rpm. The mixture was kept at room temperature for at least 15 minutes and the supernatant was recovered.

Assay procedure

Running buffer spiked with aflatoxin B1

The strips, blocked with 1 %($^w/_v$) BSA, were run on a bridge with 100 µl of running buffer spiked with a serial dilution of free aflatoxin B1 from 0 – 1000 ppb (ng/ml).

To each sample 1 µl of sonified detection conjugate was added.

Matrix influence of extract

A sample of nutpowder without aflatoxins was extracted with either acetonitrile/H_2O or methanol/H_2O. The extracts of blank sample were diluted 2, 10, 25 or 50 times in the particular running buffer and were assayed on a bridge in three different set-ups. (see table 1)

Table 1: Three different set-ups for testing matrix influence of extract

	Set-up 1	Set-up 2	Set-up 3
Blocking agent	1%($^w/_v$) BSA	1%($^w/_v$) PVP	unblocked
Running buffer	standard	0.5%($^w/_v$) PVP	1%($^w/_v$) BSA

Extract spiked with aflatoxin B1

An acetonitrile/H_2O extract of blank nutpowder was diluted 50 times in running buffer + 1 %($^w/_v$) BSA. The diluted extract was spiked with a dilution range of aflatoxin B1 from 0 – 7812 ppb (ng/ml) and was assayed on unblocked strips with 20 ng of BSA-aflatoxin B1 on a bridge.

When all of the running buffer had run into the strip, another 100 µl of running buffer was pipetted at the bottom in order to wash the strip.

Results

Running buffer spiked with aflatoxin B1

The detection limit of aflatoxin B1 in running buffer was 0.1 ppb as shown in table 2.

Table 2: Schematic presentation of the results of serial dilution of aflatoxin B1 in running buffer

Concentration aflatoxin B1 ppb (ng/ml)	0	0.01	0.1	1	10	100	1000
Test line	++	+	±	-	-	-	-
Control line	++	++	++	++	++	++	++

++ very dark line   + dark line   ± faint line   - no line

Matrix influence of extract

Results from samples extracted either with acetonitrile/H_2O or methanol/H_2O were identical (data not shown). However, it was observed that big aggregates of detection conjugate appeared at the bottom of the strips. The extracts had to be diluted at least 50 times to eliminate this effect.

In this respect, assay performance was shown to be better on unblocked as compared to blocked strips.

Extract spiked with aflatoxin B1

In a serial dilution series of aflatoxin B1 in 50 times diluted extract a sensitivity of 10 ppb (ng/ml) could be achieved in 10-15 minutes (see figure 3). This result implies that an undiluted extract should contain 500 ng/ml, i.e., 2.5 mg/kg nutpowder to be detectable in this experimental set-up.

←Control line
←Test line

0   0.5   2.5   12.5   62.5   312   1562   7812   ppb (ng/ml)
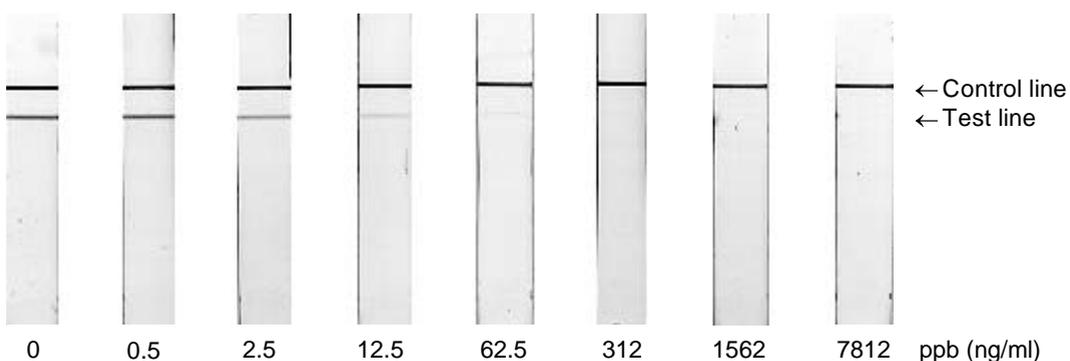
Figure 3: Results of competitive SPIA with a serial dilution range of aflatoxin B1 in 50 times diluted extract (acetonitrile/H_2O)

Discussion

The dilution series of aflatoxin B1 in buffer showed a detection limit of 0.1 ppb. This detection limit has been obtained under strictly defined circumstances and in relation to aflatoxin MRLs this assay performance meets the requirements of international agencies.

However, the experiments with extracts of sample showed a disturbing effect on the test. The reason that the detection conjugate aggregated at the bottom of the strip could be a fixation of proteins and thus clustering of antibodies, C-sol and other proteins present in the sample.

Another possibility maybe a change in membrane structure due to residual amounts of organic solvent.

Extracts of blank sample gave the best result on unblocked strips with running buffer + 1 %($^w/_v$) BSA. This is in contrast with the fact that blocked strips gave the best result with running buffer (data not shown). Probably, the blocking proteins on the strips disturb the flow of extracts of nutpowder by interaction with matrix components.

The detection limit of aflatoxin B1 in a diluted extract of blank sample was shown to be about 10 ppb (ng/ml). This result implies that a contaminated sample should contain at least 2.5 mg/kg. This is a very high concentration in relation to levels found in food products.

As a consequence, the present aflatoxin B1 SPIA detection limit is inconsistent with the officially approved extraction methods.

In a further attempt to optimize the SPIA performance, several parameters will be tested in order to increase the sensitivity of the SPIA for aflatoxin B1.

Assay performance may be increased by removal of the organic solvent from the extract.

This can be done by either evaporation of the methanol or removal of the acetonitrile with nitrogen depending on the extraction method used. After removal of the organic solvent, the sample can be diluted in buffer. It is possible that the solubility of aflatoxin B1 decreases upon removal of the solvent resulting in loss of aflatoxin B1.

In order to check the efficiency of the extraction method, a blank sample could also be spiked before the extraction. This should indicate whether aflatoxin B1 can be recovered to an acceptable level.

Alternatively, in an attempt to keep the test relatively rapid and simple, other extraction methods could be evaluated, e.g. by using Solid Phase Extraction.

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Session 2:

**Food, Nutrition
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Suitable methods for recognizing food allergens

*C. Ortolani *, E.A. Pastorello °*

*Department of Allergology and Clinical Immunology - Niguarda Ca' Granda Hospital, Piazza
 Ospedale Maggiore 3 - 20162 Milan – Italy
°Department of Internal Medecine – IRCCS Policlinico – Via F. Sforza 35 – Milan - Italy

Two highly ranked medical journals published studies (in 1988 and 1992) on cases of death due to anaphylactic shock from food allergy[1,2]. Thirteen cases were described in detail in these two studies. It is worth noting that peanut was responsible for seven of the reported cases, and that in nearly all of the cases the provoking meal was consumed outside the home (in restaurants or school canteens).

All the patients, except one, were fully aware of being allergic to the shock-provoking food, but they did not know that the particular food was present in the food they had eaten.

Some recent studies have highlighted the fact that food allergy is the most common cause of serious anaphylactic reactions [3-6]. In these studies, anaphylactic reactions due to food allergies are more prevalent than such reactions due to drugs.

A recent EC-sponsored study, SCOOP project, on the occurrence of severe food allergies in the European Union, examined all the serious anaphylactic reactions due to food that have occurred in Europe in recent years. Despite the fact that majority of the countries that participated in the study anaphylactic cases due to food are not recorded, many cases of death due to food-allergy induced anaphylaxis and numerous cases of anaphylactic shock due to food allergies were recorded. The responsible foods were: peanut, tree nuts, cow's milk, soy, fruit and vegetables, milk, eggs etc.

It is, therefore, important to remember:

- food allergy is an important cause of serious anaphylactic reactions;
- in many cases, the food origin of anaphylactic reactions is not specified;
- the threshold amounts of these foods for provoking anaphylactic shock are not known, but in some cases very small amounts are sufficient. For example 0.1 mg for peanut;
- food allergens are often present in commercial foods when they are not indicated on the list of ingredients (hidden allergens);
- the risk of anaphylactic reactions from food, especially from hidden foods, must be tackled by scientists, legislators, industry and consumers associations.

The role of the allergological researcher involves:

- identifying the foods responsible for allergic reactions;
- classifying the foods according to how dangerous these are;
- identifying the allergenic molecules of each allergizing food and characterising their physic-chemical properties;
- improving and refining diagnostic tests for food allergens;
- producing hypoallergenic foods.

In October '97 we signed a FAIR contract (FAIR CT97 3224) which was designed to fulfil some of these requirements for a selection of allergenic foods. The project tasks are: on the basis of a double-blind, placebo-controlled food challenge (DBPCFC), select a minimum of fifty patients with real food allergy. Identify and characterise the major allergens for the chosen foods. Establish reliable diagnostic tests, and finally, research the allergenic molecules in the common foods and, if present, produce hypoallergenic foods.

This project is being carried out in the following centres: Niguarda Hospital, Milan; Paul-Ehrlich Institute, Langen, Germany; BIBRA International, UK; National University Hospital, Copenhagen, Denmark; University Hospital, Zurich, Switzerland; CNR, Turin, Italy; DISTAM, University of Milan, Italy and Soremartec, Belgium.

The foods selected for this study are: Prunoideae fruits, hazelnut and celery. Prunoideae were selected because of the high number of cases of anaphylaxis reported, it is the most common cause of food allergy in the Mediterranean countries and because these fruits are used in many processed foods such as fruit juices, sweets, ice creams, teas and jams. Celery was also chosen because of the high number of anaphylaxis reported, especially in Central Europe, where this is used as a spice and flavour enhancer.

And hazelnut was chosen also on the basis of the number of anaphylactic cases reported and because this food is commonly used in the production of chocolate, ice cream and confectionery, but also because it is very often present in the form of a hidden allergen.

The rationale of this project lies in selecting patients with a real food allergy, demonstrated by the DBPCFC. Allergens are proteins, and some plants may contain more than 1000 proteins. However, only three to six of these proteins can be considered major allergens, and only 10 to 15 minor allergens. The number of allergens officially recognised by the WHO/IUIS Allergen Nomenclature Subcommittee is presently limited: 26 major allergens have been identified for about 17 food items. A better understanding of the major allergens will lead to more reliable *in vivo* and *in vitro* diagnostic measures.

The sera of patients allergic to a specific food item are necessary to prepare an allergogram by SDS-PAGE immunoblotting for the identification of the major allergens of a food. Thus, it is clear that the role of the clinical allergologist is fundamental for allergen identification, inasmuch as the sera containing the specific IgE to foods must be obtained from clinically confirmed positive patients; and in these subjects, clinically confirmed positive must be based on double-blind, placebo-controlled food challenge (DBPCFC). An imbalance between laboratory and clinical investigation can lead to the identification of major allergens that do not correspond to symptomatology.

Present technology offers several methods with which hypoallergenic foods can be produced or identified. However, it appears that food technology studies have often been performed without carrying out appropriate clinical controls. In fact, investigation in the field of food technology has often proceeded without proper identification of the major allergen/s of the food one intends to make hypoallergenic. Moreover the hypoallergenicity of the hypoallergenic foods that have been produced to date has rarely been subject o thorough *in vitro* and *in vivo* trials. It is in the latter phase of a study that the clinical allergologist has a fundamental role. A food presumed to be hypoallergenic can be confirmed so if it meets the requirements of each of the following consecutive steps:

1. preparation of the hypoallergenic food;

2. *in vitro* verification of claimed hypoallergenicity (SDS-PAGE immunoblotting);

3. *in vivo* testing of the hypoallergenic food in sensitised animals;

4. SPT with the extract of the hypoallergenic food in sensitised subjects;

5. *in vivo* verification by administering the hypoallergenic food openly and then by DBPCFC of sensitised subjects.

The DBPCFC methods for peach, hazelnut and celery were defined during the first year of the project. Eighteen DBPCFC were carried out for peach, 44 for hazelnut and 10 for celery. The major allergen of peach was identified as a 9 kD protein, whose amino acid sequence was identified. This protein belongs to the group of Lipid Transfer Proteins. This allergen was registered as a new protein in the Swiss Prot data bank and on the WHO-IUIS list as Pru p1. It was observed in a large number of commercial food products. The amino acid sequence N-terminal (20 amino acids) of the major allergen of apricot, plum and cherry revealed that these molecules are very similar to Pru p1. The complete amino acid sequence for these molecules is not yet finished.

The sera of patients allergic to hazelnut recognise a 17 kD major allergen (Cor a1) which belongs to the Pathogenesis Related Proteins; a low molecular-weight allergen was also recognised by approximately 20 % of the allergic patients. The next stage of the project will see the purification, sequencing of the amino acids and recombinant DNA synthesis of Cor a1, and the identification of the low-weight protein. Brown Norway rats were sensitised with hazelnut extract and these demonstrated extremely high immunogenic activity for this protein. Various extracts of different qualities of hazelnut were compared, demonstrating an identical allergenic composition.

The project study on celery commenced later than the Prunoideae work and thus, will have to wait until the second year of the project in order to have a sufficient number of DBPCFC-positive patients, which will lead to the study of any new allergens.

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Phytoestrogens and prevention of cancer

B. L. Pool-Zobel

Department of Nutritional Toxicology, Institute for Nutrition, Friedrich Schiller University of Jena, Dornburger Str. 25, 07743 Jena, Germany

Summary

One recent important achievement of biomedical research has been the recognition that high vegetable and fruit consumption is significantly associated with a reduced tumour risk in multiple tissues. Dietary phytochemicals are one of the plant food components suspected to be involved in the chemopreventive actions. Phytoestrogens, which structurally resemble mammalian oestrogens, may contribute to lowering risks for breast, prostate and colon tumours by modulating molecular events involved in tumor progression. The compounds may bind to estrogen receptors and thus inhibit cell proliferation in estrogen-receptor positive cells, although adverse, proestrogenic effects may also occur. Isoflavonoids are found in soy products as glycosides of genistein and daidzein, which may be metabolised in the human to equol, O-demethylangolensin and 5-hydroxy-o-demethylangolensin. Lignans (secoisolariciresinol and matairesinol, metabolised to enterodiol and enterolactone) are found in linseed, in grains and some vegetables. Genistein and equol have been shown to exert diverse antioxidative activities within mammalian cells, a property which leads to the reduction of oxidative damage. Thus there is a potential of these compounds to be protective also in cells without oestrogen receptors. This review will focus on the molecular mechanisms by which phytoestrogens act chemopreventive and how these activities relate to the anticancer action of foods in which they are contained.

Introduction

The incidence of cancer and the pattern of afflicted tissues is very much different on a global basis (1). The majority of cancers world wide is due to environmental factors causing genetic alterations in somatic tissues (2). Tobacco smoke and diet are the most important environmental factors, each responsible for 30 to 35% of all human cancers (3). The differences in dietary patterns world wide explain, in part, the differences of afflicted tumor tissues in different countries. The major dietary related tumours are located in the gastrointestinal tract (colon, stomach, oesophagus). But also other tumours originating from other epithelial tissues (lung, bladder, pancreas) may be dietary related. Tumours found in hormone dependent organs (breast, prostate) are associated with hormonal risk factors, but dietary influences probably also play an important role (4).

Individual dietary factors which may contribute to enhancing risks for the various cancers are heterogeneous. In general, diets high in total fat or in saturated/animal fat are considered causative for tissues such as breast, prostate, colon, and pancreas (5). This may explain the higher incidence rate of these tumours in countries consuming a so called "Western Style diet" (North America, Australia, Central Europe) in comparison to lower incidence rates in countries consuming diets with higher contents of plant foods (Mediterranean Countries, Asia, Africa). Moreover, it is meanwhile well recognised that vegetables and fruits are protective for many tissues. The numerous findings are exemplified by studies of Block et al. (6), or Steinmetz (7) reviewed by Hill (8) and more recently by Potter and Steinmetz (9). The dietary ingredients that are implicated as being the actual chemopreventive factors are phytoprotectants (micronutrients, secondary plant ingredients) and

products formed during the fermentation of non-digestible components in the gut. According to Wattenberg (10), the major mechanisms are to prevent carcinogens from exerting their mutagenic effects (blocking agent activity) or to prevent the initiated cell from developing into a further transformed or cancerous cell (suppressing activity).

Only few compounds have really been actually proven to be responsible for the protective activities of plant foods. They include vitamin C, carotenoids, selenium, vitamin E and Allium compounds. For these individual compounds, evidence is available for probable or possible associations with reduction of risk in stomach, lung, colon, breast, oesophagus and pancreas. Furthermore, non digestible plant ingredients (nonstarch polysaccharides, fibre) may reduce tumor risks in colon, breast and pancreas (4).

A large number of other plant ingredients are additionally considered to contribute to the protective effects of vegetables, fruit and grains. On the basis of diverse mechanistic studies directed at elucidating antigenotoxic, antioxidative, anticarcinogenic activities *in vitro*, in cell culture or in animal experiments, the substances have obtained the general nomenclature as being phytoprotectants. One special group of compounds are called phytoestrogens, on account of their origin from plant foods. Also, the structures resemble mammalian steroid hormones and some of these compounds have the ability to bind to estrogen receptors (11).

Phytoestrogens

The major plant components with estrogen binding capacity belong to the group of isoflavonoids and of lignans, both diphenols. In addition, selected hydroxylated flavonoids have been shown to interact directly with the estrogen receptor, and induce transcriptional gene activation, a property which also classifies them as phytoestrogens (see below). These compounds include flavonoids like apigenin, naringenin, isoliquiritigenin and kaempferol which are wide spread throughout the plant families (12). Moreover, indolo [3,2-b] carbazole, an analog of indole-3-carbinol found in *Brassica* vegetables is antiestrogenic in human breast cancer cells (13).

The best investigated phytoestrogens, however, are the isoflavonoids. Isoflavonoids are present in soy products and the major compounds are genistein, daidzein and the mammalian metabolite of daidzein, equol. Their occurrence in plant foods has been recently reviewed (14). In plants they are present as glycosides and are metabolised in humans by intestinal bacteria. Both metabolites and aglycones are absorbed from the gastrointestinal tract. In the liver they are reconjugated, and undergo enterohepatic circulation. In blood and urine they are mainly found as conjugates (reviewed in (15)). Genistein is also formed from biochanin A and metabolised to dihydrogenistein and 6'-hydroxy-0-demethylangolensin. 0.4-0.65 µM were found in the plasma of 3 males and 1 female after consumption of a soy meal with 78 µmoles (11). Daidzein is present in soy products and in clover. It can be formed from formononetin and is metabolised by the gut flora to dihydrodaidzein and subsequently to equol and O-demethylangolensin (16). Peak plasma levels in clover reached appr. 0.5 µM after high consumption of soy or clover (17). 0.3-0.4 µM were found after consumption of a soy meal containing 53 µmoles (11). Consuming a soy powder-beverage (60g/d, 28 d) lead to high genistein (0.9 µM) and daidzein (0.5 µM) levels as well (18). Urinary excretion levels have been measured in various studies as well, largely revealing substantial variations among individuals (19) (19) (reviewed in (20)). This latter paper describes the investigated associations between-self reported soy intake and excretion in different ethnic groups. The finding was that differences in urinary excretion of isoflavones and intake were different among the groups and may also reflect

differential intestinal absorption patterns. Intake ranged from appr. 2-13 g/day and urinary excretion from 100-400 mol/hour (20). In human milk different excretion patterns were observed than for urine. Consumption of 5-20 g roasted soy beans led to excretion of daidzein and genistein roughly in the range of 30-60 µM (21). In prostate fluid high concentrations of equol (10µM) and daidzein (1.5µM) were detected in samples of men from Hong Kong. Mean levels were several fold lower in prostate fluid of men from Britain and Portugal (22). Recently isoflavonoids have been detected in significant levels in beer (1.26-29 nM, sum of isoflavonoids (23)).

The mammalian lignans enterolactone and enterodiol are formed from precursors in plants, secoisolariciresinol and matairesinol. The precursors are found in flaxseed and in grains, where they are closely associated with the aleuron layer (24). The lignan values vary in flaxseed greatly depending on plant variety, harvest location and harvest year. Amounts of 0.96 µmol/g to 3.15 µmol/g were observed among different varieties (25). Recently, they have also been detected in coffee and tea at levels of 15.9-81.9 µmoles/kg secoisolariciresinol and 1.6-11.5 µmoles/kg matairesinol (26). Upon ingestion, they are converted by the intestinal bacteria to enterodiol and enterolactone, respectively. The excretion is closely associated with fibre intake and reaches levels of 3.86 µmoles per day (sum of lignans). After consumption of crucifercus vegetables; interesting gender differences have been observed. Men excreted more enterolactone than enterodiol, pointing to differences in intestinal metabolism (27). In premenopausal women consuming flaxseed powder urinary excretion increased from appr. 1.1 to 19.5 and from appr. 3.2 to 27.8 µmol/d for enterodiol and enterolactone, respectively. The interindividual variation was very high and not dependent on the phase of menstrual cycle. The excretion of the lignan enterodiol is higher for subjects on a high vegetable/fruit diet than on a diet high with ligume/alluim plants (28). Approximately 0.14-0.85 µM were found in the plasma after high linseed consumption by women (17). 0.4-0.65 µM were found by Barnes et al. (11).

The lignan and isoflavonoid properties which have resulted in classifying them as protectants are derived from the observation that they are contained in soy products or cereals, that populations with high soy or cereal intake have low breast, prostate cancer risk and also have higher excretion levels of these compounds, that some may compete with estradiol for rat uterine nuclear estrogen type II binding site, and that they stimulate sex hormone binding synthesis in the liver, thus reducing the biological effects of sex hormones (reviewed in (24)).

Estrogenic and antioestrogenic activity of phytoestrogens

Estrogens are being increasingly accepted as a risk factor in human breast cancer and they are implicated in the induction of uterine endometrial adenocarcinoma (reviewed in (29)). One of the involved mechanisms is expected to proceed via the induction of estrogen receptor-dependent tumour growth. The rationale is that the phytoestrogens competitively inhibit endogenous hormones from binding to their intracellular receptors. Normally, when estrogens, such as estradiol, bind to their receptor, a number of complex changes take place which ultimately lead to a change in the expression of target genes. Specifically a receptor-ligand interaction yields a complex bound receptor which will serve as a transcription factor, bind to the DNA at the estrogen response element, and activate transcription. The estrogenic actions lead to mitotic stimulation, and anti-estrogens are considered to block the mitogenic action by binding to estrogen receptors. This binding may result in conformational changes which prevent the bound estrogen receptor to recognise the estrogen-response element (complete antioestrogens). Alternatively, the binding to

the estrogen receptor may not lead to such severe conformational changes thus allowing pro-estrogenic activities to ensue (pro-estrogens). For the example of the synthetic non-steroid "antiestrogen" tamoxifen (see below) which is metabolised to 4–hydroxytamoxifen, different estrogen antagonist potencies are displayed, depending on the species and tissue examined and the end-point measured. This is due to the nature of 4-hydroxytamoxifen which acts as a partial estrogen. It is a pro estrogen or antiestrogen depending on nature of the estrogen receptor. In contrast, tamoxifen usually results in less efficient activation of transcription. Since tamoxifen-activation and nature of the estrogen-receptor may vary from cell to cell type, agonist and antagonist activities may be observed by the same compound in different tissues (reviewed in (30)). This aspect has also been partially investigated for genistein. Genistein binds to estrogen receptors and acts antiestrogenic as well as antiproliferative in estrogen- receptor positive mammary tumor cell lines, at high concentrations but stimulates growth at low concentrations (31-33).

Phytoprotectants	Compound	Blocking Activities*	Suppressing Activities*	Peak Plasma Levels
Isoflavonoids (Phytoestrogens)	Genistein	Inhibits lipoprotein oxidation at 1 µM (88). Induces quinone reductase activation in COLO205 cells at 0.1-10 µM (89)	nM-10µM stimulate growth in MCF-7 (31)	0.5-0.9 µM
	Daidzein	Inhibits lipoprotein oxidation at 1 µM (88).	>10 µM inhibit growth in MCF-7	0.5 µM
Lignans (Phytoestrogens)	Enterolactone	Reduces oxidative DNA damage in HT29 clone 19A cells at 100 µM (49) Induces quinone reductase activity at 0.1-10 µM in COLO205 cells (89)	0.5-2 µM inhibit 1 nM estradiol-induced proliferation in MCF-7 cells 0.5-2 µM act proliferative in MCF-7 cells	0.14-0.85 µM
Chemopreventive agents (Antiestrogen)	Tamoxifen	Inhibits formation of UVR-induced oxidative base damage in vitro and intracellularly at 5 µM (76). Upregulates expression of quinone reductase in estrogen receptor positive breast cancer cells (77)	0.005 – 5 µM inhibit growth of estrogen receptor free colon cells (71) 0.1-1 µM inhibit growth of MCF-7 cells	
Estrogen	Estradiol		1 nM stimulates growth in MCF-7 cells	

*for additional literature, see text

Enterolactone was studied for its potential to compete with estradiol in estradiol receptor positive breast cancer cells (MCF-7). It was found that 0.5-2 µM enterolactone inhibited the stimulatory activity of 1 nM estradiol, but on its own also acted proliferative. At concentrations above 10 µM, toxicity seemed to be apparent (34). In a reporter gene assay using Hela cells transiently cotransfected with an expression vector for the estrogen receptor and an estrogen-responsive reportergene construct, coumestrol, genistein and zearalenone increased the activity. In MCF-7 cells the estrogen responsive ps2 was induced and did not inhibit the estradiol-induced proliferation of T47 d cells. Thus, these findings are also showing that the compounds are not acting mostly antioestrogenic in vitro, implying other mechanisms should prevail if they are indeed chemoprotective in vivo (35).

Phytoestrogens and antioxidative activities

Phytoestrogens are polyphenols, which are able to act as antioxidants by virtue of the hydrogen-donating capacity of their phenolic groups or by chelating transition metals, which catalyse the ROS formation from peroxides (36) (37) (38). Some evidence has accumulated, demonstrating that flavonoids protect cells against oxidative stress(39;40). Isoflavonoids and lignans may also protect cells against oxidative stress. Especially genistein has been investigated in this respect. Thus, it has been shown that soy isoflavonoids are effective *in vitro* in inhibiting oxidation of ß-carotene linoleate. The aglycones are more effective than the corresponding glycosides and genistein is more effective than daidzein and glycitein, latter of which had no effect (41). 5-50 µM genistein were also effective in inhibiting tumour promoter-induced hydrogen peroxide formation *in vitro* and *in vivo (42).* UV light- and Fenton reaction-induced oxidative DNA damage were also inhibited by the soybean isoflavone genistein (43). Finally in mice, genistein inhibited c-*fos* expression and reduced the formation of skin tumours induced by dimethylbenzanthracene *in vivo (11;42).* In serum, 1µM genistein and daidzein, 0.1 µM equol and 0-demethylangolensin were efficient inhibitors of in vitro lipoprotein oxidation (44). Also, in an assay aimed at determining antioxidant activity in aqueous phase in vitro, genistein was more effective than daidzein > genistein > biochanin = daidzin > formononetin ~ ononin, the latter of which was not an antioxidant (45). Our own studies have shown that the mammalian lignans enterolactone and enterodiol were not efficient in preventing H_2O_2 induced DNA damage in the colon tumor cell line HT29 clone 19A (*unpublished results, interim report FAIR - CT- 95 – 0894).* For this we used a similar approach which had proven effective in demonstrating the antigenotoxic activity of flavonoids (40) (46;47). It encompasses the detection of DNA-Strand breaks in single cells using microgelelectrophoresis (Comet Assay (48)). We have modified the protocol and analysed the efficacy of phytoestrogens to prevent endogenously arising oxidised pyrimidine and purine bases. The lignans enterolactone and enterodiol as well as the isoflavonoids formononetin and dihydrogenistein (all dosed at 100µM) were most efficient in protecting the cell against endogenous products which cause oxidative base damage -(*unpublished results, interim report FAIR - CT- 95 – 0894) (49).* These mechanisms are of general importance, since oxidative DNA damage is responsible for enhancing the process of tumorigenesis by increasing the probability of tumor initiation and progression (50-53). Thus antioxidants will protect against oxidants and related compounds to which cells are exposed through extracellular and intracellular biochemical mechanisms (54-59).

In addition to interference with hormone receptor mediated growth, also the antioxidant properties of phytoestrogens may be considered to be a form of "antiestrogenic" effects. Thus, it has been shown that estrogens may be metabolically activated to reactive intermediates. 2-Hydroxylation of steroid

estrogens comprises the major metabolic oxidation pathway (reviewed in (29)). Also 4-hydroxlation to yield 4-hydroxylated estrogens comprises of another common pathway. For tumor development this is an important pathway since 4-hydroxyestradiol is carcinogenic in a hamster kidney tumor model, whereas the 2-hydroxylated estrogens did not induce tumours (reviewed in (29)). The mechanism of tumor induction by estrogen metabolites could be due to their capability of redox cycling. This consists of the formation of quinone after oxidation of the hydroxylated intermediate. The quinone may be reduced by NADPH dependent cytochrome p450 reductases to a semiquinone intermediate. The semiquinone can then react with molecular oxygen to form superoxide radicals or, via formation of hydrogen peroxide, to hydroxyl radicals (29) (60). Therefore the blocking activities of the phytoestrogens, may be just as significant in protecting against the hormone induced carcinogenic lesions in tissues as their suppressing effects are, and may be mechanisms contributing to effects observed in estrogen receptor –negative cells.

Genotoxic potential of phytoestrogens

The newer data on the carcinogenic potential of estradiol, points to their efficacy of leading to the formation of reactive oxygen species in addition to causing hormone receptor mediated cell growth. The structural resemblance of phytohormones to the mammalian steroid hormones should therefore also cause concern, in respect to posing a similar type of potential genotoxic hazard. Thus the study on the genotoxicity of mammalian lignans and isoflavonoids using mutational assays is of importance for assessing risk/benefit associations. It should be born in mind, that the ingestion of phytoestrogens with the diet leads to high endogenous concentrations, which exceed the plasma levels of physiological hormones 10 to 1000 fold. Furthermore, as a result of finding protective effects by plant ingredients, food related products or supplements containing phytoestrogens may become available, which will allow a much higher intake of these bioactive compounds than is possible with only the diet. Using short term assays of genetic toxicology, Kulling et al. have investigated enterolactone, enterodiol, and their plant precursors matairesinol and secoisolariciresinol on cell free microtubule assembly. In Chinese hamster V79 cells the disruption of the cytoplasm microtubule complex, induction of mitotic arrest, induction of micronuclei and mutagenicity at the HPRT gene locus was studied as well (61). In the cells, the compounds were tested up to 100µM and in the cell free system, up to 200µM. All compounds were devoid of aneuploidogenic and clastogenic potential under the experimental conditions. The negative results however, are not sufficient to exclude a genotoxic potential of these compounds, since the mechanism of action shown for estradiol involves metabolism by cytochrome p450 species, which are not expressed in the hamster cells. Therefore additional toxicological evaluation is necessary prior to using these bioactive compounds as supplements.

Presently, in our EC-funded project on *"Phenolic Phytoprotectants - Role in Preventing Initiation, Promotion and Progression of Cancer" (FAIR - CT- 95 – 0894)*, we are not only investigating the protective aspects of these compounds, but have also regarded potential toxic and genotoxic effects in colon cells (HT29 clone 19A tumor cell line and primary colon cells derived from human biopsy samples). Genotoxicity is being monitored with the comet assay (62) and additionally oxidative DNA damage is being detected with repair specific enzymes (46;47). Toxicity is being monitored by microscopical analysis of trypan blue exclusion. The incubation of colon cells for 30 minutes with 100 µM of each phytoestrogen has so far revealed that lignans (enterodiol, enterolactone, matairesinol, and secoisolariciresinol) and most of the tested isoflavonoids (daidzein, O-demethylangolensin, equol, dihydrodaidzein, formononetin, biochanin A, dihydrogenistein, and

genistin) induced neither strand breaks nor oxidised DNA bases. In contrast, genistein was repeatedly genotoxic and induced strand breaks (% fluorescence in tail was 1.9 ± 0.4 in the NaCl control to 10.3 ± 2.1 at 100 µM genistein, means ± SEM, n=7, ** <<0.01, paired two-tailed t-test). These results are insufficient on their own for a toxicological evaluation, but do warrant further studies to assess the impact of this genotoxicity on risk evaluation. A continuation of these studies was aimed at determining the concentration effect relationship. Therefore, genistein was also investigated at 12.5, 25, 50, 100, 125 and 150 µM. The strand break incidence increased twofold already at the lowest tested amount of the phytoestrogen, and responded in a concentration dependent manner until 100µM, which represented the highest effective concentration. These results were obtained after only 15 minutes incubation, and since time is also a factor which increases the genotoxic response, they may be of significance also for the human exposure situation, which report plasma levels only 10-20 fold less in adult men, females and infants (11).

The genotoxic potency of genistein is of importance to the reported findings of Rao et al. (63). Genistein applied at a dietary level of 250 ppm to rats enhanced azoxymethane-induced non invasive and total adenocarcinoma multiplicity in the colon, without affecting colonadenocarcinoma incidence or multiplicity of invasive adenocarcinoma. The authors conclude this effect may be related the inhibition of prostaglandin-inactivating enzymes, which were also significantly affected by genistin in this study.

Tamoxifen and it's possible resemblance to phytoestrogens

Tamoxifen is a nonsteroidal antiestrogen developed 30 years ago as a contraceptive. However, it was found to elicit opposite effects and stimulated ovulation. Later on it was found to have therapeutic efficacy in metastatic breast cancer and since 1985 it has also been widely used as an efficient auxiliary therapeutic agent after radiation and / or surgery for early breast cancer (reviewed in (64) (65)). Meanwhile the rationale for using it in therapy of breast cancer is well supported by meta analysis of 61 clinical trials, showing that 51 % of tamoxifen treated women were disease free after 10 years, compared to 45% in the control group. Also, a similar difference of 6% was found for the mortality rate after 10 years (66) (67). In another, recently published overview, information was obtained and analysed on 37000 women obtaining adjuvant tamoxifen in 55 trials. After 5 years of therapy with tamoxifen the proportional recurrence reductions produced among these women during about 10 years follow up was 47% and the corresponding mortality reduction was 26% (68). The proper terminology for this type of prevention is actually "the inhibition of non detectable breast cancers from becoming clinically evident" (64). However, the success of these trials have also led to the launch of a chemopreventive trial, the "Breast Cancer Prevention Trial" (BCPT) in April 1992. This randomised, prospective, double-blind, placebo-controlled clinical investigation, involving nearly 300 clinical sites in the United States and Canada is conducted by the National Surgical Adjuvant Breast and Bowel Project (NSABP) and is being primarily funded by the National Cancer Institute and also by the National Heart, Lung and Blood Institute (reviewed in (64) It has been performed in women who were eligible on account of belonging to non-diseased but high risk groups. They received 20 mg tamoxifen or placebo in the form of two tablets once a day. Intervention was planned for 5 years with a follow up period of 7 years, which is still ongoing. Meanwhile in 13,388 women, a 45 % reduction in breast cancer incidence has been noted among high risk participants who took tamoxifen http://cancertrials.nci.nih.gov). In contrast, other similar tamoxifen chemoprevention trials from the Untied Kingdom and from Italy, respectively, showed no positive results. This is being discussed as being maybe due to differences in study size, or to differences in the type of subjects.

E.G., the European subjects were not as high in risk as the Americans and some were taking hormone replacement therapy, which could have masked protective effects (commentary (69)). In any case, the contradictory results and the promising outcome of the first BCPT trial has now led to much support for the decision to begin a fourth "International Breast Cancer Intervention Study (IBIS), which is presently recruiting a target of 7000 women.

From a mechanistic point of view, tamoxifen has similar properties as those described above for some of the phytoestrogens. It is considered to be an antiestrogen, since it acts by binding to estrogen receptors in ER-positive cells and thus inhibits cell growth (60). However, in estrogen receptor-negative breast cancer cells (MDA-MB-435) tamoxifen and it's active metabolite 4-hydroxytamoxifen also inhibit growth (70). These findings are in accordance with the results of the clinical trials demonstrating that 30% of estrogen receptor-negative patients also respond to tamoxifen. Tamoxifen also inhibits growth of estrogen receptor-free colon cells (0.005 – 5 µM) (71), which may mean that it binds to other receptors involved in growth control. The clinical trials, however failed to observe a significant tamoxifen-related reduction or enhancement of colorectal cancer incidence (68).

One of the mechanisms of ER-independent growth inhibition by tamoxifen and analogs could be the inhibition of protein kinase C (PKC), which plays a critical role in growth regulation (72). The type of inhibition may be different for different structural analogues, and includes a slow interaction with the regulatory domain of PKC as well as with phosphatidylserine vesicles (73). The pathway of PKC-inhibition in estrogen receptor-negative breast cancer cells at high concentrations (>100µM) is apparently independent of it's ability to inhibit growth in these cells, since only 1-10µM are needed for antiproliferative activity. Studies by Gundimeda reveal that tamoxifen does not directly support the membrane association of PKC and that there is a release of arachidonic acid which is correlated with the PKC membrane translocation. Various antioxidants can inhibit the cellular effects of tamoxifen, indicating that the compound, by initially partitioning into the membranes, induces a generation of transmembrane signals and an oxidative stress to elicit the membrane association of PKC. This is then followed by an irreversible activation and subsequent down-regulation of this enzyme which in part may lead to cell growth inhibition (74). The modulation of PKC by phytoestrogens has not been investigated extensively for genistein and instead there are data showing that genistein inhibits protein tyrosin kinase phosphorylation (75).

In colon cells, we had also seen a physiological response by equol, and by the lignans, indicating they may also bind to yet unknown receptors and cause signal transduction (49). We are presently investigating the consequences of this biological property, which may affect signal transduction of the MAPK kinase cascade in our work for the EC project *"Phenolic Phytoprotectants - Role in Preventing Initiation, Promotion and Progression of Cancer" (FAIR - CT- 95 – 0894).* The results, however, do support the observation that equol, matairesinol, enterolactone and enterodiol potently interfere with as yet unknown receptors in estrogen-receptor free colon cells.

Contrasting the findings on the role of oxidative stress in PKC modulation in breast cancer cells, in some way, is the observation that tamoxifen also acts as an antioxidant in that it Inhibits formation of UVR-induced oxidative base damage in vitro and intracellularly in cells at 5µM (76). Similar mechanisms had been presented above for genistein. Tamoxifen, however, upregulates expression of QR in breast cancer cells (estrogen receptor-positive) (77), whereas isoflavones equol and genistein do not induce xenobiotic-metabolising enzymes in mouse and in human cells (78). This is an important property antioxidants may have (79). The mechanisms of induction are thought to

proceed via an antioxidant response element which is activated upstream by the antioxidants via a redox sensor and the MAPK-kinase or Jak/Stat signalling pathways (80;81). Alternatively, the change of oxidative/reductive cellular environments may enhance gene expression and activate transcription of proteins of the chemoprevention system by leading to an enhanced binding of certain transcription factors to the DNA (82). No information is available on whether lignans (which in part do reduce oxidative DNA damage as described above) alter expression of drug metabolising enzymes. However, we are presently elucidating the potential of enterolactone to induce GSTπ protein using an enzyme linked immunoassay *(EC project "Phenolic Phytoprotectants - Role in Preventing Initiation, Promotion and Progression of Cancer" (FAIR - CT- 95 – 0894))* (83).

Tamoxifen not only prevents the recurrence and incidence of breast cancer in clinical trials, but also poses a significant risk for cancer at another site. In the update analysis of tamoxifen efficacy as an adjutant therapy agent, the incidence of endometrial cancer was approximately doubled in trials of 1 or 2 years of tamoxifen and approximately quadrupled in trials of 5 years of tamoxifen. However, the number of cases was small and the ratios were not significantly different from each other (68). Tamoxifen induces DNA adducts (single injection of 20 mg/kg in rats) after one treatment or daily treatment for 3-6 days (84), has genotoxic potential also in the liver of mice (45 mg/kg/day, 4 days) and in a human cell line, which expresses cytochrome p450 enzymes and epoxid hydrolase (85). In rats, the chronic administration of tamoxifen results in the development of high incidences of hepatic adenomas and hepatocellular carcinomas (86).

Therefore the antiestrogen/proestrogen properties and chemopreventive/genotoxic potentials of tamoxifen are of similar quality as those observed so far for some investigated phytoestrogens. The beneficial aspects of tamoxifen, so far out weigh the risks, thus enabling the 2nd chemoprevention study to be launched (see above).

Cancer preventive potential of phytoestrogen-containing foods

The type of risk benefit evaluation posed for synthetic drugs like tamoxifen or for isolated phytoestrogens to be used as drugs (like resveratrol or ß-carotene) will be of different quality than the evaluation of dietary phytoestrogen intake. All evidence accumulated so far showed that high intake of plant foods will reduce risks associated with development of tumours in many tissues. When assuming an otherwise nutritionally well balanced diet, a high intake of a variety of vegetables and fruits will pose no toxicological risk and under normal circumstances should be no issue for cancer risk expectation. Therefore an evaluation of phytoestrogens in plant foods interests more on the basis of: Do they contribute to the cancer protective potential of plants? To which extent? Which compounds are most effective? And, will a high intake of these specific plant foods containing isoflavonoids or lignans actually ensure a risk reduction of tumours e. g. in breast, prostate or colon?

Support for the assumption that phytoestrogens (which from a mechanistic point of view have protective potential, especially at concentrations >10-100µM see above) are responsible for cancer prevention of plants foods comes from the observational studies. Populations that have a high intake of soy products have a lower risk for breast, uterine, prostate and colon cancer. They also have strikingly higher exposure to isoflavonoids. Their urinary excretion of genistein and diadzein is e. g. 10-100/old higher than that of American or Finnish people (34). Also plasma levels are higher and reach in Japanese people levels up to 276 nmol/l of genistein. This is, however still far removed from the concentrations found to be antiproliferative in cell culture systems. In fact proliferative activities have been observed at concentrations near the plasma level ranges (31).

In spite of the circumstantial relations and mechanistic data at high phytoestrogen doses, the actual epidemiological associations do not prove phytoestrogen containing foods to lead to risk reduction. In a review by Messina et al. (87), the risk associations of soy products were examined by evaluating 21 case control studies and 26 cancer sites. In 15 studies, no statistically significant difference in risk was found for consumption of soy products and tumour incidence at 9 cancer sites (including breast, prostate and colon). 10 reported a decreased risk (for stomach and rectum) and in one study (consumption of fried beans) an increased risk was observed for developing oesophageal cancer. The 25 studies with fermented soy products revealed no significant difference in 18 studies, decreased risks in 3 and increased risk 4 studies. Additional studies by Yuan et al. found no relationship between breast cancer incidence and soy protein intake. Tofu was associated with a decreased risk for stomach cancer (reviewed in (9)).

The failure of finding an unequivocal protection by isoflavonoid-containing foods in epidemiological studies may have its roots in the lacking power of such associations – or in the fact that phytoestrogens – available as they are from the food – are not sufficient in quality and quantity to exert the effects they have shown in some *in vitro* or in animal chronic cancer studies. The *in vitro* effects of growth inhibitory actions, for which phytoestrogens originally were most studied is observed in concentrations far above the physiological plasma levels. At physiological plasma concentrations phytoestrogens tend to enhance proliferation. Genistein even acts genotoxic to colon cells between 12.5-100 μM. However, it remains to be determined what the actual "physiological" concentrations are in the potential tumor target tissue (e. g. breast or prostata or colon epithelial cells). At least fluids associated with these tissues have shown higher levels (e. g. > 10μM) in prostate fluid and breast aspirate (22). Nevertheless it should be born in mind that consumption of these products is also associated with a lower intake of dietary fat and higher intake of starch, non starch polysaccharides, fibre, vitamins, minerals, and a host of other plant ingredients with protective potential. Together, with the other beneficial aspects of Asian diets (less red meat, more vegetables, etc.) these may be the actual protective aspects for risk reduction of breast, prostate and colon cancer, thus leaving the evidence for phytoestrogens in cancer prevention still unresolved.

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Antioxidants and their role in healthy nutrition

G. Rechkemmer

Institute of Nutritional Physiology, BFE, Haid-und-Neu-Str. 9, D-76131 Karlsruhe, Germany

1 Introduction

Oxidation processes in the metabolism are essential to generate the energy necessary for the life of higher aerobic species, however, during these processes also highly reactive oxygen (ROS) and nitrogen (RNS) species are produced. These reactive molecules potentially react with lipids, proteins, carbohydrates (glycoproteins) and DNA and thus interfere with the functions of cellular membranes, cell metabolism, cellular signalling, cell growth and differentiation.

During evolution endogenous protection systems have developed to counteract the deleterious effects of cellular oxidation. Protective antioxidant enzyme systems consist primarily of superoxide dismutase (SOD), glutathione peroxidase (GPO) and catalase. In addition to the endogenous antioxidative mechanisms exogenous antioxidants provided with the diet may help to prevent oxidative stress, e.g. the imbalance between prooxidative and antioxidative processes. Oxidative stress has been implicated as a causative process in the development of a vast number of degenerative diseases and also as a major contributor to aging and photooxidative stress of the skin.

Besides these negative effects recently it has been demonstrated that ROS play a role in cellular signal transduction and evidence is accumulating that important intracellular processes are influenced by the redox status of the cell (1;2).

The reactions of ROS with DNA and the resulting oxidative modification of purin or pyrimidin bases may lead to mutations. These mutations, if not corrected through specific DNA repair mechanisms, may finally lead to carcinogenesis.

Reaction of ROS with lipids may cause lipid peroxidation of low density lipoproteins (LDL). The oxidized LDL particles are not recognized by cellular LDL receptors and thus may be deposited on and within the vessel walls and cause atherosclerotic plaques. LDL oxidation appears to be one of the primary events in the development of atherosclerosis and thus seems to be associated with the development of hypertension, stroke and coronary heart disease.

In this brief review some of the basic aspects of the relation between dietary antioxidants and health will be discussed. This issue has been covered in greater detail and depth in recent publications (3-5).

In summary, an increased consumption of dietary antioxidants, primarily contained in fruit, vegetables and whole grain products, is of great importance for a healthy nutrition and very likely beneficial for the prevention of degenerative diseases.

2 ROS and free radicals

In biological system only few radicals are of physiologic and pathophysiologic importance. ROS are generated by energy input (singlet oxygen) or by a series of redox reactions starting from molecular oxygen (FIGURE 1).
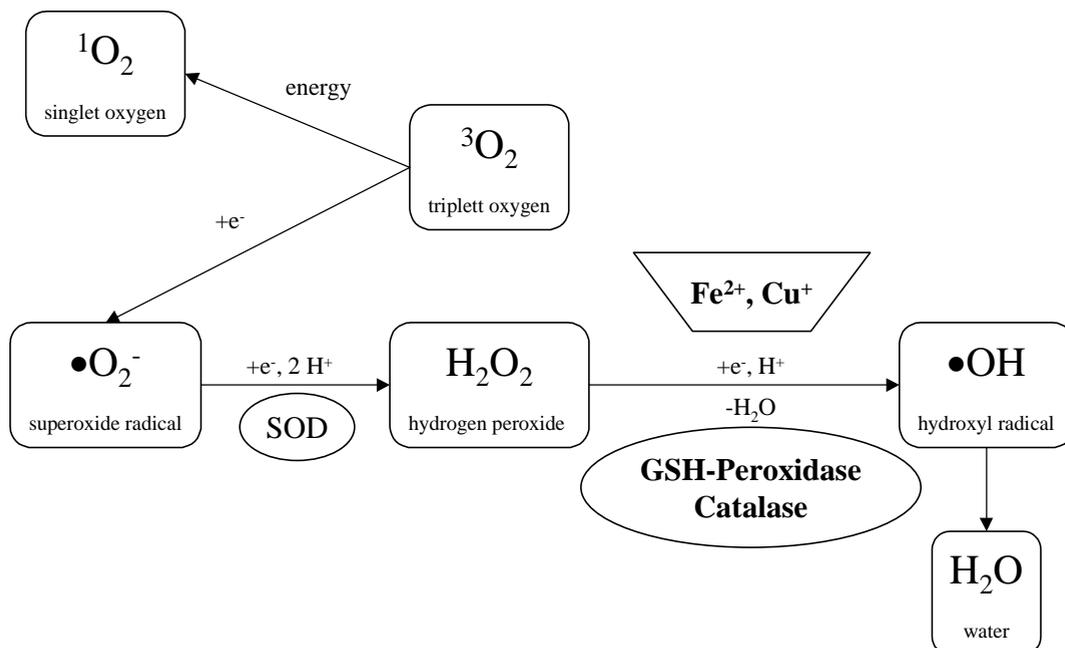
Figure 1: Oxygen and reactive oxygen species. SOD: superoxide dismutase; GSH-Peroxidase: glutathione-peroxidase

Free radicals are generated endogenously but also originate from external sources (TABLE 1). Free radicals play an important role in non-specific immune functions, during phagocytosis oxidative bursts are produced to destroy bacteria and viruses.

Table 1: Some sources of free radicals (6)

Endogenous sources	*Exogenous sources*
• Mitochondria	• Cigarette smoke
• Phagocytes	• Environmental pollution
• Reactions involving iron and other transition metals	• Radiation
	• Ultraviolet light
• Arachidonate pathways	• Certain drugs, pesticides, anaesthetics, and industrial solvents
• Peroxisomes	
• Excercise	• Ozone
• Inflammation	
• Ischaemia/reperfusion	

The radical with the highest reactivity and thus potentially the most deleterious molecule is the hydroxyl radical (TABLE 2). However, on the other hand the hydroxyl radical reacts very quickly and indiscriminately with other molecules crossing it's way and thus those reactions are limited to the immediate vicinity where hydroxyl radicals are produced. Hydroxyl radicals are generated during Fenton-type reactions involving free iron (Fe^{2+}). Any condition leading to an increase in free iron causes an increase in oxidative stress.

Table 2: Lifetimes of free radicals occurring in biological systems (3)

Species	Symbol	Half-life at 37°C, s
Superoxide	$O_2^-\bullet$	1×10^{-6}
Hydroxyl	$\bullet OH$	1×10^{-9}
Alkoxyl	$RO\bullet$	1×10^{-6}
Peroxyl	$ROO\bullet$	1×10^{-2}
Singlet oxygen	$^1O_2$	1×10^{-6}
Molecular oxygen	$^3O_2$	$>10^2$

3 Association between free radicals, ROS, oxidative stress and disease

It has been suggested that oxidative stress is related to a number of degenerative diseases. Free radicals appear to be involved in the pathogenesis of these diseases (FIGURE 2).

Brain and nervous system
Parkinson's disease
Amyotrophic lateral sclerosis
Hypertensive cerebrovascular injury

Lung
Bronchopulmonary dysplasia
Cigarette smoke effect
Effects of oxidant pollutants (O_3, SO_2, NO_2)

Red blood cell
Sickle cell anemia
Malaria
Protophorphyrin photo-oxidation

Eye
Cataract
Adult macula degeneration
Photic retinopathy

Inflammatory disorders/ immune functions
Age-related decline in immune function
Rheumatoid arthritis
Glomerulonephritis

Heart and cardiovascular system
Atherosclerosis
Keshan disease (selenium deficiency)
Alcohol cardiomyopathy

ROS

Cancer

Aging

Kidney
Metal-ion mediated nephrotoxicity
Autoimmune nephrotic syndromes

Radiation injury

Exercise-induced oxidative stress

Reproductive functions
Sperm abnormalities
Hypertensive complications of pregnancy

Leprosy

Gastrointestinal tract
NSAID-induced GI tract lesions
Oral iron poisoning
Endotoxin liver injury

Ischemia-reperfusion
Stroke/myocardial infarction
Organ transplantation

Iron overload
Nutritional deficiencies
Dietary iron overload
Idiopathic hemochromatosis

Figure 2: Examples of the possible relation between reactive oxygen species to certain clinically relevant diseases and aging.

Besides these diseases the aging process also appears to be related to increased oxidative stress, because of a age-dependent decrease in antioxidant defence systems. Currently two alternative hypothesis of the aging process are discussed, firstly the oxidative stress and secondly the telomerase hypothesis.

The hypothesis relating oxidative stress to aging results from the observations that the amount of oxidative damage increases as an organism ages (7). Caloric restriction causes lower metabolic

rates and consequently a lower production of the free radicals that produce oxidative damage to DNA and other parts of the cell. This hypothesis is further supported by the observation that the overexpression of antioxidative enzymes extends the maximum life-span of transgenic animals and the fact that the variations in longevity among different species correlates inversely with the rates of mitochondrial generation of the superoxide anion radical and hydrogen peroxide. The restriction of caloric intake lowers the steady state levels of oxidative stress and damage, retards age-associated changes, and extends the maximum life-span in mammals (7). An alternative hypothesis of the life-span increasing effect of caloric restriction is related to the hormonal effects associated with it. Growth hormones and particularly insulin levels are decreased and thus tumor growth may be retarded.

The telomerase hypothesis of aging is related to the observation that the telomers, repeated base sequences at the ends of chromosomes, shorten with every cell division. Expression or overexpression of telomerase increases the life span of organisms and prevents the shortening of the telomers and thus the number of cell divisions is greatly increased (8;9).

Most likely both mechanisms discussed above participate in the mechanisms of aging, but whereas without gene therapy it does not appear to be possible to affect the telomer associated mechanisms of aging, it is highly probable that the aging process could be delayed by a healthy lifestyle including eating a diet rich in antioxidants, e.g. primarily consisting of fruit and vegetables, and by reducing the energy intake, e.g. caloric restriction.

4 Dietary antioxidants

The best known and best studied dietary antioxidants are the classical antioxidative nutrients, e.g. the vitamins C and E (the tocopherols and the tocotrienols). Vitamin C (ascorbic acid) serves as a water-soluble antioxidant, whereas the tocopherols (especially the α-tocopherol) are incorporated into the lipid membranes of cells and have a chain-breaking activity against the propagation of lipid peroxidation. Vitamin C and E complement each other and act synergistically in their antioxidant activity (10-12).

In the diet, however, particularly in those consumer groups with a high consumption of fruit and vegetables other constituents besides the classical antioxidative vitamins may also play a significant role as functional antioxidants. In particular some phytochemicals like the carotenoids, the flavonoids and the catechins have been examined in recent years with respect to their antioxidative potential and their possible link to the prevention of degenerative diseases. Primarily large scale epidemiological studies have provided indications that a diet rich in vegetables and fruit and thus in dietary antioxidants is associated with a decreased risk for coronary heart disease, stroke (13) and certain types of cancer (14;15). In a recent dietary intervention study with nonsmokers it was demonstrated that the consumption of controlled diets high in fruit and vegetables significantly increases the plasma antioxidant capacity (16).

The polyphenolic compounds (flavonoids and catechins) in general show a much higher antioxidative potential *in vitro* compared to vitamin C or the synthetic water-soluble vitamin E analogue, trolox . However, the bioavailability of these compounds, e.g. their absorption from the gut into the systemic circulation, is only poorly studied and thus their importance for and contribution to the total antioxidant status is unclear. Hypothesis like the "French paradox" have been put forward to explain the lower incidence of coronary heart disease despite a high consumption of saturated fats

in southern France by the high consumption of flavonoid-rich red wines and the subsequent increase in oxidative capacity of the serum (17).

Table 2: Some food that contain nonnutrient antioxidants (modified from (6)

Product	*Antioxidants*
Soybeans	Isoflavones, phenolic acids
Green tea, black tea	Polyphenols, catechins
Coffee	Phenolic esters
Red wine	Polyphenols, anthocyanins
Rosemary, sage, other spices	Carnosic acid, rosmaric acid
Citrus and other fruit	Flavonoids, chalcones
Onions	Quercetin, kaempferol
Carrots, tomatoes, green leafy vegetables	Carotenoids
Olives	Polyphenols

4.1 Carotenoids

Carotenoids have been shown to be effective singlet oxygen quenchers, especially at low oxygen partial pressure. However, their role and their contribution as dietary antioxidants is still discussed controversially (18;19). In vitro carotenes and xanthophylls are capable to scavenge the ATBS$\bullet^+$ radical in the order of lycopene>β-cryptoxanthin>β-carotene>lutein>zeaxanthin>α-carotene. In this system the tested carotenes and some xanthophylls were better antioxidants than vitamins C and E, however, the xanthophylls canthaxanthin and astaxanthin were ineffective reductants in this system (20;21). The cancer-preventive properties of carotenoids has been attributed not only to their antioxidative potential but also to their ability to induce gap-juntional communication. It has been demonstrated that these two properties of carotenoids operate independently of each other (22). In a dietary intervention study with carotenoid-rich vegetable juices a significant reduction in DNA damage in lymphocytes was observed during the intervention period as compared to a control period (23).

For a long time interest in the physiological activity of dietary carotenoids was almost exclusively focused on β-carotene and it's action as a vitamin A precursor (24;25). However, among the approximately 600 naturally occurring carotenoids less than a tenth have provitamin A activity. In the human diet only a few carotenoids contribute significantly as vitamin A precursors, e.g. β-carotene, α-carotene and β-cryptoxanthin. Other major carotenoids in the human diet are lycopene and lutein/zeaxathin. Lycopene recently has gained increasing interest because of an association found between a high consumption of tomato-products, the primary source of lycopene in the human diet, and prostate cancer (26;27).

In recent years the proposed reduction of lung cancer by β-carotene in high risk groups like heavy smokers and asbestos-exposed workers was of particular interest. Therefor two large intervention trials were conducted in recent years, the α-tocopherol and β-carotene intervention trial (ATBC) in Finland and the carotene and retinol efficacy trial (CARET) in the USA. In both studies heavy smokers were supplemented for several years with 20 or 30 mg β-carotene daily, respectively

(28;29). Both studies, however, resulted in an increase in lung cancer incidence in the supplemented group and the CARET study was prematurely terminated. In the Physicians Health Study on the other hand no effect of long-term supplementation with 50 mg β-carotene every second day had no effect on the incidence of malignant neoplasms and cardiovascular disease (30).

The xanthophylls lutein and zeaxanthin are of specific importance because of their specific enrichment in the macula lutea of the eye (31). Age-related macula degeneration is the most significant reason for blindness in the elderly and appears to be related to a decrease in carotenoid concentration and thus a lower protection against photooxidative damage in the retina. Long term lutein supplementation has been shown to increase the lutein concentration in the retina considerably and thus may offer an additional protection against blue light damage (32). Also in the human lenses lutein and zeaxanthin are the only carotenoids detected, but these carotenoids do not appear to be linked to cataract formation (33)**.**

4.2 Flavonoids

Flavonoids constitute a large group of phytochemicals present in varying concentrations in fruit and vegetables (34). The flavonoids are efficient antioxidants *in vitro*. Flavonoids are much more potent to inhibit oxidative DNA damage to human lymphocytes than vitamin C (35). The structural aspects of the *in vitro* antioxidant activity of flavonoids has been investigated in detail recently (36). However, their antioxidant potential *in vivo* is not studied in great detail. Two recent dietary intervention studies with foods containing high concentrations of flavonoids an increase in plasma antioxidant capacity was demonstrated (16;37).

The flavonoids constitute a large group of compounds, appr. 5000, with the common structure of polyphenolic ring systems with different substituents. In plants the flavonoids are usually present as glycosides. The nature and the number of the sugar moieties varies and this is the major reason for the chemical diversity of the flavonoids. Only a few compounds have been thoroughly investigated so far, among them quercetin. Quercetinglycosides are present in high concentrations in onions but also in apples. The bioavailability of quercetin has been measured indirectly by the disappearance from the intestinal contents in ileostomy patients and by measuring quercetin plasma concentrations (38;39). The potential health effects of quercetin have been reviewed recently (40).

An important group of flavonoids present in the human diet are the anthocyanins. Anthocyanins are water-soluble red-violet plant pigments (for an in depth review see (41)) and are also present in plants exclusively as the glycosides. The aglycones are named anthocyanidins. Of all flavonoids anthocyanins are peculiar because the bear a positive charge at the C-ring structure and thus in the dissociated form are cations. Nevertheless they interact with other cations, e.g. trace elements, and their color is affected by this interaction. Anthocyanins are present in high concentrations especially in berries like black currant, elderberry, aronia, blue berries and red grapes but also in some vegetables like red cabbage, lentils and egg plant. The anthocyanins are pH-sensitive and the color change with pH can be used as an indicator. The presence of anthocyanins in grapes and wine has attracted the attention of researchers with respect to the "French paradox". In two recent studies it has been demonstrated that red wine polyphenols and alcohol-free red wine leads to an increase in plasma antioxidant capacity and reduces LDL oxidation *in vivo*, respectively (42;43).

With respect to tea catechins two controversial studies have been published recently. In one study neither black nor green tea increased the resistance of LDL to oxidation (44), while in the other

study jasmine green tea epicatechin isomers inhibited LDL-oxidation (45). Thus the importance of tea compounds as dietary antioxidants has to be studied in more detail, particularly with respect to the catechin uptake necessary to achieve physiological effects.

5 Summary

Dietary antioxidants derived primarily from fruit and vegetables appear to have important physiological functions. The scientific evidence presented so far is indicating an important contribution of these compounds to the prevention of cardiovascular disease and cancer. There also some indications that the mechanisms underlying the aging process are affected by the antioxidant status. However, so far we have insufficient information about the relative uptake and thus the importance of the different antioxidant compounds contained in our diet. Studies on the bioavailability of these compounds from different food matrices have just started and the cellular studies aimed at a mechanistic understanding of the physiological actions of these dietary constituents are just beginning. In relation to the development of functional foods, the dietary antioxidant phytochemicals are certainly the prime candidates for thorough investigation. A better understanding of their actions can potentially lead to the development of new foods with scientifically proven functional or even health benefits.

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Session 4:

Technological Methods
to Improve Food Quality

Fundamental trials to clean UHT tubular modules

A. Graßhoff

Federal Dairy Research Centre Kiel, Department of Process Engineering, D-24121 Kiel, Box 6069, Germany, E-mail address: grasshoff@bafm.de

Abstract

UHT treatment of milk and dairy products is increasingly practiced. Due to the possibility of longer running time tubular UHT modules are more widely used than plate heat exchangers . UHT heaters exhibit significantly higher fouling than common pasteurizers do. In severe cases, fouling may lead to complete plugging of individual flow tubes in tubular UHT modules. Regulations stipulate at least daily cleaning for UHT modules. However, to maintain proper and efficient function of UHT modules, more frequent cleaning is commonly required. Although the cleaning mechanisms for milk pasteurizing equipment have been intensively investigated, there is a lack of fundamental information concerning cleaning of UHT milk heating equipment.

An experimental plant especially designed for investigation of cleaning UHT tubular modules has been installed at the Institute of Process Engineering at the Federal Dairy Research Centre, Kiel/Germany. The plant consists of four steam-heated stainless steel vessels (40 litres each) and a circulation loop (containing the UHT module) with computer controlled valves. The UHT modules are fouled by normal operation at a Westphalian dairy plant. The deposits on the test modules represent processing of up to 200.000 litres of milk and dairy products. Temperatures in each vessel and at the module's inlet and outlet, the flowrate in the circulation loop, the electric conductivity and the pH value of the cleaning solutions are continuously monitored and recorded by a computerized data acquisition system. Deposit removal kinetics are monitored indirectly using a sensitive differential pressure measuring device (resolution better than 1 millibar) installed across the UHT module. The progress of deposit removal in a pre-selected flow tube of the UHT module is determined by combining flowrate and differential pressure data and calculating frictional losses across the tube. This data is collected, relevant parameters computed and information displayed on a computer screen once per second. After a cleaning trial, the flow tubes are dried, inspected and photographed with a rigid endoscope.

The objective of the initial trials with the experimental UHT cleaning plant was to develop measurement techniques to observe the cleaning process and to define the extreme process parameters for further experiments. The overall objective of the investigations is to define optimal cleaning parameters for cleaning UHT plants, for a wide variety of different deposits (e.g. cream, fruit milk etc.).

1 Introduction

Long-life milk products are continuously on the advance. There is hardly a larger dairy to be found which does not produce UHT products. While the process of heat exchange has widely been well understood and the UHT processing plants are highly sophisticated, the cleaning procedure of these plants seems to rest on a permanent experimental stage. And, there seems to be a considerable lack of scientific investigations in this field. There are several major questions about applicability of fundamental information developed for pasteurizing equipment to the design of UHT cleaning

regimes. A doubling of the cleaning rate with a temperature increase of 15 K has been observed in the temperature range between 50 and 90 °C for cleaning pasteurizers (*Bulletin No. 328/1997 of the International Dairy Federation*). One objective of these studies is to determine if this relationship continues for temperatures above 100 °C. Another objective is to determine, whether an optimal caustic concentration exists for UHT deposit removal, as has been identified for cleaning pasteurizers. The optimal flow dynamics of the cleaning fluids in cleaning the heavy deposits encountered in UHT modules will have also to be investigated.

2 Description of the experimental plant
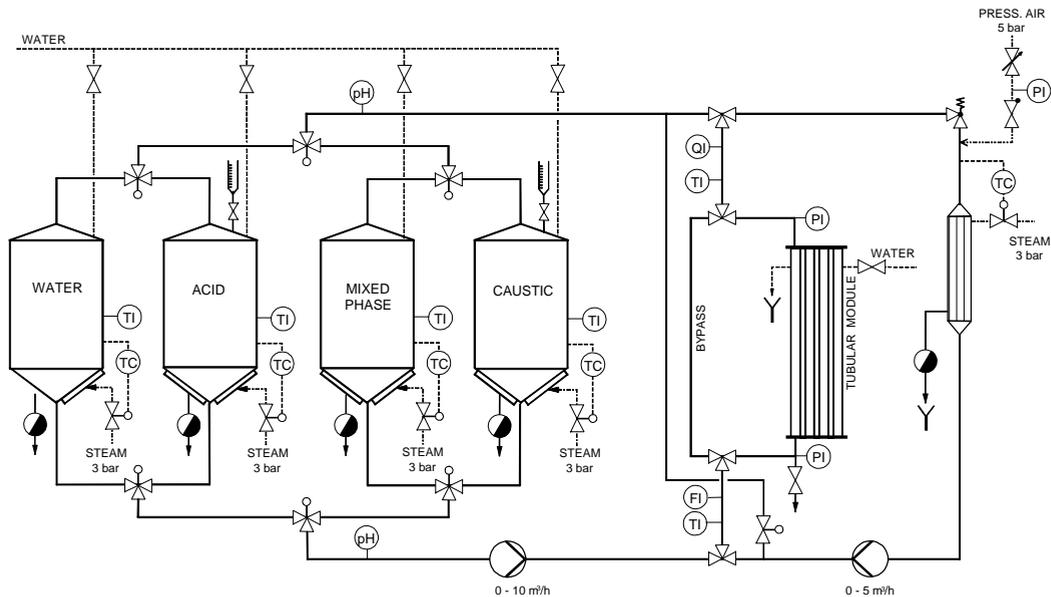
Fig. 1: Schematic diagram of the experimental plant.

Four steam heated stainless steel vessels (40 litres each - fresh water, acid, mixed phase and lye) are connected by an arrangement of pipelines and computer controlled valves so that the cleaning fluids can be pumped in a circuit through a test section which contains the UHT tubular module to be cleaned. The tubular module consists of 4 bundles with each 16 parallel flow paths with 12 mm inner diameter and 3 m in length. The modules are fouled by normal operation in a Westphalian dairy plant. After the production run and before the normal plant cleaning the test UHT module is interchanged with an identical one. The fouled module is transported to the institute and serves as an experimental object for cleaning trials. In this way, investigations are performed with UHT deposits of the same type and scale as they normally occur in dairy plants. The deposits on the test modules represent processing of up to 200.000 litres of milk and dairy products.

The temperatures as well as the volume flow of the circulating solutions were measured at several significant points in the cleaning loop of the experimental plant. Moreover, pH electrodes as well as inductive conductivity probes have been installed in the liquid feed and return lines. All readings are centrally collected by a PC and suitably processed via the data acquisition software DASYLAB 3. The process temperature in the circulation loop can be controlled in the range between room temperature and 135 °C.

3 Fundamental trials to establish the flow path condition

Due to the lack of a better criterion for on line monitoring of the deposit removal kinetics the pressure loss in the testsection was measured with a resolution of 1 millibar. To reduce the measuring error hysteresis caused by adhesion of viscous liquids in the connections between measuring point and the differential pressure sensor, a very slight steady flow of compressed air (about 0.2 millilitres/second) was injected at both sides of the sensor into the lines. The air exits the lines at the measuring orifice. Thus, the lines are free of liquid at every time.

Basic measurements have been performed with water in a clean tubular module, impacting different numbers of parallel flow path tubes with variable fluid flows.
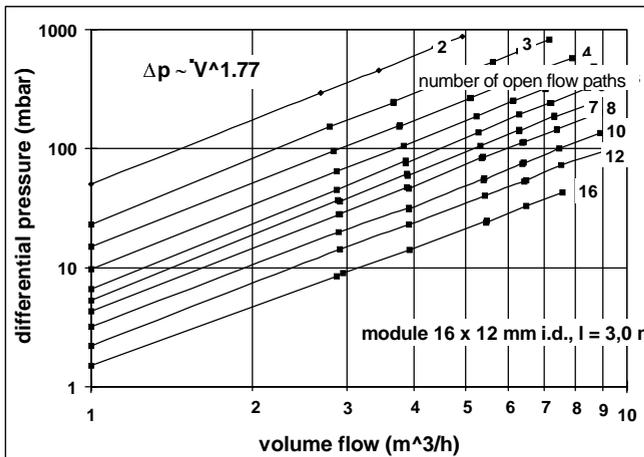
Fig. 2: Differential pressure as a function of the volume flow and the number of parallel flow paths.
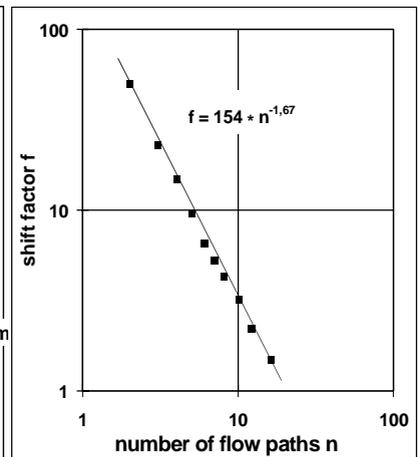
Fig. 3: Shift factor for the family of differential pressure curves of fig. 2.

Combining both diagrams delivers the differential pressure as a function of the volume flow V and the number of free flow paths, n:

Equation 1: $\qquad \Delta p = 154 * n^{-1,67} * V^{1,77}$

Rearrangement of Eq. 1 results in

Equation 2: $\qquad n = \left(\dfrac{\Delta p}{154 \cdot V^{1,77}} \right)^{-0,6}$

As Δp and V are monitored continuously with DASYLAB the number of free flow paths can be computed one per second and displayed in a separate window on the computer screen. By dividing this number with the number of the theoretically available flow paths a relative value in the range between 0 and 1 indicates the cleaning extent and thus a possible way to on line monitor the deposit removal kinetics in the flow paths of the tubular module.

4 Results of initial cleaning trials

Due to the processed products, the process temperatures and the running time of the UHT plant, there can arise extremely different deposits with very different removal characteristics. That's why it is not possible to establish a universally applicable cleaning procedure for UHT plants.

In a first trial the module had been soiled by processing 120.000 litres of evaporated milk at about 125 °C. The deposit formation was not excessively bad, all flow paths were still free. A sufficient cleaning (no organic residues at the pipe surfaces) was achieved by a 2 phase CIP procedure, starting with the alkaline phase (1 % related to sodium hydroxide, with addition of complexing agents) at 85 °C and finishing with an acid run (0. 5 % nitric acid) at 75 °C and a clear water rinse. After drying by blowing air through the pipes the cleaning result was monitored with a rigid endoscope. There were considerable amounts of mainly inorganic matter (a grey, powdery scale) which had resisted the acid impact. A solving test of this matter in a test tube established its complete solubility in a slightly alkaline solution containing active chlorine. Thus an alkaline active chlorine solution (0.25 % NaOH, 500 ppm Cl_2O) at 80 °C was circulated for 15 minutes through the flow paths with the result of metallic clean surfaces.

Appreciable more difficulties arose at the cleaning of a tubular module in which at first 120.000 litres evaporated milk and then 80.000 litres protein enriched milk had been heated. The deposit formation was considerably stronger. Some flow paths were completely plugged. An initial water rinse did not any noticeable deposit removal, but treatment with 2 % NaOH solution with complexing agent NTA (nitrilo triacetic acid) at 90 °C resulted in rapid deposit removal. Deposit removal was such in this case that some of the measuring device (pH and temperature probes, pitot sensors) were inactivated by sheets of deposit which had been detached from the surface but not dissolved and subsequently collected at obstacles in the flow loop. Some tubelike deposit sheets proved as so firm, that suspended wet pieces of about 0.5 meter length did not tear under their considerable weight.
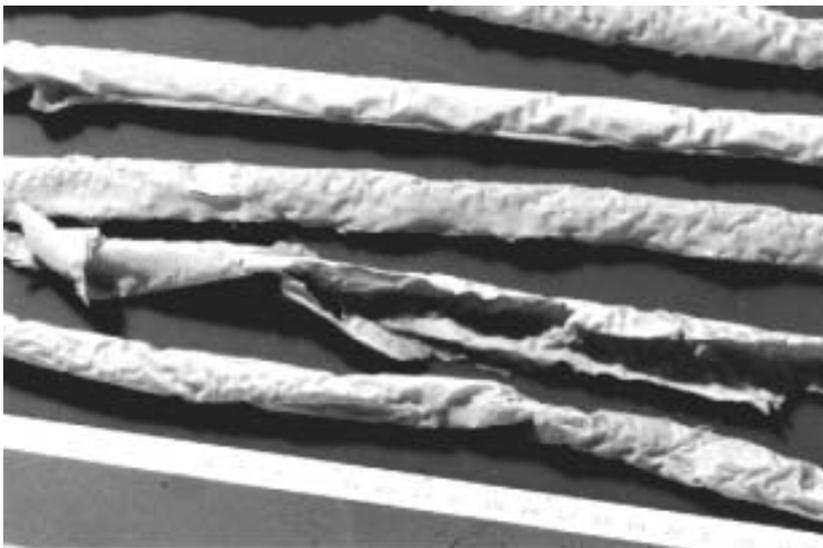
Fig. 4: Sheets of the deposit
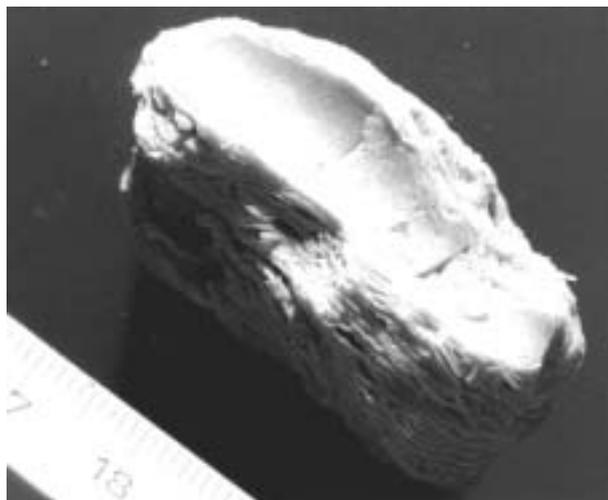
Fig. 5: Deposit matter, which had built up at the pH probe

At the end of the two phase circulation cleaning procedure several flow paths were still plugged. Some of them could be opened by water pressure of about 10 bar before another complete cleaning run was performed. (How to open tenaciously plugged pipes see below). To achieve metallic clean surfaces of all flow paths a final active chlorine run (0.25 % NaOH, 500 ppm Cl_2O) at 80 °C proved to be necessary.

A relative strong deposit formation had been noticed in a tubular module after processing of 150.000 l chocolate milk. But, surprisingly, to clean this module did not exhibit too much difficulties. Blowing room air for 72 hours through the flow paths resulted in drying up the scale so that it got an extremely briddle structure with a low adhesion force to the metallic pipe surface. The removal of the spoilage was nearly completely affected by pumping tap water through the pipes. An acid treatment (0.5 % nitric acid, 75 °C) completed the cleaning of the chocolate milk module.

5 Plugged flow paths

A danger in the cleaning of a heating device with parallel flow paths is that single paths may be plugged and the circulating cleaning solutions will find the way of minimum resistance. That means that the cleaning solutions will exclusively run (according with a higher velocity) through the free passages. As a matter of fact the circulation cleaning can work only if the chemical cleaning agents get free access to the entire soil surface. The penetration ways of the cleaning chemicals into the interior of the soils have to be kept as short as possible. If the penetration depends exclusively on diffusion zones of a tube plug deeper than some millimetres cannot be reached - finally resulting in a plug, that cannot be removed by chemical dissolution. Its removal has to be done mechanically.

In an initial stage of the plug development a pressure impact up to some bars for pushing it out may work. (The usual pressure difference across a single flow path ranges by some hundred millibars which is not sufficient to push out the rigid plug). But - if the plug has survived some cleaning procedures with subsequent product heating cycles, its structure and its wall adhesion properties may have changed considerably, so that - having reached a length of some meters - it resists pressures up to several hundred bars. A way to remove such a tenacious plug would be to push it

out by pressure impact up to 1 000 bar . Apart from the risk of a pipe burst this service (expensive!) can be done only by special companies. It may not be regarded as a regular way to open plugged flow paths. For opening the plugged flow paths of the test object in the institute a special twist drill was developed and applied with some success. It had to be guaranteed that the inner pipe walls by no means would be scratched or damaged at the whole length of 3 m.

At one end of a 2 m stainless steel tube (10 x 2 mm) a twist drill tip of 20 mm length was welded on in a way that its channels for the cuttings removal remained open. Tap water with a pressure of 6 bar was induced into the tube by a special rotary transmission leadthrough, which has been constructed in the institute. The actuation was done by a commercial portable drilling machine. Water exits the drill immediately at the tip, where it cares for appropriate cooling and lubrication, but first in line for drawing away the peeled drill cuttings. An important feature was the centring of the drill tip by a wire spiral of 30 mm length, which was welded on the hollow shaft 20 mm from the tip.

By means of this special drill the plugged flow paths could be opened for the subsequent chemical cleaning procedure in a short time without damaging the inner pipe surfaces.

Fig. 6: Twist drill tip for the damageless drilling up of plugged flow paths

6 First results of chemical cleaning trials

Though comprehensive experimental series have already been performed with various chemicals and under various process conditions a critical analysis of the cleaning results proves all of the trials to be still in an initial stage. A universal standard procedure, by which all the differing UHT deposits could be removed without any residue could not yet be established. But finally in each case it was possible to return the test module to the dairy for a new 'soiling run' with metallic clean surfaces.

The tubes were dried by blowing room air through the flow paths. At the dried surfaces the cleaning condition was evaluated visually by means of an 1.5 m rigid endoscope. Objects down to a size of less than 1/10 mm could be detected with this endoscope. For documentation endoscope photographs have been made.
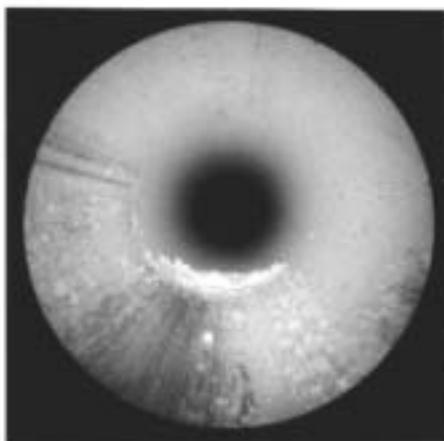
Fig. 7
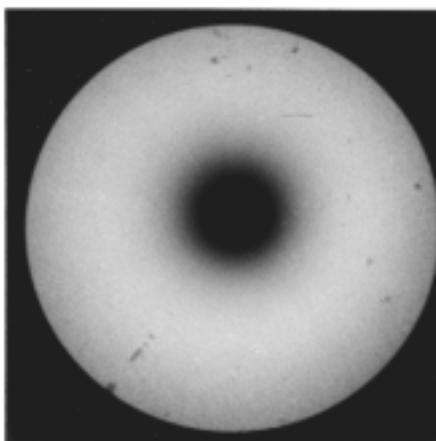
Fig. 8
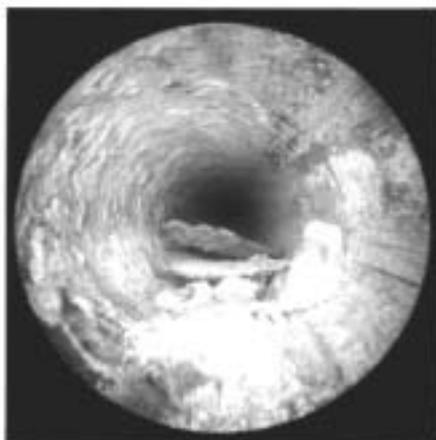
Fig. 9
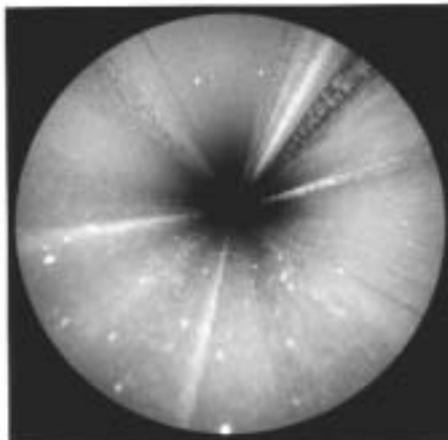
Fig. 10

Fig. 7: Module processed with 50.000 l protein enriched milk at 125 °C. Inner pipe surface after exposition to by 0.25 % NaOH without additives, 30 min at 80 °C; flow velocity 1.5 m/s. Uniform powdery layer, mechanically damaged by the endoscope.

Fig. 8: Module processed with 50.000 l protein enriched milk at 125 °C. Inner pipe surface after being exposed to 0.5 % NaOH without additives, 30 min at 80 °C; flow velocity 1.5 m/s. Uniform powdery layer.

Fig. 9: Module processed with chocolate milk. Inner pipe surface after exposition to 1.0 % NaOH without additives, 60 min at 80 °C; flow velocity 1.5 m/s - 1.0 % HNO_3, 15 min at 80 °C - 1.0 % NaOH, 60 min at 120 °C. Considerable residues, which could not be removed by the 3-phases impact. To get this tube free of any residue, it was processed 5 hours with 4 % NaOH with addition of 1 % EDTA at 90 °C. Subsequently it was impacted for 2 hours by 2 % NaOH with addition of active chlorine at 92 °C and a flow velocity of 3.0 m/s. Final residues were removed by the aforementioned chemicals at a circulation velocity of more than 6.0 m/s.

Fig. 10: Module processed with 150.000 l evaporated milk and 50.000 l protein enriched milk. Inner pipe surface exposed to 0.5 % NaOH with addition of EDTA, 30 min at 120 °C; 1.7 m/s. This tube exhibits metallic clean surfaces!
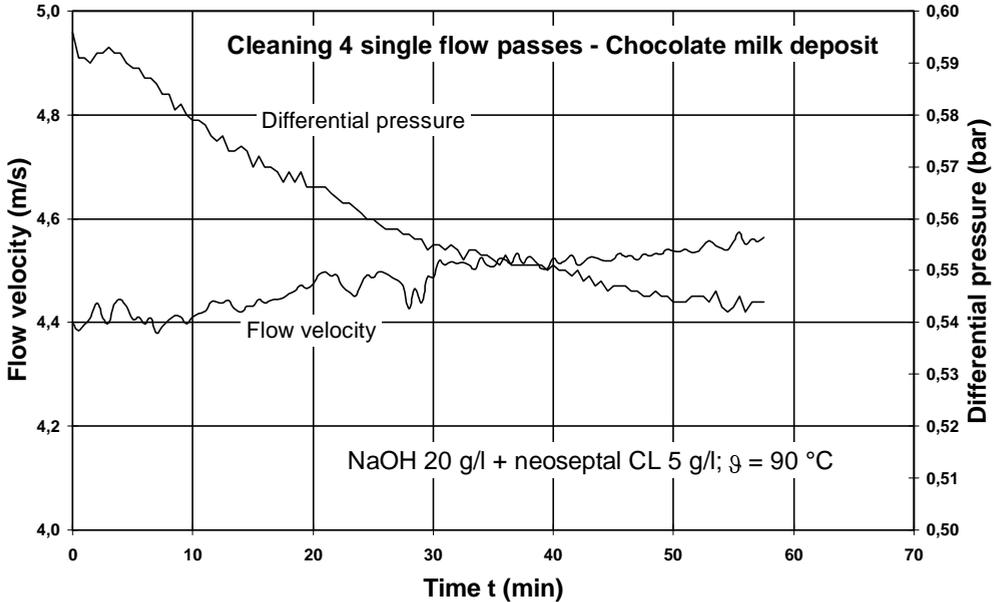
Fig. 11: Differential pressure and flow velocity during the impact of single flow paths

7 Pressure jet spray cleaning

Chocolate milk deposits have proved to be very difficult to chemically clean. Observations indicated that these deposits could be removed with a cotton swab so these critical flow paths were exposed to extremely high mechanical shear stresses. A high pressure jet spray lance with a cone nozzle was inserted into the tube. The spray medium was cold water at 100-120 bar. The result was a metallic clean surface - without application of chemicals within few minutes. Trials in this connection will be continued.

8 Conclusions

Fundamental trials to clean UHT tubular modules have been performed in the lab of the Department of Process Engineering of the Federal Dairy Research Centre in Kiel, Germany, where a computer controlled CIP plant equipped with a computerized data acquisition system has been installed. An original tubular module was fouled in a dairy UHT plant, removed before its cleaning and transported to the institute for experimental purposes. No universal standard CIP procedure has yet been established, which was appropriate to remove all different types of deposits resulting from the UHT heating of different milks and dairy products in a sufficient manner. The CIP process parameters (type and concentration of the cleaning chemicals, temperatures, flow velocity, phases sequence, processing times etc.) had to be specifically adjusted to the various types of deposit. Completely plugged flow paths had previously to be opened with a special water flushed twist drill before exposed to a CIP procedure. Some promising experience has been made with pressure jet spray impact of those deposits, whose removal by a conventional CIP procedure turned out problematic. The trials will be continued.

Analytical models of apples drying processes

E. Kaminski, S. Peron, R. Kramkowski, M. Szarycz, L. Janowicz

The Agricultural University of Wroclaw, Institute of Agricultural Engineering, Poland

Key Words: apple, kinetics, convection drying, microwave drying, inert drying

1 Nomenclature

m	- mass of the sample	[kg]
m_s	- mass of dry sample	[kg]
u	- water content	[kgH$_2$O/kgd.m.]
u_o	- initial water content	[kgH$_2$O/kgd.m.]
u_e	- equilibrium water content	[kgH$_2$O/kgd.m.]
u_k	- critical water content	[kgH$_2$O/kgd.m.]
U_{red}	- reduced water content $\quad U_{red} = \dfrac{u(\Theta) - u_e}{u_o - u_e}$	[-]
Θ	- drying period	[min]
T, t	- temperature	[°K], [°C]
a_m	- coefficient of intrinsic diffusion for water	[mm^2/min]
K	- coefficient of drying	[min^{-1}]
φ	- relative humidity	[%]
G	- sample thickness	[mm]
G_o	- initial sample thickness	[mm]
ψ	- coefficient of shape	[-]

2 Introduction

Apples are featured with high initial moisture content, complex chemical composition and significant change in shape during drying (drying shrinkage). Mathematical description of the run of such process is often very complex and differs from general equations resulting from the theory of mass and heat transfer (for example Lewis equations) describing the run of drying curves.

3 The aim of investigations

The investigations aimed at description of drying process in form of mathematical equations. Three methods of drying were analysed:

- convective drying (natural and forced convection)
- convective drying aided with microwaves
- drying in slow circulating bed of inert material.

The influence of the following factors has been considered:

- in the case of convective drying: rate, temperature and moisture of air as well as the shape and dimension of particles
- in convective drying aided with microwaves: the temperatures of heated material and of the air carrying away the moisture and the power of microwaves,
- in the process of drying in inert bed: the influence of thickness of raw material enveloping the inert balls.

4 The method of investigation

The methods of investigations was presented in papers [1, 2, 3, 4 and 5].

5 Results of investigations

5.1 The kinetics of apples drying in natural convection conditions
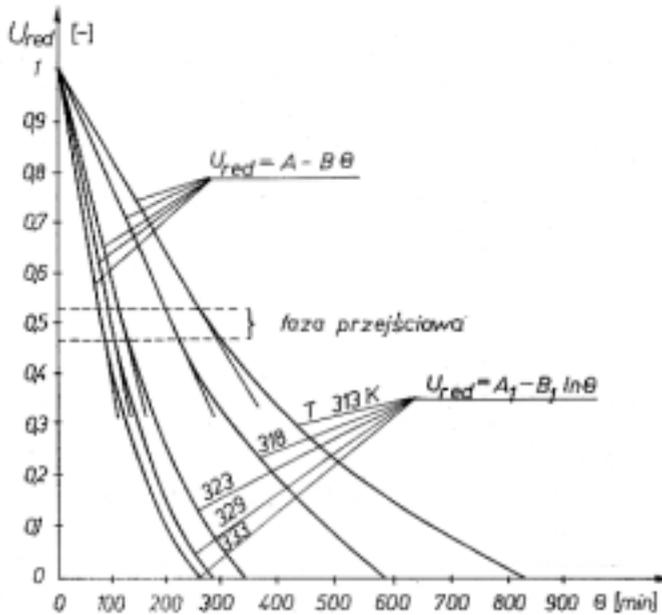
Fig. 1: Drying curves for apple slices plotted on the basis of assumed equations

In Fig. 1, an exemplary drying curve of apple slices (5 mm thick) of Idared variety in the temperatures of 313 - 333 K (40 - 60°C) has been shown.

The curves have been shown in coordinate system $U_{red} = \dfrac{u(\Theta) - u_e}{u_o - u_e} = f(\Theta)$

For the first period of drying, the formula $U_{red} = A - B\Theta$ and for the second one, the equation $U_{red} = A_1 - B_1 \ln\Theta$, have been proposed. The two periods are separated with the transition zone $u_k = 3 - 3.5$ kg H_2O/kg d.m. Coefficients A, B, A_1, B_1 are shown in the table 1.

Table 1: Coefficients A, B, A_1, B_1

No.	Temperature		A	B 10^3	A_1	B_1 10^3
	K	°C				
1	313	40	1	1.80	3.085	0.459
2	318	45	1	2.39	3.082	0.483
3	323	50	1	4.24	2.776	0.477
4	329	56	1	5.21	2.700	0.482
5	333	60	1	6.26	2.456	0.446

It has been stated that the change in thickness of slices in effect of drying process can be approximated with the equations $G/G_o=0.26+0.75u/u_o$ or $G/G_o=A_2\exp(-B_2\Theta)$. where the coefficients A_2 and B_2 depend on temperature. Introducing the changes in apple slice thickness G, into the well know formula for drying of plate with infinite dimensions: $U_{red} = \dfrac{8}{\pi^2}\exp\left(\dfrac{-\pi^2 a_m}{G^2}\Theta\right)$ it was possible

to determine the effective coefficient of water diffusion a_m. The values of the coefficient have been collected in table 2.

Table 2: a_m coefficient

No.	Temperature		$a_m 10^3$
	K	°C	mm^2/min
1	313	40	1.89
2	318	45	1.50
3	323	50	1.92
4	329	56	3.56
5	333	60	2.95

5.2 Kinetics of drying in forced convection conditions

In forced convection conditions, the process - similar to natural convection - can be also divided into two periods, separated with water content u_k = 3.5 kg H_2O/kg d.m. The coefficients A, A_1, B and B_1 depend on the temperature and rate of air what has been shown in Figs. 2 and 3.

The changes in slice thickness and effective diffusion coefficient in effect of drying are shown in Fig. 4 and Fig. 5 respectively.
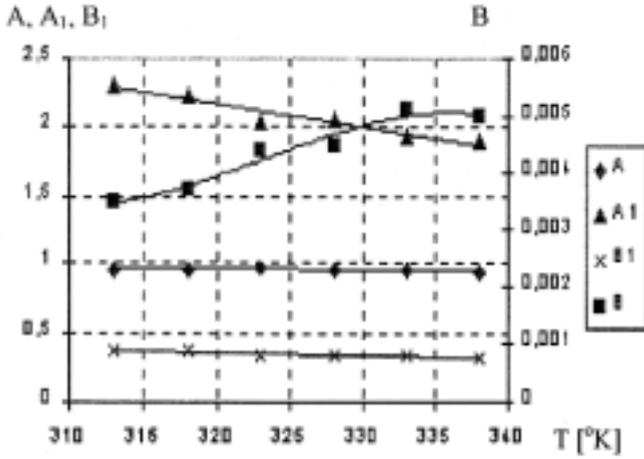
Fig. 2: Coefficients A, A$_1$, B and B$_1$ versus air temperature
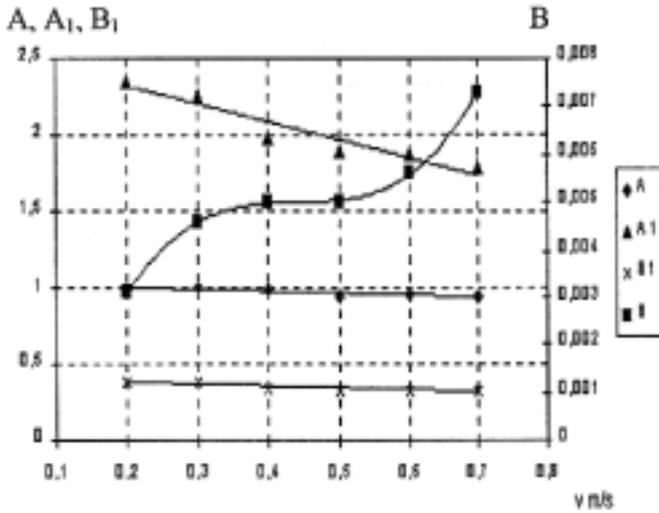
Fig. 3: Coefficients A, A$_1$, B and B$_1$ versus air rate
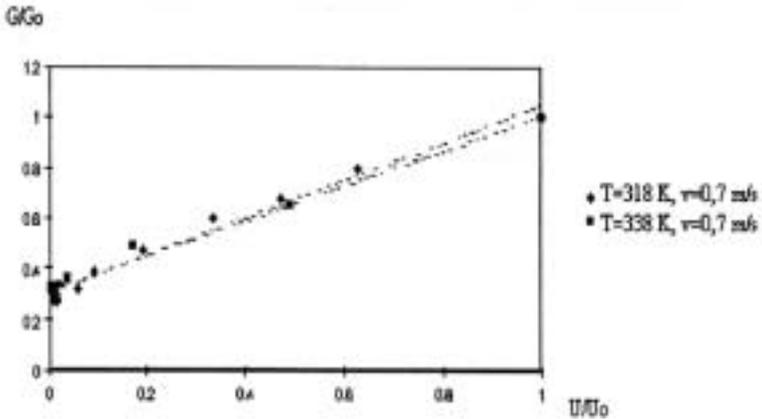
Fig. 4: Changes in equivalent dimension $G/G_o = f(u/u_o)$

$a_m = 0,0105-0,0012t$ v=0,7 m/s
$a_m = 0,0068-0,0009t$ v=0,5 m/s
$a_m = 0,0051-0,0006t$ v=0,3 m/s
t [°C] 40<t<65
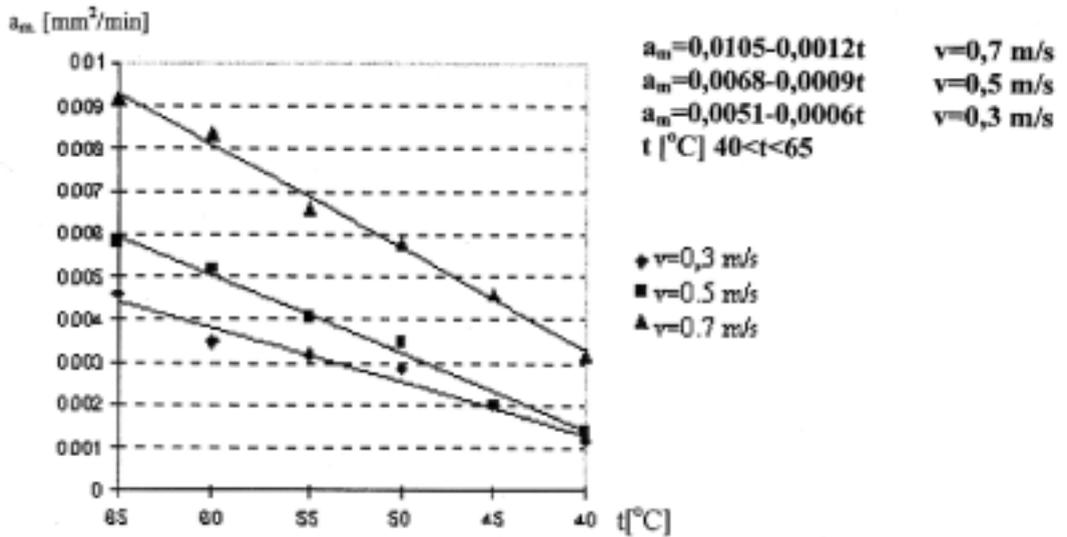
Fig. 5: Changes in effective coefficient of water diffusion versus temperature and rate of air

The influence of relative moisture of air on the coefficients in the equations of kinetics of drying is shown in Fig. 6.

$A = 0,001\varphi + 0,9678$
$B = 0,0042 - 0,00004\varphi$
$A_1 = 0,0132\varphi + 1,6938$
$B_1 = 0,0007\varphi + 0,2952$
20%<φ<80%
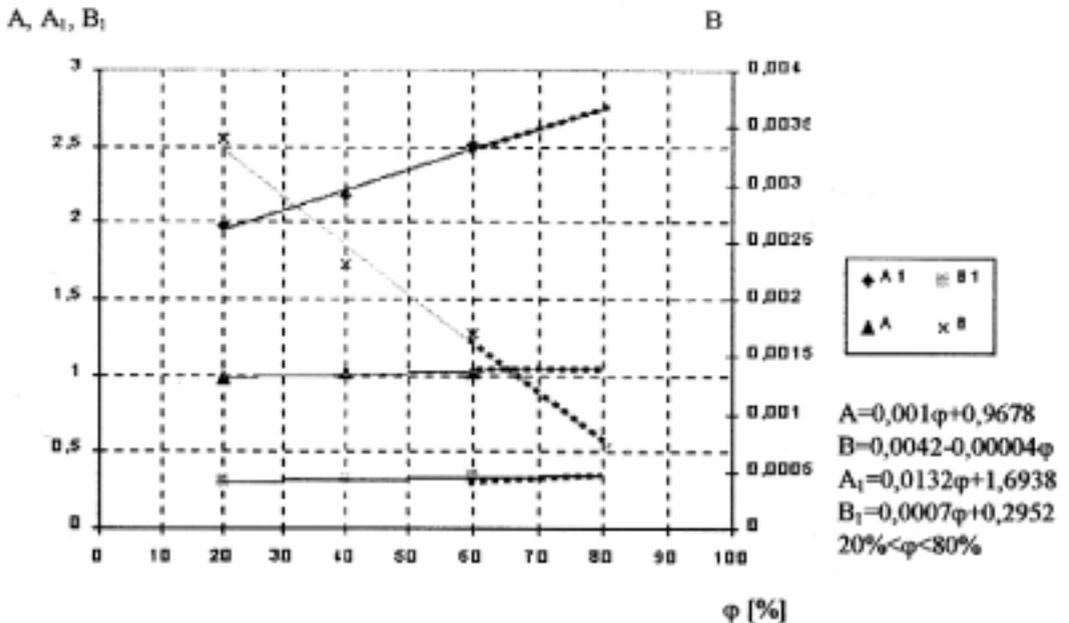
Fig. 6: Changes in coefficients - A, A$_1$, B and B$_1$ versus relative moisture of air - φ, for T = 333K

Comparison of drying of slices and cubes with 5 mm and 10 mm thickness respectively, shows considerable higher drying rate in the second case. It has been illustrated in Fig. 7.

u [kg/kg]
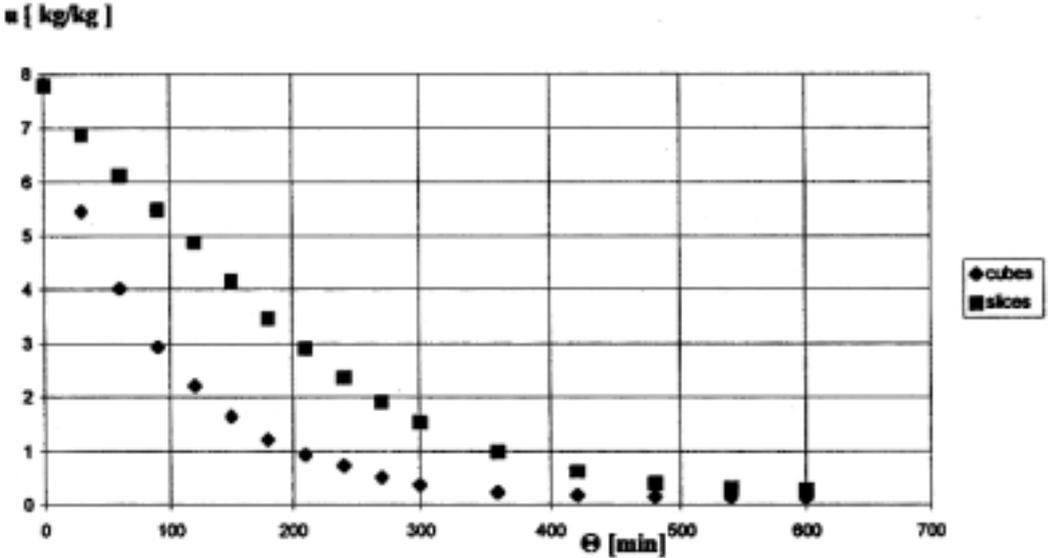
Fig. 7: Comparison of drying curves of apples prepared in form of cubes and slices

Higher water flux in the case of cubes follows from the different ratio of the surface - F to the volume - V mass transfer.

$k = F/V = 6$ cm^2/cm^3 for cubes

$k = F/V = 4$ cm^2/cm^3 for slices

5.3 Kinetics of apple drying in microwave and convective process

In the case of heating aided with microwave energy, it is also possible to distinguish two periods of drying one with the constant and the second one with changing drying rates. The change in the character of drying process takes place at the critical water content $u_k = 4.9$ kg H$_2$O/kg d.m. Straight section of the curve can be described by the equation (Idared variety):

$$u = u_o - a\Theta$$

whereas the second period can be approximated by:

$$u = u_k \exp(-b\Theta_1) \qquad \text{where } \Theta_1 = \Theta_o - \Theta_{uk}$$

The coefficients a and b are related with temperature by the following equations:

$$a = 0.0052T - 4.5319$$

$$b = 0.0017T - 0.502$$

In Fig. 8 an exemplary difference of the runs of convective drying and convective drying aided with microwave energy at the same thermal conditions has been shown.

The power of magnetrons emitting microwaves influences the first and second period of drying. In Fig. 9, the influence of microwave power on the drying rate in the first period of drying has been shown.
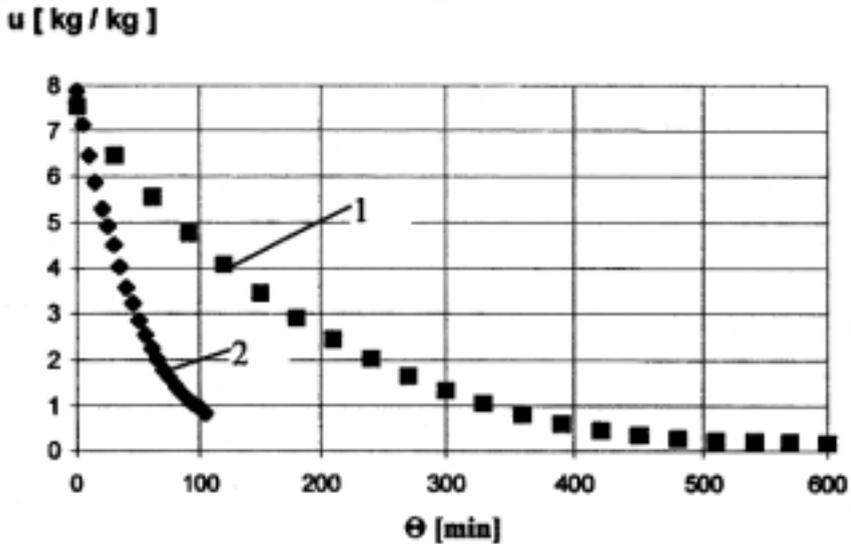
Fig. 8: Comparison of drying curves for apples of Idared variety.
1 – drying in forced convection conditions; T=318K, v=0.4 m/s
2 – convective drying aided with microwave energy; T=318K, v=0.1 m/s

$$du/d\Theta = -0.4648E^3 + 1.0822E^2 - 0.6578E + 0.1898 \quad R^2 = 1$$
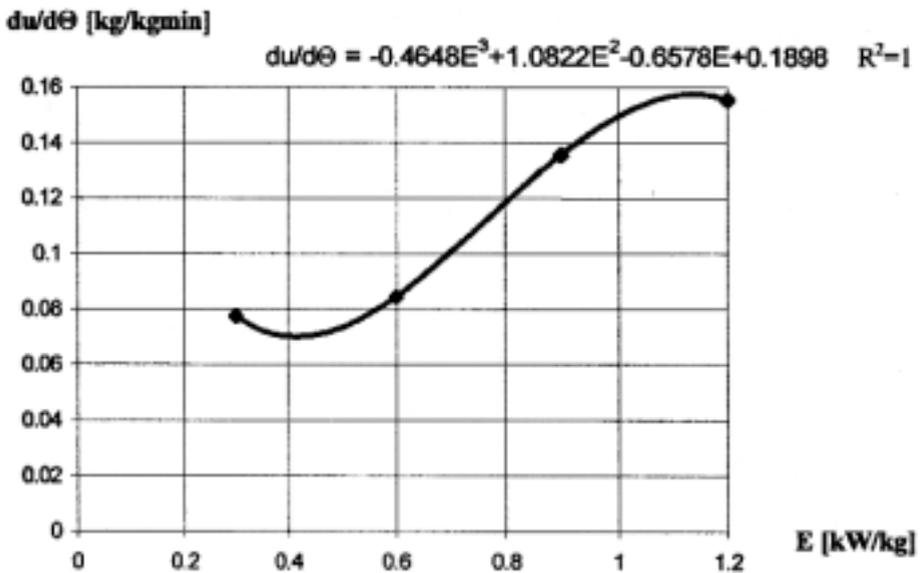
Fig. 9: Drying rate of apples versus power of magnetrons in the first period of drying

The period of decreasing drying rate can be divided into three sub-periods, described by the equation (Bankroft variety):

$$u = \psi_x \exp(-K_x \Theta_x)$$

The values of u_k and K_x for particular sub-periods have been collected in table 3, for different microwave power E.

Table 3: The values of coefficients of drying and critical points

E [kW/kg]	K_1 [1/min]	K_2 [1/min]	K_3 [1/min]	u_{k1} [kg/kg]	u_{k2} [kg/kg]	u_{k3} [kg/kg]
0.3	0.0185	0.0286	0.0187	4.18	1.99	0.49
0.6	0.0282	0.0396	0.0319	4.05	1.78	0.44
0.9	0.0338	0.0383	0.0327	3.92	1.49	0.39
1.2	0.0380	0.0401	0.0247	4.04	0.95	0.40

5.4 Drying of apple pulp in the inert bed conditions

Exemplary curves of drying versus the thickness of envelope and drying rate in the second period of drying have been shown in Figs. 10 and 11 respectively.
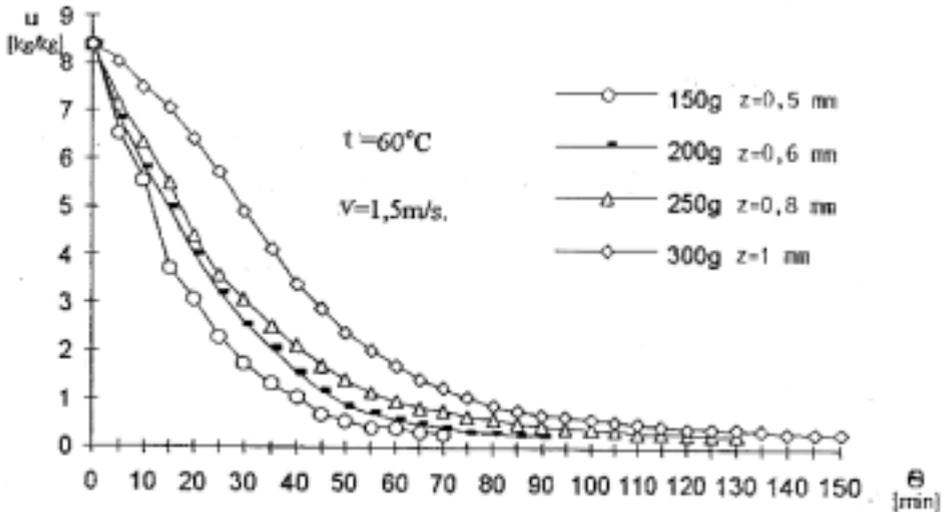
Fig. 10: Dependence of water content u on time Θ for different thickness of the wet material envelope
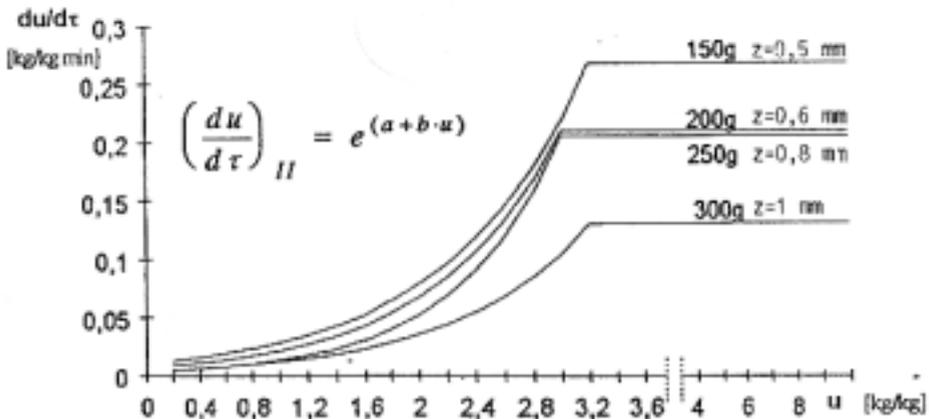
Fig. 11: Diagram of drying rate du/dΘ versus water content u for various thickness of envelope on the ball

The drying rate can be approximated with the equation:

$$\left(\frac{du}{d\Theta}\right)_{II} = e^{(a+bu)}$$

The values a and b for various thickness of envelope are shown in table 4.

Table 4: Coefficients a, b and correlation coefficient r for various thickness of the envelope on the balls

Coeff.	Z=0.5 mm (150 g)	Z=0.6 mm (200 g)	Z=0.8 mm (250 g)	Z=1 mm (300 g)
a	-4,37538	-4,74133	-5,40738	-5,2848
b	1,0186	1,13543	1,3664	1,08582
r	0,80	0,83	0,86	0,88

6 Conclusions

- The proposed empirical equations enable mathematical description of the drying curves in various drying conditions including pure convective drying, convective drying aided with microwave energy and inert bed drying.

- On the basis of the equations it is possible to determine the effective coefficients of water diffusion versus the drying process parameters.

- The thickness of the envelope of drying pulp on inert balls influences the drying process in slow circulating bed conditions.

- Application of microwave heating causes acceleration of the process of apples drying in comparison with conventional methods.

7 Literature

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Treatment of food systems with high intensity electric field pulses

A. Angersbach, V. Heinz, D. Knorr

Department of Food Biotechnology and Food Process Engineering
Berlin University of Technology, Königin Luise-Str.22, D-14195 Berlin
Tel: +49 30 314 71250, Fax: +49 30 832 7663, e-mail: foodtech@mailszrz.zrz.tu-berlin.de

Abstract

High electric field pulses have been applied especially for the permeabilization of biological membranes with major emphasis on the irreversible permeabilization of microbial and on reversible or irreversible permeabilization of plant membranes.

Current efforts in our laboratory dealing with high intensity electric field pulses (HELP) concentrate on cultured plant cells as model systems, on intact plants and size reduced plant tissues as well as on the inactivation of microorganisms. The aim of this work is to aid and optimize the release of valuable metabolites (i.e. pigments, flavours, bioactive components, enzymes) from plant cells and tissues and to affect mass and heat transfer for subsequent unit operations such as dehydration, extraction, freezing, to improve food quality (i.e. fruit and vegetable juices), to understand stress and wound kinetics of plant cells and tissues as induced by HELP treatment and to possible develop a novel non-thermal food preservation method. This paper deals specifically with means to quantify irreversible permeabilization of plant membranes, energy requirements and metabolite yield as well product quality of HELP treated plant foods.

Introduction

Treatment of food systems with high intensity electric field pulses as a reemerging technology after the early attempts by Doevenspeck [1], Flaumenbaum [2], Gilliland and Speck [3], Sale and Hamilton [4] is mainly concentrating on the inactivation of microorganisms [5 - 8]. However, the irreversible permeabilization of plant membranes has also become a focal point of attention [9 - 11] and real food systems have been treated successfully [12, 13]. Limited data are still available as to the engineering aspects of high electric field pulse (HELP) treatment [14, 15]. General food related reviews have been provided by Vega-Mercado et al. [16], Knorr et al. [17], Barbosa-Canovas et al. [18] and resources regarding the impact of HELP on biological membranes are available [19 - 22].

Key research needs, besides engineering aspects such as treatment chamber design, pulse characteristics and process optimization, include the understanding of the impact of HELP on plant membranes, plant cells and plant tissues on a cellular as well as on a macromolecular level. Results of such activities will be exemplified in this review.

Irreversible permeabilization of plant membranes

The application of an external electric field which induces a critical or supercritical electrical potential across the membrane leads within short time (in the nano- and microsecond range) to electrical breakdown and local structural changes of the cell membrane. This first field effect results in a drastic increase in permeability due to the appearance of pores in the membranes. For example, the critical externally electric field strength for potato tissue is between 0.1 and 0.4 kV/cm (Figure 1).

The data were accumulated by using the sample conductometric analysis during the field application.
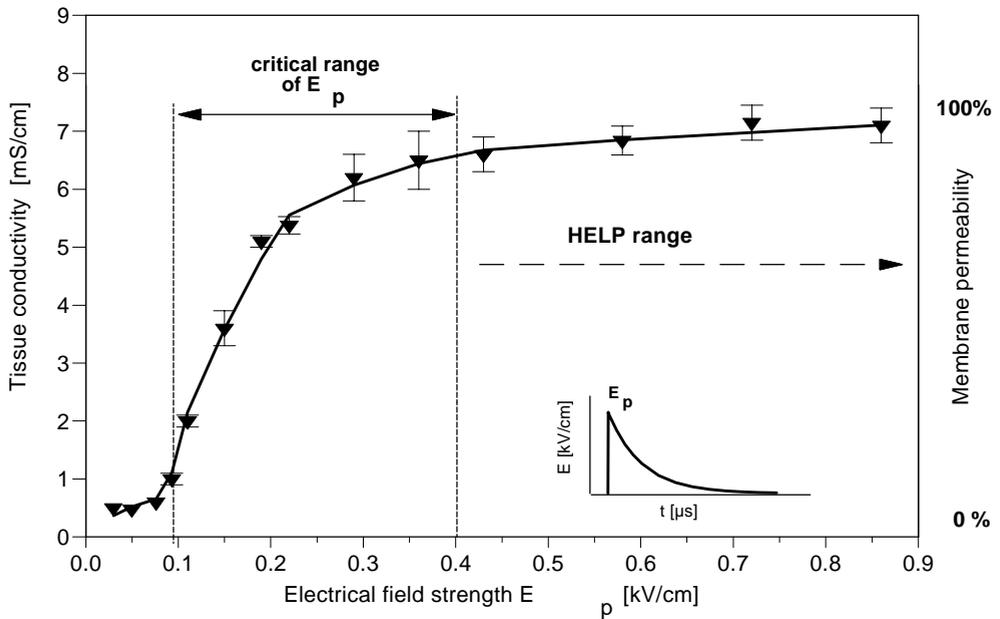
Figure 1: Relationship between effective electrical conductivity of potato tissue (during the pulse) and electric field strength E_p (peak value).

Based on previous work dealing with the frequency dependent changes of conductivities of biological materials [21] a index for cell disintegration was developed:

$$Z_p = 1 - b * \frac{(K_{HF}' - K_{NF}')}{(K_{HF} - K_{NF})}; \quad b = \frac{K_{HF}}{K_{HF}'}; \qquad 0 \leq Z_p \leq 1 \quad \text{with:}$$

K_{NF}, K_{NF}' = electrical conductivity of untreated and treated materials, respectively, in a low- frequency field (1-10 kHz); K_{HF}, K_{HF}' = electrical conductivity of untreated and treated materials, respectively, in a high-frequency field (3 - 50 MHz). This disintegration index characterizes the proportion of damaged (permeabilized) cells within the cells system. For intact cells, $Z_p = 0$; for total cell disintegration, $Z_p = 1$.

Using the cell disintegration index Zp work towards optimizing and maximizing cell rupture via HELP treatment was carried out.

As expected, each individual HELP process parameter (field strength, E; current density, I; and pulse duration, t) is important for irreversible cell membrane disintegration. For example, the larger the magnitude of E (with t= constant), I(with E and t= constant) and t (with E and I= constant), the more markedly the cell disintegration index increases after pulsing (Fig. 2).

Variation of the conductivity of the immersion medium surrounding the plant tissues during HELP treatment (Figure 2) clearly indicated an inverse relationship regarding the effectiveness of HELP

induced permeabilization between medium and product conductivity. These data also most likely help to explain the low yield improvements of apple juice [2, 12]) as compared to carrot juice [17] since the conductivity at HELP frequency is approx. 1 mS/cm for apples as compared to 4 mS/cm for carrots. In addition the importance of pulse duration could also be demonstrated (Figure 3) indicating the interrelationship of field strength, current density and pulse duration as well as the complexity and process variability of HELP processing.
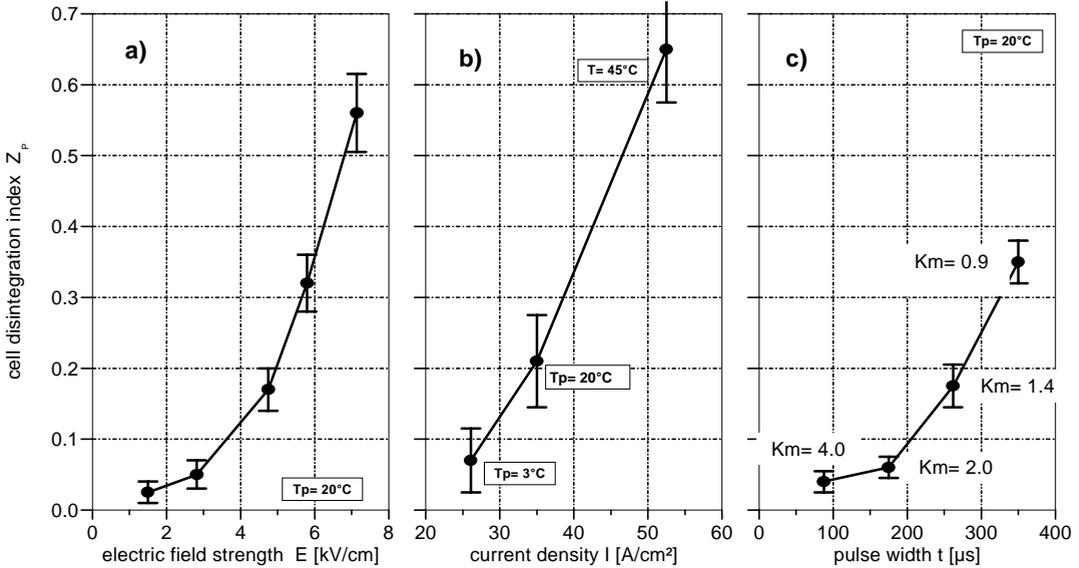
Figure 2: Cell disintegration of potato tissue after application of single pulse (exponential decay) as affected by: a) variation of electric field strength (E) and constant pulse duration (t= 510 µs); b) variation of current density (I) and constant field strength (E= 4.7 kV/cm) and pulse duration (t= 510 µs); c) variation of pulse duration t (by variation of conductivity of immersion medium K_m [mS/cm]) and constant field strength (E= 7.1 kV/cm) and current density (I= 50 A/cm²). Peak pulse voltage used for estimation of electric field strength (E) and current density (I).

Variation of the conductivity of the immersion medium surrounding the plant tissues during HELP treatment (Figure 2) clearly indicated an inverse relationship regarding the effectiveness of HELP induced permeabilization between medium and product conductivity. These data also most likely help to explain the low yield improvements of apple juice [2, 12] as compared to carrot juice [17] since the direct current conductivity for intact apple tissue is approx. 0.25 mS/cm as compared to 0.45 mS/cm for carrots. In addition the importance of pulse duration could also be demonstrated (Figure 3) indicating the interrelationship of field strength, current density and pulse duration as well as the complexity and process variability of HELP processing.

Energy requirements for membrane permeabilization

It is interesting to note that the application of variable electric field strength (in supercritical field strength range, for potato tissue $E_p > 0.4$ kV/cm, Fig. 1) and pulse duration, but constant electrical energy density Q [J/kg sample] per pulse, resulted in the same degree of cell disintegration (no significant difference at the 95% confidence level, Figure 3). This parameter characterized the

energy input per volume/mass unit of the cell materials and is as the integration of $V^2(t)/R_p$ over the pulse duration (where $V(t)$ and R_p is the time depends voltage at the treatment sample and resistance of the sample) determined. For example at Q = 0.45 kJ/kg, HELP treatment of E_p = 1.12 kV/cm and t = 490 μs achieved the same Z_p as at E_p = 2.5 kV/cm and t = 100 μs. Based on these findings, we suggest the specific energy requirement as a dominant HELP process parameter for plant membrane permeabilization in the supercritical field strength range.
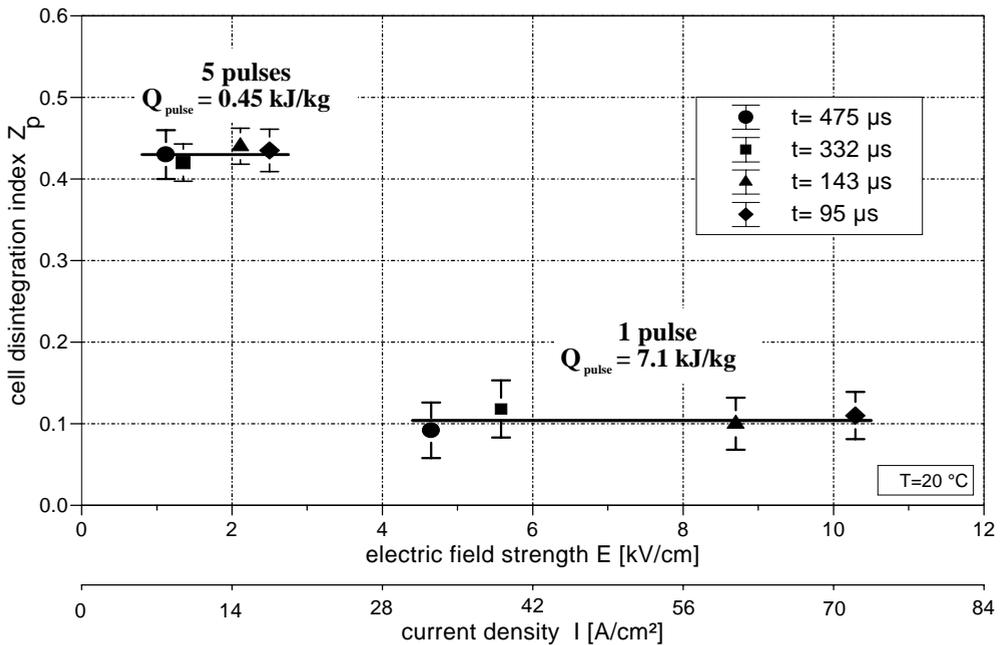
Figure 3: Relationship between cell disintegration index (Z_p) variable field strength (E), current density (I) and pulse duration (t) at constant specific energy input per pulse (Q) after one or five HELP pulses.

Using the specific energy input as critical process parameter the entire range between zero effect to maximum disintegration could be achieved with one pulse treatment (Figure 4, a).

These data suggest that at a low energy input of approx.12 kJ/kg maximum permeabilization of plant tissues can be achieved. As shown in Figure 4, b the optimum energy input per pulse is within the range of approx. 10^2 and 10^3 J/kg. At lower levels of energy input low cell permeabilization resulted even at high numbers of pulses. Energy inputs beyond 10^3 J/kg did not increase the process effectiveness and can consequently be considered as excess energy. Again the complexity of HELP processing becomes evident considering the dependence of the degree of cell disintegration on the number of pulses applied. It should be noted again that treatment in the low energy input range allows processing without temperature increase while high energy input enables - if desired - combined heat and HELP treatment.
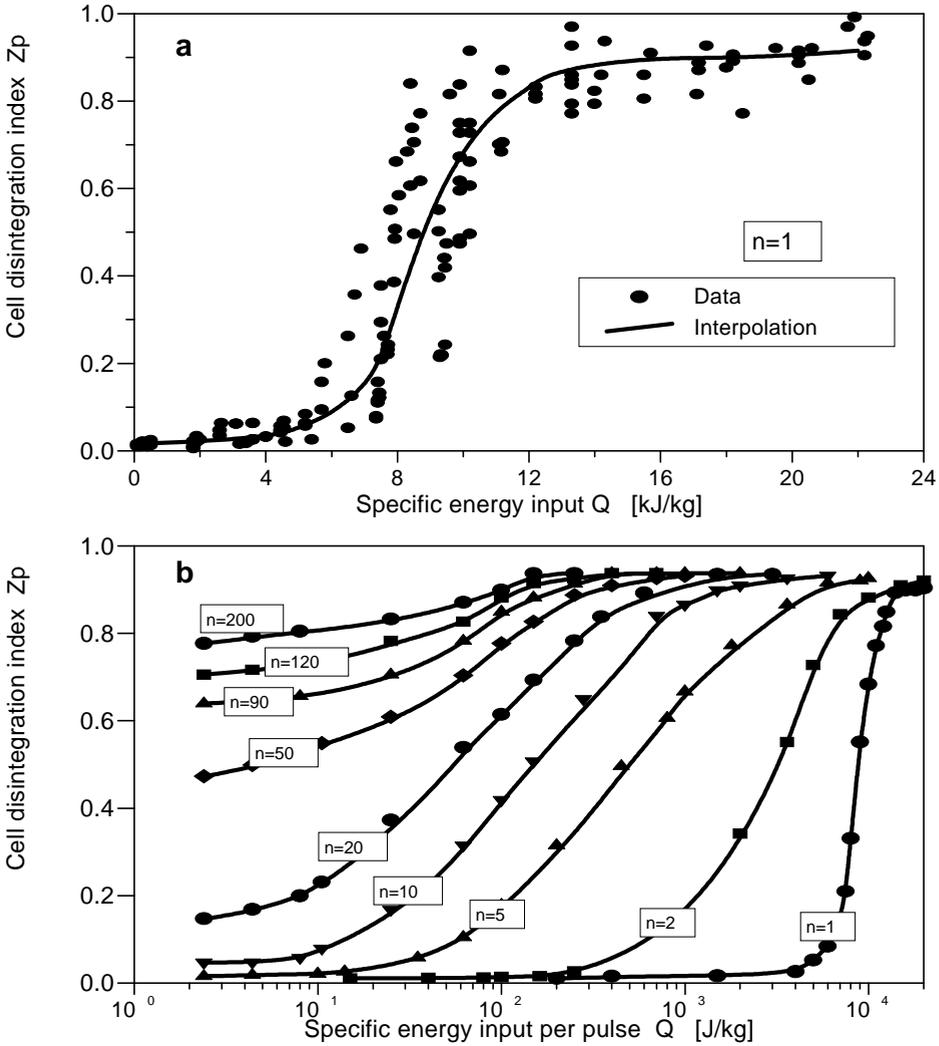
Figure 4: Relationship between specific energy input per pulse Q of (a) one and (b) 1-200 pulses and cell permeabilization of potato tissues. A: primary data and interpolation curve, b: interpolation curve. Disintegration index was identified 10 min after treatment. The field strength was varied from 0.6 to 26 kV/cm, pulse duration from 10 to 800 μs using a pulsing rate of 1 Hz. Initial sample temperature was 20°C.

The effectiveness of the total energy input with regards to cell disintegration is demonstrated in Figure 5.
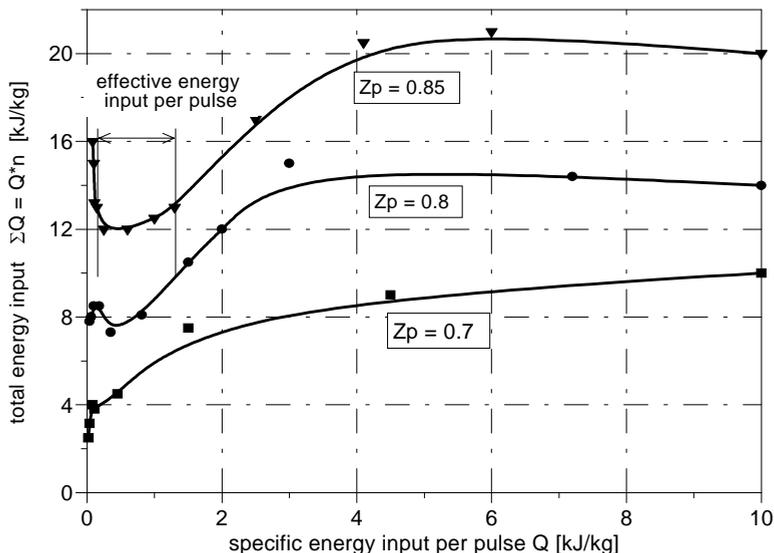
Figure 5: Total energy input (Σ Q) vs specific energy input per pulse (Q) as related to degree of cell disintegration (Z_p).
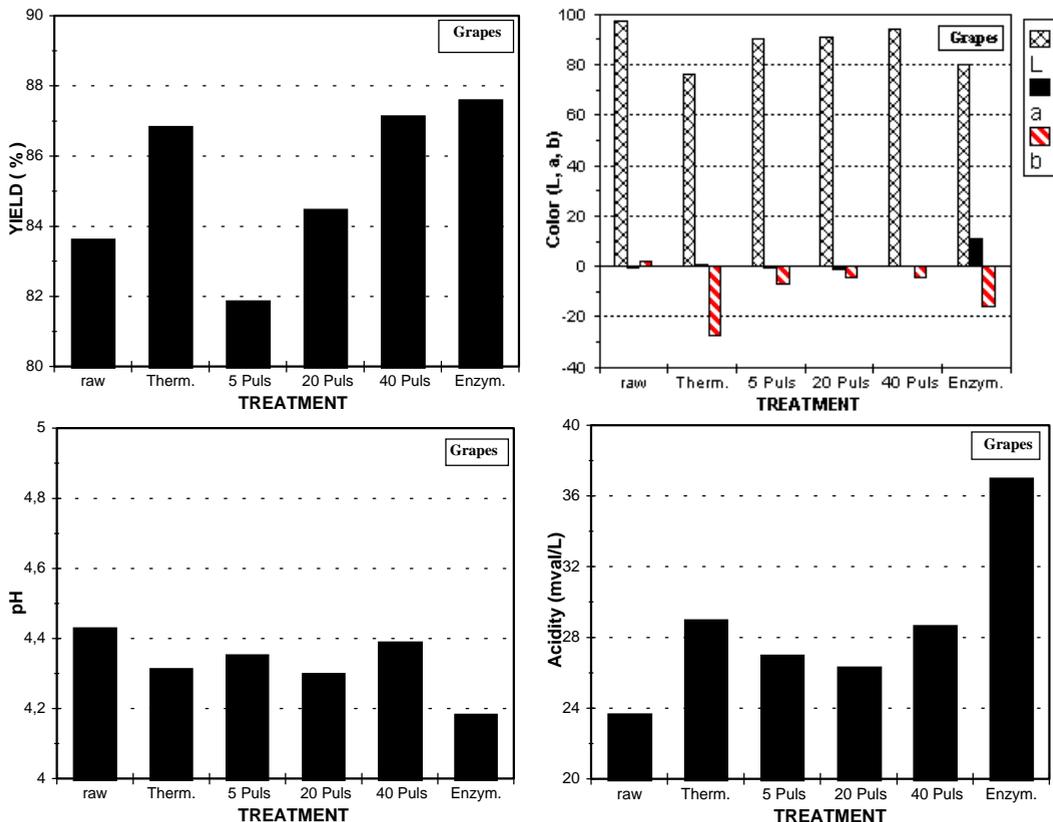
Figure 6: Juice yield and quality criteria of HELP treated grapes (after Esthiaghi and Knorr, 1997). Raw :freshly squeezed; therm: 90°C, 30 s; HELP: 5, 20, 40 pulses (E= 1.5 kV/cm, t = 214 μs, Q_{pulse}= 200-250 J/kg); enzym: Pectinex 100-L, 50°C, 30 min.

Metabolite yield and product quality of HELP treated plant foods

Grape juice yields and quality indicators are presented in Figure 6 demonstrating that HELP treatment provides yields comparable to enzymatically or thermally permeabilized plant cells with product qualities of the HELP treated juices resulting in closer resemblance to the untreated („freshly squeezed") juices than to the enzyme treated products. This was especially evident for colour, pH and acidity (Fig. 6).

Inactivation of microorganisms

Reduction of vegetative organisms in food systems can be achieved [5]. Moreover, data in our laboratory suggest that at pH lower than 5.5 germination of *Bacillus subtilis* spores can be induced by HELP [23]. This offers unique aspects for combination processes.

High electric field pulses: Food safety, quality, and critical process parameters

(FAIR CT 97-3044) (three year project, starting date Nov. 1, 1997)

The general objective of this project is to address and overcome specific scientific and technological hurdles, which is necessary to make an informed judgement on the **relevance** of **high electric field pulse (HELP) technologies**, as well as to realize and to deliver their full benefits. This overall objective will be achieved by systematically exploring the scientific questions relevant to the effects of HELP of various characteristics, on the **safety** and **quality** of food materials, and to use the information generated as the basis of proposing new **process options.** Crucial areas to be evaluated are the effects of HELP on **food safety** and on **food quality**, supported by the identification and evaluation of **critical process parameters** and the exploration of HELP as processing technology or pre-processing steps (**process concept development)**.

It consists of the following partners:

Berlin University of Technology (coordinator), Unilever Research Vlaardingen, University of Montpellier II, Katholic University Leuven, ICE-TEC, CPC Europe, SIK, Pernod Ricard, University of Zaragoza and Tetra Pak.

Conclusions

It is hoped that this brief review could outline the potential for novel process and product design and development. However, more scientific evidence is required regarding the electro-chemical properties of food systems, the complex engineering requirements and demands as well as the interaction between HELP processing and product safety, quality and functionality.

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Inactivation of *Salmonella typhimurium* in liquid egg by ionizing radiation

E. Mayer-Miebach, W.E.L. Spiess

Federal Research Centre for Nutrition, Institute of Process Engineering
Haid-und-Neu-Strasse 9, D-76131 Karlsruhe, Germany
Phone/Fax: +49 721 6625-345 /111, ivt@bfe.uni-karlsruhe.de, http://www.dainet.de/bfe

Abstract

As indicated by national surveillance programmes, the incidence of infectious gastrointestinal diseases caused by microbially contaminated food is still growing world-wide. According to the Federal Institute for Health Protection of Consumers and Veterinary Medicine (bgvv), the number of cases of 'Enteritis infectiosa' in Germany, 90,704 in 1989, was more than doubled until 1997, when 211,084 cases were registered. Salmonella sp. was responsible for about one half (105,340 cases). Eggs, egg products and egg containing dishes contributed to about 50 % of the outbreaks and to about 65 % of individual cases of illness in 1991. As ionizing radiation is a suitable method to inactivate Salmonella sp., comprehensive data are available. These data obtained from different matrices are not transferable in general. If samples of Salmonella typhimurium DSM 554, added to liquid egg and cultivated under laboratory conditions are irradiated in liquid egg at doses between 0.5 and 3.5 kGy, non-linear inactivation curves are obtained. However, when cells of the same microorganism grown in buffered peptone water, a medium frequently used for cell cultivation, are irradiated in liquid egg, the inactivation curves are linear and the inactivation rate is higher.

Introduction

The incidence of 'Enteritis infectiosa' caused by microbially contaminated food is still growing worldwide. In Germany, the number of diseases more than doubled from 1989 until 1997. *Salmonella sp.* was responsible for about 50 % of cases (1).

- 105,340 cases of 'Salmonellosis' in 1997
- eggs and egg products caused 50 % of outbreaks and 65 % of individual cases in 1991

Inactivation of *Salmonella sp.* by ionizing radiation was investigated in liquid egg and in a synthetic matrix.
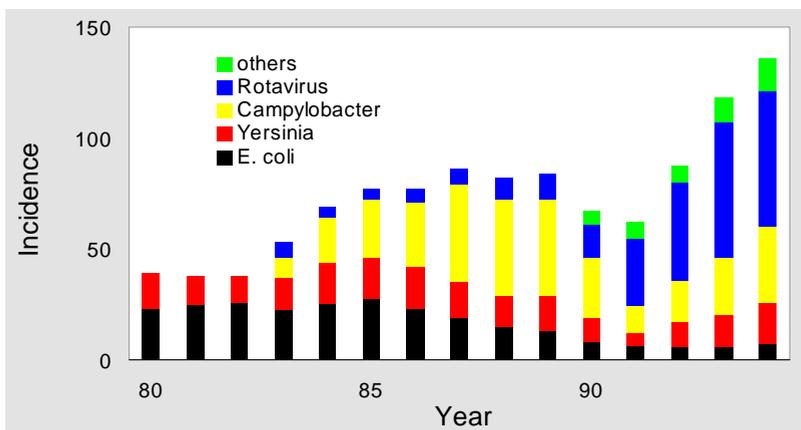
Figure 1: Incidence of 'Futeritis infectiosa' without Salmonellosis

Material and methods

Salmonella typhimurium DSM 554 was cultivated under laboratory conditions in liquid egg and in buffered peptone water. Bacteria from the synthetic growth medium were centrifuged, resuspended in peptone water twice and finally resuspended in oxygen free liquid egg or peptone water. All samples were divided and irradiated at different doses between 0.5 and 3.5 kGy. Counts of survivors were detected using an Agar produced from buffered peptone water and Rambach- Agar, a growth medium specific for *Salmonella sp.* Non-irradiated samples were used to determine initial counts.

Results

Semilogarithmic inactivation curves of *Salmonella* are linear, if the bacteria cultivated in buffered peptone water had been irradiated in liquid egg without the metabolic products released during growth (fig. 2). A D_{10}-value of 0.52 kGy was calculated.
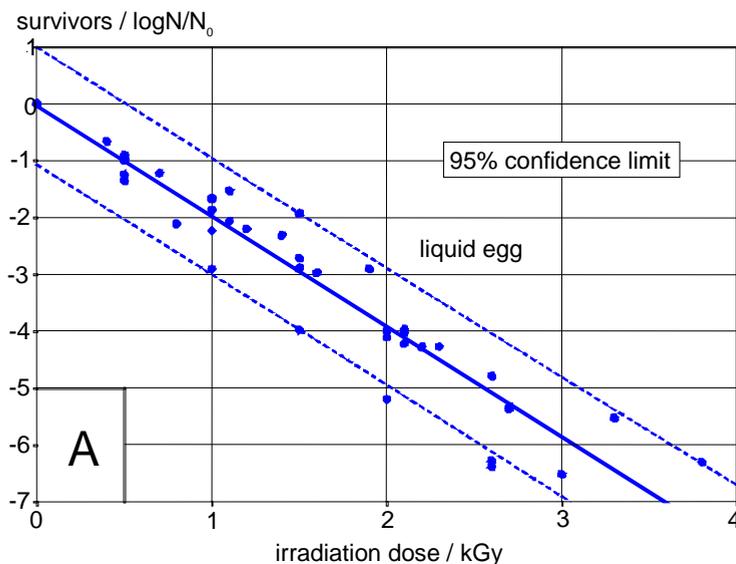
Figure 2: Inactivation of *Salmonella typhimurium* DSM 554 in liquid egg cultivated in buffered peptone water

Inactivation curves of bacteria grown in liquid egg and irradiated directly were non-linear as were the inactivation curves of bacteria grown and irradiated in buffered peptone water with and without metabolic substances released during bacterial growth (fig. 3).

The higher inactivation rate within the synthetic matrix is due to matrix compositions. D_{10}-values calculated by a model based on linear data were 0,62 and 0,2 kGy respectively.
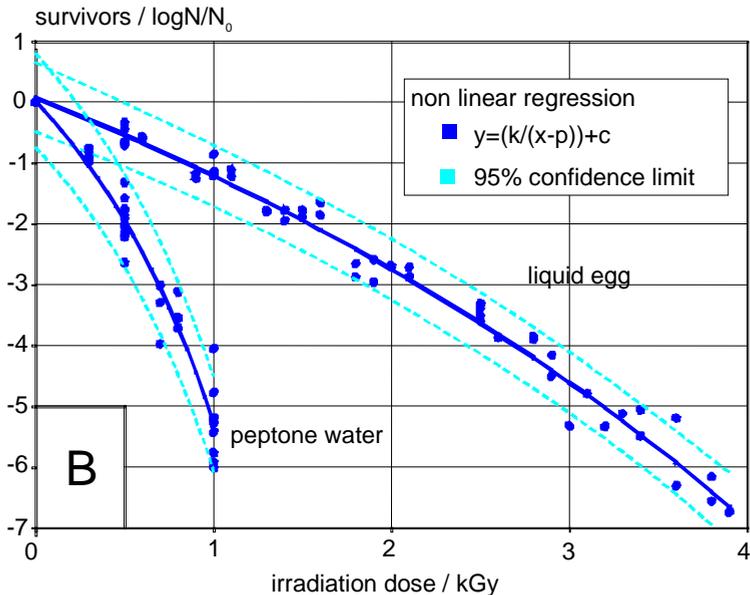
Figure 3: Inactivation of *Salmonella typhimurium* DSM 554 within the growth matrix (liquid egg, peptone water)

Conclusion

As the presented results show, D_{10}-values measured under different conditions are not transferable in general. In order to calculate minimal doses for complete inactivation of *Salmonella sp.* in a specific food matrix, the real course of inactivation curves measured in this matrix should be taken into account. In this case, mathematical modelling does not replace practical experiments.

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Factors affecting sulphur dioxide absorption in tomatoes prepared for sun drying

F. Pazir, Ü. Yurdagel, A. Ural, Ö. Babalik

Ege University Eng. Fac. Food Eng. Dep. Bornova-Izmir, Turkey

Summary

Effects of dipping time, metabisulphite concentration, pH and mixing of the solution on the absorption of sulphur dioxide by halved Riogrande variety tomatoes prepared for sun drying have been studied. Solution concentration, mixing of the solution and increasing of the dipping period during mixing were found very effective on total sulphur dioxide content of sun dried tomatoes. Significant linear and quadratic relations were found between each of investigated factors. pH of the solution and dipping period without mixing in the solution were found effective only at the beginning of the dipping process.

1 Introduction

The tomato is an extremely valuable raw material for a very wide range of processed foods and the demand by the food industries of the world for tomatoes continue at a high level.

In the last years sulphured or salted sun dried tomatoes found a great market opportunities beside the traditional tomato products like juice, paste, ketchup and canned peeled tomatoes.

Dehydration is a method of evaporation of water from food material to a water activity where there's no or minimum chemical and microbiological reactions take place (Meier, 1985). The most unfavourable side of dehydration is browning of food material. Browning can occur before, during and after drying process (Baloch et.al. 1973; Cemeroglu ve Acar, 1986). Some fruits and vegetables expose to SO_2 or its' derivatives before drying for the inhibition of browning. SO_2 can be used in apple, apricot, tomato etc.. drying for the inhibition of browning, control and inhibition of microbiological growth and as an antioxidant and reducing agent for readily oxidizable compounds such as carotenoids (Gilbert and McWeey, 1976; Wedzicha, 1987).

In the preparation of fruits and vegetables for dying, sulphur dioxide is added for the preservation of colour and flavour, to prevent enzyme catalyzed oxidative changes, to prevent microbial deterioration and to facilitate drying by plasmolyzing the cells.

Sulphurization of fruits and vegetables can be achieved by burning of powder sulphur and exposing the fruit and vegetable to an atmosphere rich in SO_2 (Gökçe, 1973) or by dipping into sodium or potassium metabisulphite solutions (Heydenreich, 1967). There are some advantages of dipping into sodium or potassium metabisulphite solutions. First of all air pollution will be avoided, the sulphurizing time will be decreased because of faster absorption in liquid phase and finally the losses of SO_2 during drying will be diminished because of the better penetration of SO_2 into the fruit by dipping process (Stafford and Bolin, 1972a,b; Rosello et.al., 1993).

In this study, halved Riogrande tomatoes which will be sun dried were dipped into sodium metabisulphite solution. The effect of concentration, pH, circulation of the solution and dipping time on the absorption of SO_2 by halved tomatoes were investigated.

2 Materials and methods

2.1 Material

Riogrande variety tomatoes which were harvested in 1995 were used. Fully ripened red tomatoes in standard length (63 mm average) were used in trials. After washing, tomatoes were cut into half perpendicularly. Processed tomatoes were sun dried until the humidity reaches to 13-14 per cent. Dried tomatoes were packed in high density polyethylene bags and bags were thermally sealed. Samples were stored at -20°C till they were analyzed.

2.2 Methods

2.2.1 Processing method

The experimental planning was based on the concentration, pH, circulation of the solution and dipping time into solution. In every trial 10 kg tomatoes were used. Tomatoes and dipping solution ratio was 1/3. After drying 550-600 g dried tomatoes were produced from 10 kg of fresh tomatoes and analyzes have been performed on these samples.

Effect of dipping time

Effect of dipping time in circulated sodium metabisulphite solution

Dipping solution : 8% sodium metabisulphite
Dipping time   : 2.5, 5, 7.5 and 10 min.

Effect of dipping time in still (without stirring) sodium metabisulphite solution

Dipping solution : 8% sodium metabisulphite
Dipping time   : 5, 10, 15 and 20 min

Circulation type of the solution

Dipping solution : 8% sodium metabisulphite

Dipping time   : 10 min

1. Continuous circulation
2. One circulation in every 2.5 min intervals
3. One circulation in every 5 min. intervals
4. No circulation

Effect of solution concentration

Dipping solution : 3%, 6%, 9% and 12% continuously circulated sodium metabisulphite solution

Dipping time   : 5 min

Effect of solution pH

Dipping solution : 8% continuously circulated sodium metabisulphite solution

Dipping time   : 5 min

      a) pH 4.5
      b) pH 4.0
      c) pH 3.8

2.2.2 Analyze methods

Refroctometric dry matter, pH, acidity analyzes in tomatoes were performed according to Cemeroglu (1992).

Total sulphur dioxide was determined using modified Reith Williams method (Ural et.al., 1990) and the statistical analyzes were performed according to Alvey (1977) in GENSTAT programme.

3 Results and discussion

Raw material analyzes were carried out in washed tomatoes.

Mean measures and some quality characteristics of the tomatoes were as follows:

length :	73.1±7 mm
width :	49.6±6 mm
weight :	115.3±25 g
refractometric dry matter :	4.9±1
pH :	4.2±1
acidity :	0.35±0.05 g / 100 g

3.1 Effect of dipping time

3.1.1 Effect of dipping time in circulated sodium metabisulphite solution

Halved tomatoes were dipped into 8% circulated sodium metabisulphite solution for 2.5; 5; 7.5 and 10 min. SO_2 content of these tomatoes after drying were shown in Figure 1.
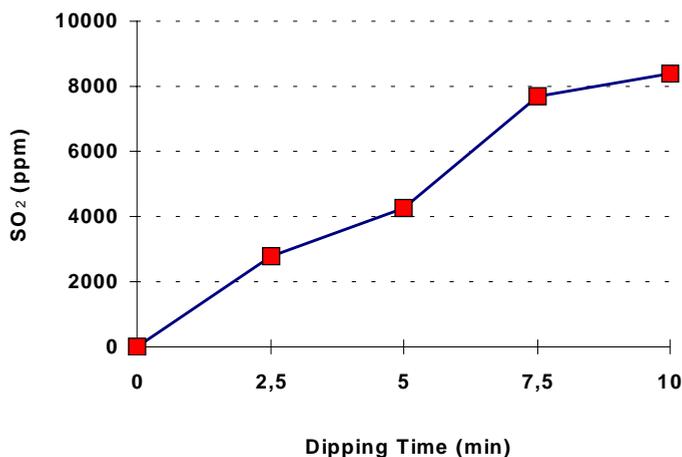
Figure 1: Effect of dipping time on the absorption of SO_2 (circulated)

A linear relation (p=0.01) was found between SO_2 absorption and dipping time in dried tomatoes dipped into circulated solution before drying. As the dipping time increased SO_2 absorption was also increased. 2.5 and 10 minute dipped tomatoes reaches to 2785 and 8395 ppm SO_2 content after sun drying, respectively. Final SO_2 contents of dried tomatoes were found statistically different for each dipping period stage.

3.1.2 Effect of dipping time in static (still) metabisulphite solution

The effect of dipping time of tomatoes in 8% static metabisulphite solution on the SO_2 content of dried tomatoes can be seen in Figure 2. While dipping tomatoes into the solution for 5 min resulted 1631 ppm SO_2 content in dried tomatoes, in 10 min dipping 2464 ppm, in 15 min dipping 2825 ppm and in 10 min dipping 2480 ppm SO_2 were found in dried tomatoes.
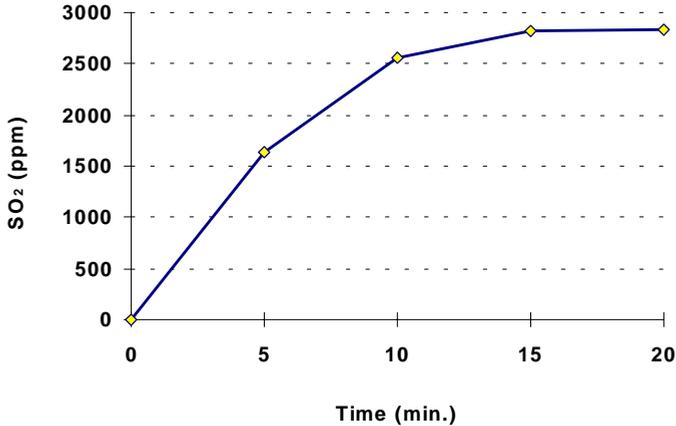
Figure 2: Effect of dipping time on the absorption of SO_2 (static)

Similar results were also presented with fresh apricots (Stafford and Bolin, 1972a; Rosello et.al, 1989). In the other research performed with the dried apricots, it was found that the absorption of SO_2 was higher in few seconds of dipping period and was slow down in further times (Stafford and Bolin, 1972b). In these researches there were no explanation about the circulation of the dipping solutions. In a research done on two different hybrid of mushrooms, 31.1 and 71.8 ppm SO2 have been found after dipping into 1000 ppm sodium sulphite solution for 3 min. (Beelma et al., 1988).

3.2 Effect of circulation type on the absorption of SO_2

Circulating of the 8% sodium metabisulphite solution for different time intervals was found effective on the absorption of SO_2 in samples (Figure 3).

As a result of variance analyse, linear and quadratic effects of dipping solution circulation was found significant at a p=0.01 level. According to a 5% LSD level, the samples which were circulated continuously form one group (a) and they absorbed higher amounts of SO_2 (a), while 2,5 and 5 min circulated samples (b) and noncirculated samples (c) formed the other two groups with lower SO_2 amounts. It may be said that dipping into 8% circulated metabisulphite solution for 2.5 min was enough to maintain the dried tomatoes with 2500 ppm SO_2. For that aimed limit the period must be increased to 10 min in non circulated solution. Continuous circulation of the metabisulphite solution increased the SO_2 content of sun dried tomatoes approximately 3 folds, namely from 2480 ppm to 8246 ppm, for 10 min application.
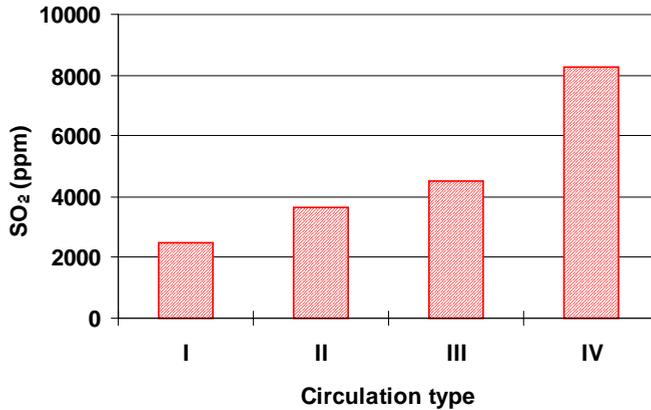
Figure 3: Effect of circulation type on the absorption of SO_2
        I) No circulation
        II) One circulation in every 5 min. intervals
        III) One circulation in every 2.5 min. intervals.
        IV) Continuous circulation

3.3 Effect of solution concentration on the absorption of SO_2

Significant linear and quadratic relations were found between the dipping solution concentrations and SO_2 absorption amounts of sun dried tomatoes. As the concentration increased SO_2 absorption was also increased (Fig. 4).
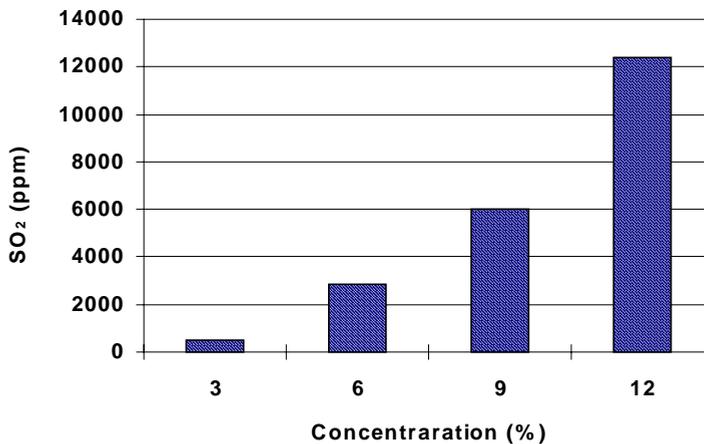
Figure 4: Effect of solution concentration on the absorption of SO_2

For a 5 min dipping period the sulphur dioxide content of sun dried tomatoes reached to 500 ppm level for 3 percent metabisulphite concentration treatment. Increasing of the metabisulphite concentration to 6, 9 and 12 percent , increased the final SO_2 content to 2850, 6000 and 12370 ppm, respectively. Each concentration level resulted statistically different amounts of SO_2 according to LSD level of 5 percent. With fresh apricots, linear relation was observed between the concentration of the solution and the absorption of SO_2 (Stafford and Bolin 1972; Rosello et.al. 1993).

3.4 Effect of solution pH on the absorption of SO_2

For finding the effect of pH on the absorption of SO_2 , 0.25% and 0.5% citric acid was added to 8% sodium metabisulphite solution. The pH of the 8% sodium metabisulphite solution was normally 4.5. It was decreased to 4.0 when 0.25 % citric acid was added and to 3.8 when 0.55 citric acid was added.

The absorption rate of SO_2 in these continuous circulated solutions for 5 min. were given in Figure 5.
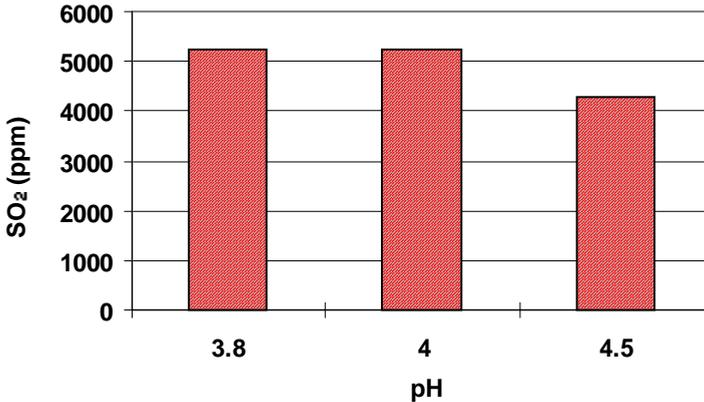
Figure 5: Effect of solution pH on the absorption of SO_2

Significant linear effect was found with the decreased pH levels or with citric acid addition for SO_2 uptake by the tomatoes. However no significant effect was found on SO_2 uptake between the acidified groups with 4.0 and 3.8 pH values.

Stafford and Bolin (1972b) could not find any effect of decreasing solution pH by citric acid addition on the absorption of bisulphite by dried apricots. In an other research performed by these researchers (Stafford and Bolin 1972a) it was found that the low pH of the solution was effective on the absorption of SO_2 in fresh apricots and they concluded that this was caused by the increased of the higher sulphate concentration in the solution at low pH. At pH = 2.5 the sulphurous acid concentration of bisulphite solution is 20% and sulphurous acid is in equilibrium with SO_2 and H_2O, at pH 4.5 there is no sulphurous acid in the medium (Stafford and Bolin 1972a). Absorption rate of sulphurous acid is higher than bisulphite ion (Heydenreich, 1972a). However with the decreasing pH of the solution, the loss of SO_2 increasing during drying (Stafford and Bolin 1972a).

As a result, factors effecting the SO_2 content of dried tomatoes can be summarized as follows;

- As the concentration of the solution increased, the absorption of SO_2 also increased. Both linear and quadratic relation was found between the concentration and the absorption of SO_2.

- Circulation of the dipping solution was found very effective on the absorption of SO_2. This relation was both linear and quadratic.

- Decreasing the pH level from 4.5 to 4 was found effective on the absorption of SO_2 but statistically no difference was found between 0.25% and 0.5% citric acid added solutions with pH of 4.0 and 3.8 respectively.

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Review and Transfer Congress, Congress Centre, Karlsruhe, Germany
October 18-20, 1998

Session 5:

Consumer Perception
and Transfer Strategies

Consumer assessments of food texture: importance of oral breakdown of food to texture perceptions and preference

W.E Brown

Institute of Food Research, Earley Gate, Reading, RG6 6BZ, UK

Abstract

This presentation reviews recent progress in the application of mastication studies to understanding consumer sensory perceptions of, and preferences for, food products. The methods for recording mastication patterns from human subjects, and linking these with sensory assessments of a variety of food products, are described. The importance of understanding how foods are progressively broken down in the mouth to avoid linking perceived attributes to inappropriate instrumental measures, is described in the case of the perceived hardness and crunchiness of biscuits. The importance of individual differences in patterns of oral breakdown of foods in underlining differences in consumer sensory assessments is highlighted for both perceived texture and flavour. In order to fully understand sensory properties of foods it is necessary to recognise that our perceptions are dynamic and generated by dynamic changes wrought in the products during eating, and to use dynamic methods to examine them.

Introduction

The texture and flavour of foods remain the principle determinants of consumer preference for, and choice of food products. Modern diets and lifestyles are changing the way we prepare and eat foods. In producing foods to cater to these consumer demands it is essential that texture and flavour qualities, as perceived by the consumer, are recognised as the ultimate mark of success.

- Consumer awareness of the links between diet and health provide an intention to eat a healthy diet, however this may be frustrated by dissatisfaction with the sensory qualities of new "healthy" alternatives to traditional products. Development of products to replace or supplement traditional foods must consider these sensory aspects. Recent developments in this direction have provided products with improved sensory quality and acceptability, but further progress is necessary to encourage more consumer uptake of these products.

- Trends in food preparation have seen an increase in the use of fast cooking methods and convenience foods, for use in family meals and for entertaining, alongside a re-emergence of the pleasures of traditional cooking. Modern consumers are increasingly discerning of the sensory quality of the foods they eat, and consequently convenience foods which are destined for home use should provide the expected and appreciated sensory qualities of traditional products, or indeed, desirable novel textures and flavours. Efficiency in the development of products to satisfy these demands requires an understanding of the criteria used by consumers in making these judgements.

- Whilst commonly-used methods to measure texture and flavour instrumentally provide information about the mechanical properties of foods, and flavour composition, they do not address the dynamic aspects of food breakdown in the mouth which determine how these qualities are perceived by consumers.

By understanding where the sensations come from, and how they are linked to different aspects of the oral breakdown process, we can predict texture and flavour qualities from objective data, and be more able to manipulate them at will. This will provide greater understanding of individual products, and improve efficiency in product development.

In this paper I will review some of our work examining the oral breakdown of food as people chew, and the perceptions they report as a result.

The methods

The methods we use are principally dynamic. They are aimed at recording changes in the way the sample is chewed, and its consistency and flavour, over time.

Mastication patterns

The vigour and rhythm of chewing is measured using electromyography (EMG) to record the activity of the masseter and temporalis muscles which operate to close the jaw during closing. Small electrodes placed on the skin surface overlying these muscles record the electrical signals which are transmitted in muscle fibres during contraction. The area under the EMG signal corresponding to each chew provides a measure of the muscle effort involved in the chew. The method is painless and does not interfere with mastication and is described in full in Brown (1994), and Brown et al., (1994).

The movement trajectory of the jaw during chewing provides information concerning the type of actions involved in pulverising a food sample during mastication. These may be vertical chopping actions, or lateral grinding actions, but many foods involve both actions to varying degrees, and it is usual for the type of action to change as a result of changes in the state of the food bolus over the course of the chewing sequence. Jaw movement may be recorded by monitored the position of a small magnet, attached using a medical adhesive gum to the lower incisors, using a set of sensors held by a headframe secured around the subject's forehead. This is light and allows the subject to move the head during recording without interfering with the chewing process. The method for combining EMG and kinematic (KI) recording, data acquisition, and analysis, is fully described in Brown et al., (1998a). It allows the muscle work involved in the vertical (chopping) and lateral (grinding) aspects of each chew to be calculated separately.

Consumer segmentation

Dentists have long been aware of differences between individuals in the efficiency and effectiveness with which they are able to break down foods during chewing, whilst we are commonly aware of differences between individuals in the time they take to process a mouthful of food before swallowing. In view of the fundamental relationships between the way a food is processed and the sensory perceptions derived from it, it is useful to understand the extent to which differences in the way consumers chew a food, affect their judgements of its sensory properties and their food preferences. Consumers may be more or less able to break down different foods by virtue of their masticatory apparatus and the sensory feedback they receive. We have compared subjects' chewing efficiencies with a brittle non-adherent product (nuts) and a viscous cohesive product (chewing gum), and the differences in oral breakdown patterns for a number of food categories among subjects exhibiting high efficiencies with both products (nuts and gum), low efficiencies with

both, or high efficiency with one and low with the other. Subjects for whom the median fragment size of an almond nut expirated after 10 chews was low, were classified as being highly efficient at comminuting nuts. This presumably arises from the subjects' ability to position and apply appropriate forces to the nut to achieve maximum structural disintegration at each chew. Subjects for whom the weight loss in a stick of chewing gum after 100 chews was high, were classified as highly efficient at dissolving sucrose from the gum matrix. This probably arises from subjects' high salivation rates and ability to manipulate (knead) the gum to achieve maximum extraction of the sucrose. We have demonstrated differences among these subject groups in terms of flavour (Brown et al., 1996a) and texture (Braxton et al, 1996) perception and suggested influences of chewing efficiency on food preferences (Brown et al., 1999).

Sensory assessments

Conventional methods of sensory assessment generally require the assessors to score distinct attributes of a product despite the fact that the attribute may change in quality or intensity during the course of the oral breakdown of the product. Such sensory assessments in combination with mastication studies may highlight aspects of the oral breakdown which are important in making judgements about particular sensory attributes. Alternatively the use of dynamic methods for recording sensory information, such Time Intensity (TI), in conjunction with mastication studies can reveal relationships between the chewing process and sensory perceptions. We have undertaken several studies which combined the measurement of mastication patterns with dynamic sensory assessments. For example, to determine how changes in the effort involved in chewing meat over the course of the chewing sequence correspond to dynamic perception of the tenderness of the sample (Brown et al., 1996b), or how the action of saliva during the progressive comminution of biscuit results in moistening of the sample as perceived by the subject, Brown et al., (1999). The same technique has been applied to examine how the perception of flavour intensity relates to sample breakdown during chewing, swallowing, and subsequent elimination of flavour-laden saliva from the mouth (Wilson and Brown, 1997).

Results and discussion

Influence of the dynamics of oral breakdown of food on perceived texture attributes

Some textural characteristics of food products may be related to how they break down progressively during chewing. In a study of a series of 10 "Rich Tea" type biscuits, the texture was modified by flour type and additive content. They were assessed by 19 consumers, untrained in traditional sensory analysis, for "hardness", "crunchiness" and "crumbliness", on 3 occasions. The subjects' mastication patterns were also recorded using EMG and kinematic measurement of jaw movement on 3 separate occasions. Analysis of the sensory results indicated a strongly significant negative correlation between hardness and crumbliness, ($r = -0.95$, $n = 10$), and a significant correlation between hardness and crunchiness ($r = 0.84$, $n = 10$).

Principal component analysis involving the sensory results and a number of instrumental measures of the mechanical properties of the samples indicated close links between the perceived hardness (and inversely, the perceived crumbliness) of the biscuit samples, and the results of compression and penetrometer tests. However such tests did not reflect the perceived crunchiness of the samples.

Principal component analysis involving the sensory attributes and parameters measured from the consumers' mastication patterns provided evidence of discrimination between the hardness and the crunchiness characteristics of the samples. For this purpose the parameters calculated from the mastication records were;

CW   total chew work = area under the EMG curve for all chews in the chewing sequence

CWVert   the work involved in the vertical closing of the jaw for all chews in the chewing sequence

CWHoriz   the work involved in the horizontal closing of the jaw for all chews in the chewing sequence

F5CW   chew work for the first 5 chews

F5CWVert   chew work for the vertical jaw movement for the first 5 chews

F5CWHoriz   chew work for the horizontal jaw movement for the first 5 chews

N5CW   chew work for the next 5 chews (chews 6-10)

N5CWVert   chew work for the vertical jaw movement for the next 5 chews

N5CWHoriz   chew work for the horizontal jaw movement for the next 5 chews

L5CW   chew work for the last 5 chews

L5CWVert   chew work for the vertical jaw movement for the last 5 chews

L5CWHoriz   chew work for the horizontal jaw movement for the last 5 chews
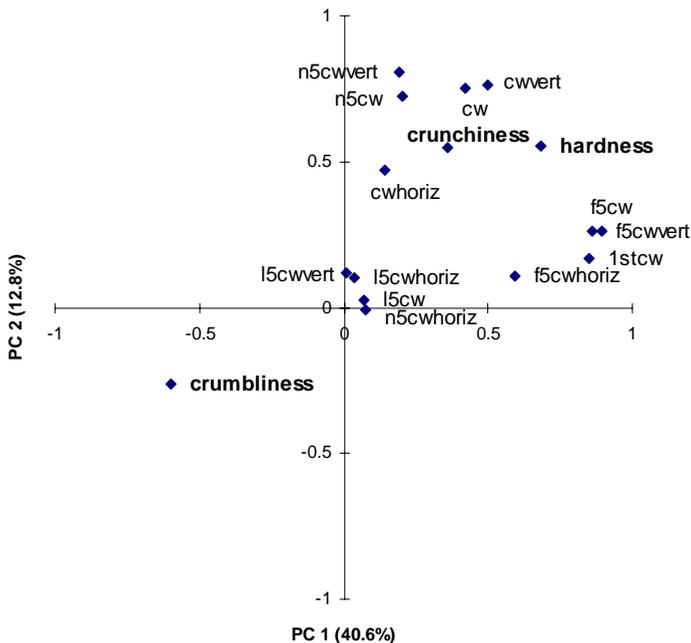
Fig 1: Principal component analysis of sensory scores and mastication parameters for biscuits

Figure 1 indicates separation of the hardness and crunchiness attributes, with hardness being more related to the effort involved in the first few chews of the chewing sequence and crunchiness being more related to the effort involved in later chews in the sequence (chews 6-10) and the total work necessary to break down the sample for swallowing. These results suggest that a crunchy biscuit need not necessarily be hard, in terms of requiring high forces to bite through in the initial bites, but should exhibit a sustained resistance over the course of the later chews, and consequently a higher work input overall. Such information should be of use to biscuit manufacturers keen to create a crunchier biscuit without it being unacceptably hard. Full details of these results are given in Brown et al., (1998b).

Influence of chewing strategies on the dynamics of oral breakdown of foods

The way in which a food material disintegrates during mastication depends on the level and type of forces applied to it by the teeth and oral surfaces (hard palate, tongue etc.), and how the fragments so generated are affected by the moisture and thermal conditions in the mouth. That individuals differ in the strategies they employ to break down a food material is indicated in Figure 2, which shows the level of chewing work at various parts of the chewing sequence (first 5 chews, next 5 chews and last 5 chews) for subjects who differ in their chewing abilities. The results are from the same study of "Rich Tea" type biscuits already mentioned. Nineteen subjects were divided into the following chewing efficiency (CE) groups; CE1, low efficiency for both nuts and gum, n=4; CE2, high efficiency for nuts, low efficiency for gum, n=6; CE3, low efficiency for nuts, high efficiency for gum, n=5; CE4 high efficiency for nuts and gum, n=4.

a   b
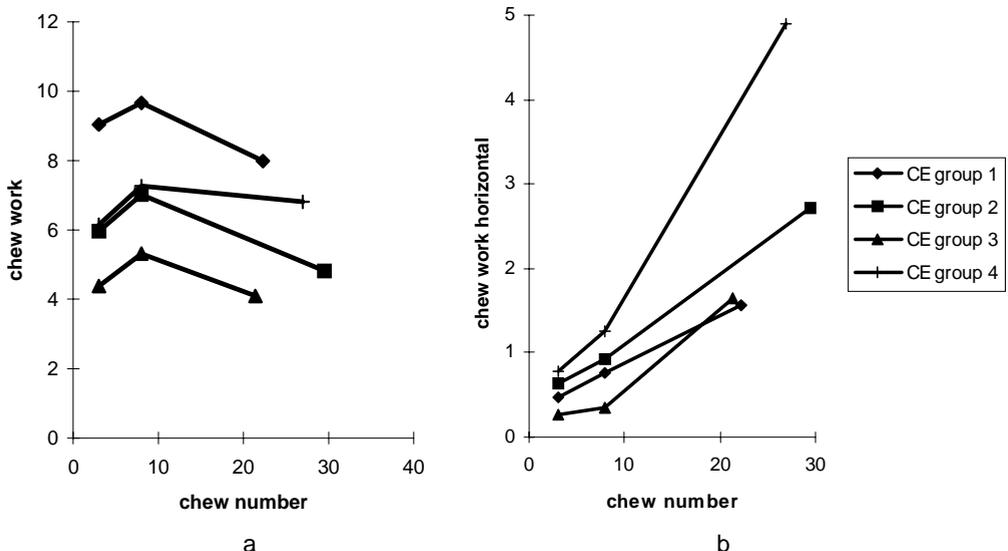
Fig 2: Changes in work associated with mastication of biscuits for subjects within different chewing efficiency groups. Values are calculated as the sum of chew work for the first 5 chews (plotted at chew number 3), chews 6-10 (plotted at chew number 8) and the last 5 chews (plotted at total number of chews minus 2). Fig 2a indicates total chew work, Fig 2b indicates work for the horizontal jaw movement only.

Figure 2a indicates that CE groups 1 and 3 exhibited short chewing sequences although CE1 expended significantly more effort during this time. Both CE 1 and 3 expended similar, and low, work

input in the grinding (horizontal) activity indicating that the difference in overall work occurred in the vertical jaw closing action (Fig. 2b). As a result of the low capacity for comminution of CE groups 1 and 3 they are likely to produce a small number of large fragments as they process the biscuits. The relatively small total crumb surface area so created may be readily moistened with saliva to allow swallowing after only a short chewing sequence. The relatively low work input by group 3 may result from greater saliva flow in these subjects (as suggested by their high chewing efficiency with gum) resulting in a softening of the biscuit matrix. In contrast CE groups 2 and 4, with their high comminution ability, required more chews to prepare the samples for swallowing. Such subjects would rapidly create large numbers of fine crumbs from the samples, requiring more saliva to "wet" and reform into a soft cohesive bolus prior to swallowing. Figure 2b provides evidence of the greater effort devoted to the horizontal grinding of the samples, especially towards the end of the chewing sequence, for groups 2 and 4 in relation to 1 and 3. Indeed this effort is most noticeable for group 4. On the hypothesis that this would tend to increase the crumb surface area even more for CE4 than for CE2, the absence of an even longer chewing sequence for group 4 may be attributed to greater salivation in these subjects.

These results emphasise differences between individual consumers in the way a product responds as they are eating it, which may underlie differences in the way they perceive the sensory characteristics of foods. Moreover, individuals appear to differ in the samples that they find easiest to process for swallowing, and such variations may underlie differences in food preferences. Full details of these results are given in Brown et al., (1999).

Influence of oral breakdown patterns on perceived flavour

Flavour components of food are released from exposed surfaces of the product while it remains in the mouth, and from residues in the saliva remaining in the oral cavity after swallowing the bulk of the sample. The total surface area available for release, and the physical nature of the surface, affect the rate and total amount of flavour released from a product during eating. As a consequence, individuals who break down a sample in different ways during mastication may derive different perceptions of its flavour, or of flavour differences among a series of similar samples. Figure 3 shows the patterns of perceived flavour intensity over time for soft (5%) and hard (25%) gelatin flavoured with 1% commercial banana flavouring for 2 different subjects. The subjects were asked to record the perceived intensity of flavour from the time they placed the sample in the mouth until they no longer perceived any flavour. Marked differences occurred between the subjects in the time course of perception and in the differences in perception between the 2 samples. For these samples (aqueous samples with relatively low sugar content (10%)) the maximum perceived intensity of flavour tended to occur at time the sample was swallowed. Consequently differences among the subjects in the peaks of perceived intensity reflect differences among the subjects in their chewing times for the 2 samples. Differences in the maximum perceived reflect the total surface area of the sample in the mouth just prior to swallowing. Consequently differences among the subjects in the relative maximum intensity of the 2 samples reflect the extent to which they have increased the surface area of the samples before swallowing. Such results demonstrate the problems in determining which of 2 samples is the more highly flavoured from taking averages of sensory scores from a number of assessors, and that the dynamics of flavour release and perception may be just as important in understanding consumer responses to food flavour as simply the flavour intensity. These results are reported in full in Brown and Wilson (1996) and Wilson and Brown (1997).
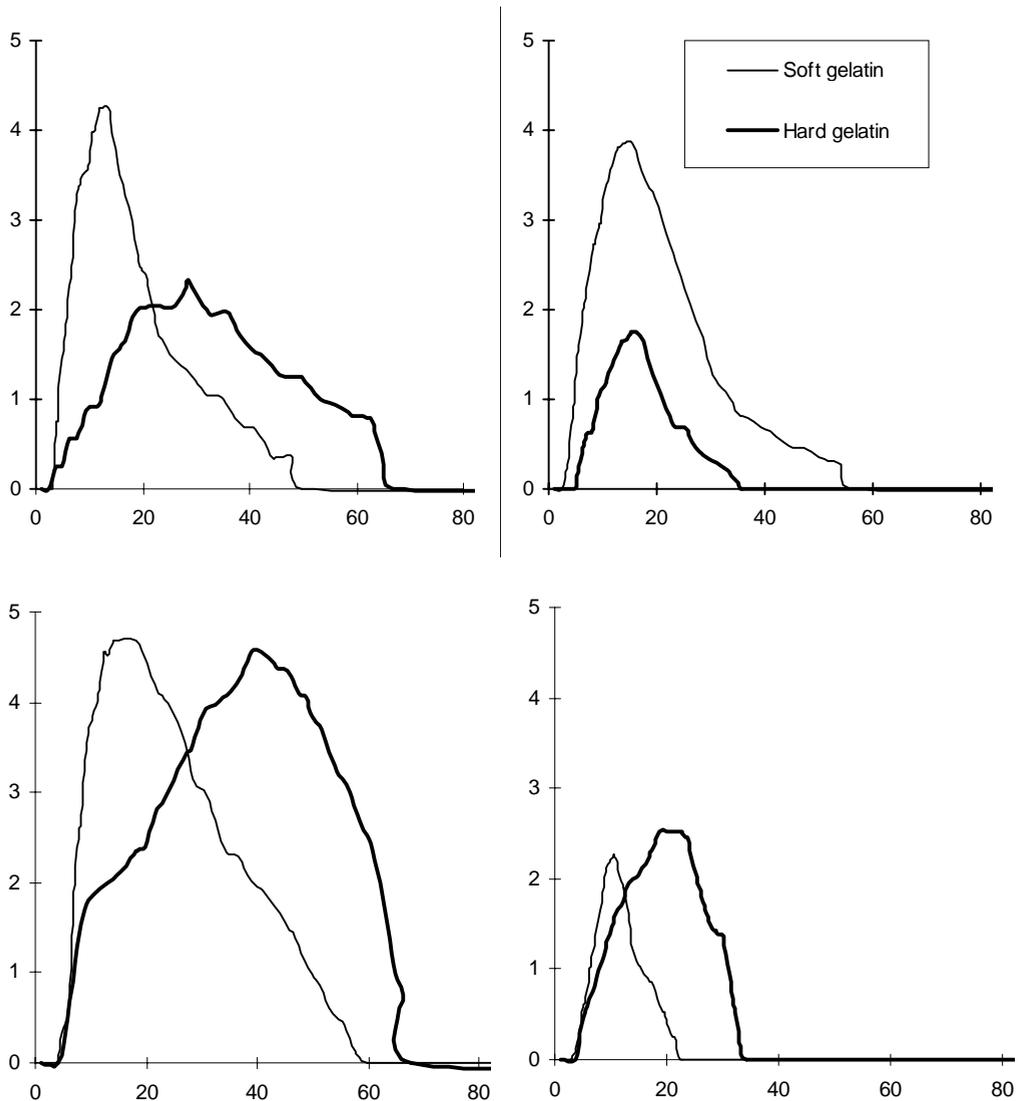
Fig. 3: Averaged Time Intensity curves for banana flavour in soft (5%) and hard gelatin (25%) containing 10% sucrose for 4 different subjects, (n=3 for each curve).

Acknowledgement

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The Impact of EU R&D programmes in industrial food research in Europe

R.B. Leslie

Unilever Research Colworth, Sharnbrook, Bedfordshire MK44 1LQ, UK.

Abstract

Over the last decade the European Commission has supported a range of activities falling within the general description of "Food R&D". The early programmes were of a tentative nature, and attracted participants primarily from academia. More recent programmes (eg ECLAIR, AIR, FAIR) have seen a significant increase in industrial participation and genuine industry: academia collaboration in pre-competitive research.

This contribution seeks to address three questions:

- What is the motivation for industrial participation in EU-sponsored Food R&D programmes?
- How are EU programmes contributing to the EU Food Industry?
- How successful and effective have EU Food R&D programmes been?

EU Food R&D programmes have facilitated an effective dialogue between the research needs of industry and the scientific creativity of academia; this has contributed to the European Food Industry enjoying a globally competitive potential. In some areas (eg Food Safety, Food Biotechnology) the stimulation provided by the EU programmes has been extremely significant. Difficult to quantify, but of high value to the Food Industry, has been the influx of new people - from students undergoing training to distinguished professors - whose introduction into the Food Industry has undoubtedly been facilitated by various EU schemes or programmes.

An overall conclusion is, that following a hesitant start, EU Food R&D programmes are now major contributors to the scientific underpinning and *innovative potential* of the European Food Industry. The challenge for the future is to further enhance the excellence of European Food Science and Technology, and to translate the undoubted European *inventive potential* into improved *innovative performance* for the European Food Industry, against the constantly evolving demands of the consumer. The available outline of Framework 5 suggests that the omens are good!

Introduction

In this contribution essentially three issues will be addressed:

- The motivation for industrial participation in EU sponsored Food R&D programmes.
- How EU Food R&D programmes contribute to the European Food Industry.
- An assessment of the success and impact of various EU programmes on the European Food Industry.

A Motivation for industrial participation

A.1 General characteristics of the European food industry

Before the impact of EU Food R&D programmes can be meaningfully discussed it is perhaps helpful to outline some general characteristics of the European Food Industry. The European Food Processing Industry is exceedingly diverse, being polarised into a small number of very large multinationals, with strong in house R&D capability and a large number of very small companies, with limited, or no, in house R&D capability.

Irrespective of the size of the specific company, a number of common research needs can be identified:

– safety assurance

– product innovation

– market awareness and consumer understanding

– raw material : processing : product formation

– awareness of, and compliance with, the legislative and regulatory framework

Accepting that some, or all, of these common requirements have a degree of research sensitivity, the question arises:

– where does industry obtain its research information in order to remain viable and, hopefully, prosper by being competitive?

Four sources of R&D input can be identified, the extent to which they are used being influenced by many factors. Principal sources for large multinationals would be in-house R&D, universities, research institutes and associations, with lesser inputs from suppliers and other company acquisitions. For the smaller companies, with limited or no in-house capability, research input might come more prominently from supplier and research associations.

Irrespective of their size, all companies have an ongoing need for technically competent and creative people. New people entering a company can be a rich source of new ideas and stimulus which ultimately underpins all company survival and viability.

Questions which all food companies, irrespective of size, must address are:

– do we need to have the input of research-based information?

If the answer is positive, a number of subsidiary questions immediately follow:

– what research information <u>must</u> we have?
  (eg to ensure the safe distribution of our products?)

– what *options* are there to progress the necessary research?
  (eg sponsored at a local university or via a collaborative pre-competitive multi-partner project?)

– is the proposed research acceptable from a cost vs chance of success vs potential pay-off judgement?

A.2 European food R&D – Recent developments

– realisation that the Food Industry <u>is</u> research sensitive

– "outsourcing" of research is becoming more normal, rather than exceptional

– acceptance that working with competitors can be managed and mutually beneficial

– impact of consumer/customer sophistication and demands

– proliferation of national, EU schemes to encourage industry : academia interaction

– realisation that to exploit research, one has to be actively involved in it - to be aware from the literature is necessary, but not enough.

These developments help to explain, and in part rationalise, why, European Industry does participate or should consider participating in EU Programmes viz:

– sharing of risk, cost for new areas

– to leverage its own in house intellectual capability

– to increase the <u>rate</u> of, and chance of, achieving technical success

– to maintain awareness of ongoing, or emerging, important research areas

– exposure to new marketing, geographical perspectives

– to enhance its recruitment opportunities

– to encourage its own scientists to maintain their professional scientific competence

– to leverage its own R&D budget.

The following question is often raised:

Is involvement in EU R&D programmes only relevant to large companies, with strong in-house R&D capability?

The answer should be an emphatic "NO"! The EU has evolved a series of special measures to facilitate the participation of SMEs. Moreover, these measures seem to be working well, as judged by the increasing level of SME involvement. In this context a number of national research associations are playing an effective role in facilitating SME involvement. However, participation and deriving usable benefits are not synonymous. An overall message is probably that the benefits to be taken out from EU R&D programmes are strongly coupled to the <u>level of involvement</u> by industry, large and small.

B How are EU Programmes contributing to the food industry?

B.1 Growth and scope of EU food R&D programmes

All the factors and developments discussed in the previous section have been running in parallel with the growth – in terms of size, diversity and sophistication - of the various EU programmes. The EU programmes have facilitated some of these developments, and each successive set of EU Framework programmes has, in turn, been influenced, by and has evolved in response to the changing needs of industry and new scientific opportunities. There has been a quantifiable and dramatic increase in the EU financial commitment to support the 'agrifood' sector over the last decade or so, via the ECLAIR, AIR, and FAIR programmes. A corresponding evolution of ways

("modalities") of accessing these research funds has also occurred (eg Concerted Actions, Shared Costs projects, CRAFT, Thematic Networks, Workshops, Training Grants etc).

A demonstrable increase in the level of industrial participation (both by large companies and SMEs) is noteworthy, as is the very high level of subscription to various programmes.

At this point, therefore, it is clear that the EU has facilitated a significant quantity and diversity of food-related R&D to which the European Industry and academia have contributed. But has it been successful Food R&D, and has it impacted upon the well-being and competitiveness of the European Food Industry?

B.2 An assessment of the impact of EU-funded food R&D

At the outset it has to be admitted that to analyse, and, particularly, to attempt to quantify the deliverables arising from the EU-funded Food R&D programmes is a challenging, if not impossible task. A possible approach is to attempt to make some judgements and comments related to the generally accepted needs of effective Food R&D. In the following paragraphs an industry requirement is first identified followed by (in italics) a comment attempting to assess the possible impact of EU Food R&D programmes.

– People with appropriate skills and training

The EU can be proud of what it has achieved in this area. The EU facilitation of student exchanges, post-doctoral fellowships, project consortia, networks concerted action workshops etc has introduced the scientific challenges of the Food Industry to a wider spectrum of people with undoubtedly beneficial effects. The European Food Masters programme is a specific example.

– Sharing best-practice, benchmarking etc which is generally considered helpful to industry

Both in its explicit dissemination projects (eg FLAIR-FLOW) and via the many informal EU networks much best practice and benchmarking have been facilitated. HACCP and the general high level of awareness of food safety issues owe much to EU involvement and stimulation.

– Appropriate research tools and measurement capabilities

All research – except that of a purely theoretical nature - is concerned with developing and testing new hypotheses. Implicit in this statement is the need for appropriate measurement techniques and specialist expertise. Again the EU Food R&D programmes have contributed much in this area. One could cite projects such as "Development of novel spectroscopic methods to authenticate wine quality" to illustrate this point.

– Awareness of emerging threats and opportunities

When times are economically hard, it is very easy for industry to focus on the problems of today and pay too little attention to the threats and opportunities just over the horizon. Although understandable, this is a dangerous situation to be in. The statistics describing the small percentage of major corporations which were prominent twenty years ago and do not exist today are well known. This "Window on the Future" is an area where the EU has been of significant benefit to the European Food Industry. Typical illustrative examples would be:

- Ultra High Pressure Technology
- Functional Foods (FuFose)
- Food Polymer Science
- Lactic Acid Genetics.

– Creating a powerful European food scientific potential

The creation of impressive networks of food scientists throughout Europe, largely attributable to the participation in Concerted Actions or Shared Cost projects, is another major achievement, due almost entirely to EU stimulation. This impressive inventive potential is certainly much envied by people outside Europe, if not fully appreciated and capitalised on by those inside Europe.

– A holistic approach to the AgroFood Industry

Until relatively recently, it was inappropriate to talk of the AgroFood Industry as a single entity - the reality was more a series of conflicting and often competing industries, failing to recognise and capitalise on their synergies and mutual interdependencies. Although there is still a long way to go, the EU Food R&D programmes do deserve credit for beginning to replace previously impenetrable barriers with much more open communication and discussion of the Food Chain.

Again, until fairly recently, scientists practising in the Food or AgroFood Industry were very fragmented, compartmentalised and generally perceived as 2nd class citizens in the spectrum of scientific disciplines. The strong encouragement by the Commission that research objectives should be tackled by integrated, multi-disciplinary approaches, and the requirement for consortia to have partners from different disciplines, training background and countries have done much to elevate the status of Food Science and Technology in Europe.

Similarly, the integration of objectives from the different DGs (Food, BIOMED, Biotechnology, Fisheries etc) has also had a major impact on a more integrated, less fragmented research capability in Europe.

– Stimulating Innovation by the EU Food Industry

Particularly with more recent EU programmes, the strong suggestion that industrial partners, especially SMEs, should be involved to facilitate exploitation has greatly reduced the previous tensions between basic and applied research, relevant to food. There are today many excellent EU-funded consortia, involving academia and companies, large and small, which simply do not recognise the relevance of the terms basic and applied - they much prefer the judgements to relate to "good science, likely to underpin future innovation".

As noted in the introductory comments, the European Food Industry has a high level of complexity and diversity. Europe is home to both multinationals, and subsistence farmers - both having an equal claim to be treated as Europeans and to have the opportunity to benefit from EU research programmes. Detached examination will confirm that the EU has managed to reflect the needs and opportunities of all this diverse user community, whether it be the fruit grower in Southern Europe seeking to harness biotechnology for improved product quality, or those concerned with fish stock sustainability in Northern Europe.

This is clearly a positive endorsement of the contribution of the EU Food R&D programmes but is it objective? Overall, in the opinion of this author, the European Food Industry is extremely well served by the science-base available in Europe, to which the EC has contributed considerable added value through its mechanisms to integrate the inputs of various academic and industrial players.

C Maximising the success and impact of EU Food R&D programmes?

Despite the positive endorsement in the previous section it is still meaningful to explore how successful and effective EU Food R&D programmes have been in terms of their impact upon the European Food Industry. A widely held view would be that the actual impact of EU Food R&D programmes has been considerably less than the potential impact. Three reasons could be:

– Potential beneficiaries are not adequately aware of the benefits! The various dissemination activities sponsored by the Commission eg FLAIR-FLOW are excellent and highly effective, but additional measures to alert potential beneficiaries could be needed.

– The "Administrative Focus" in the Commission is inevitably on the input side of the equation - soliciting proposals, evaluating them and establishing contracts etc. Almost certainly attributable to insufficient resources there seems to be too little emphasis on the output side - eg has this project delivered what it promised, and has the scientific progress been carried forward to real innovation? There seems to be little penalty for failing to deliver what consortia promised in their applications - other than that their next application may be less successful. A more certain approach would be to fund, in principle, projects from the research phase to much closer to the exploitation phase, with stage payments being paid along the way, as each stage is successfully completed.

– Many of the most innovative, entrepreneurial people and embryonic companies are "culturally uncomfortable" with the modus operandi of the Commission, which they perceive as overly bureaucratic and complex to access. These entrepreneurial people are looking for support as much from venture capitalists as from in their perception – principally funders of academic research.

As mentioned above, the EU Food R&D programmes have stimulated positive interaction and synergy between different elements of the food chain (agriculture, food processing, nutrition, consumer quality etc). This has been very beneficial. Greater integration between the various Commission Services, such that there is more integration of the R&D priorities with the regulatory, legislative, consumer perspectives, would undoubtedly be helpful to European industrial competitiveness. There is the real possibility that excellent, potentially innovative science becomes real innovation outside Europe, simply because scientific publications are globally accessible and globally studied. The Commission could, and should, do more to establish eg workshops, discussion fora at which all the elements of successful innovation can be discussed, and consensus reached. Whilst a delicate balance must obviously be struck, there is a perception that the Commission prefers to adopt a reactive stance dealing with issues when they arise rather than perhaps being adequately proactive. Initiatives by the Commission to stimulate more informed debate between the developers of novel technologies, legislators and consumers are likely to be welcomed by the European Food Industry at large.

Another issue, which is well recognised, is the extent to which EU Food R&D programmes are competing with, or genuinely complementing, national programmes.

There is clearly the potential for, if not the reality of, conflict between EU and national Food R&D programmes. Of course, the statement that EU projects must have a genuine European dimension is understood, but more explicit evidence of complementarily, rather than competitiveness, between EU and national programmes would be helpful.

Accepting that a principal motivation is the support and stimulation of genuine innovation in, and competitiveness of, the European Food Industry suggests that efforts to close the gap between actual, as opposed to, potential impact are needed. Is further customisation, and perhaps even new modalities, by which industrial users can access the powerful food science base in Europe needed? No one will dispute the statement that the large multinationals, and SMEs alike, depend ultimately upon new science as the basis of their innovation. However, what is new and appropriate for the large multinational may not be so for the local SME. The recent dramatic increase in the subscription of SMEs to the Food CRAFT programme demonstrates the intrinsic enthusiasm of SMEs to access EU programmes, providing the mechanisms are appropriate. This reinforces the need for customisation of the programmes, coupled with effective targeting.

Overall conclusion

EU Food R&D programmes have facilitated an effective dialogue between the research needs of industry and the scientific creativity of academia; this has contributed to the European Food Industry enjoying a globally - competitive potential. In some areas (eg Food Safety, Food Biotechnology) the stimulation provided by the EU programmes has been extremely significant. Difficult to quantify, but of high value to the Food Industry, has been the influx of new people - from students undergoing training to distinguished professors - whose introduction into the Food Industry has undoubtedly been facilitated by various EU schemes or programmes.

An overall conclusion is that following a hesitant start EU Food R&D programmes are now major contributors to the scientific underpinning and *innovative potential* of the European Food Industry. The challenge for the future is to further enhance the excellence of European Food Science and Technology, and to translate the undoubted European *inventive potential* into improved *innovative performance* for the European Food Industry, against the constantly evolving demands of the consumer. The available outline of Framework 5 suggest that the omens are good!

Foresight for innovation strategy at the beginning of the 21st Century

After dinner presentation

H. Grupp, K. Menrad

Fraunhofer Institute for Systems and Innovation Research (Fraunhofer-ISI), Karlsruhe, Germany

Introduction

The last five years or so in Europe and elsewhere have witnessed an upsurge of interest in technology foresight. Prior to 1990, there was comparatively little technology foresight in the United States or European countries. The first foresight initiatives in Europe were taken in collaboration with Japan, which was then having a longer record in this matter. Among several possible scientific methods in technology foresight, the so-called Delphi Survey is only one. But in the eyes of many observers, the Delphi technique seems to be more prominent than other, comparable approaches. This might be due to the magic word 'Delphi' and the association with the ancient Greek oracle.

So why not start with hindsight and consider briefly what the function of the priestess in the old Delphi temple was, whether historical research found any impacts on politics or society in those days and whether there was a lasting impact on the progress of mankind in prehistory. I will then pose the same queries to our present society, '*what is'* or better '*what could be'* the function of technology foresight for innovation strategy in economy, policy and technology development? We will conclude by discussing possible benefits of technology foresight for our societies, that march soon into the next millennium.

The foundation of Delphi and its oracle took place before recorded history. Thanks to archaeologists and historians we have extensive knowledge on the functions and benefits of the oracle (Parke and Wormell, 1956). For a thousand years of recorded history the Greeks and others, sometimes as private individuals, sometimes as official ambassadors, came to Delphi to consult the prophetess, who was called Pythia. Her words were taken to reveal the wills of the Gods. These prophecies were not usually intended simply to be a foresight of the future as such. The Pythia's function was to tell the divine purpose in a normative way in order to shape coming events.

One should consider that the Delphic monastery was one of very few spots on earth where knowledge was accumulated, ordered and preserved. The information came in from the ambassadors through their queries and the answers were written down on metal plates, several of them found by archaeologists. The temple was the locus of knowledge, or, if we put it more mundanely, the Delphic oracle was probably the largest data base of the ancient world. The priests could read and write; who else could do so in Greece? If due allowance is made for these circumstances, modern psychology will find no special difficulty in accounting for the operations of the Pythia and of the priests interpreting her utterances. Knowledge was used and disseminated to make the world better.

Certainly, the consultations were religious in form and not mere inquisitive speculations on the future or attempts to obtain practical shortcuts to success, but at least in earlier periods religion entered into every aspect of Greek life and there were few subjects on which the advice of Apollo was not sought (Parke and Wormell, loc. cit.). There is no doubt, the oracle acted as an international arbitrator. It shared the rise of Hellenic civilisation to which it contributed no small part. It is no

wonder that a witness of that time, Socrates in his 'Phaidros', around 400 years before the year zero, judged: 'The prophetess at Delphi (...) turned many good things towards the private and public affairs of our country'.

Thanks to the oracle the Greek people learned over many generations to abstain from bloody vendetta, to apply to courts when quarrelling in private life occurred and to solve disputes in a fair way. It can be traced back to the oracle that one should not poison the well of one's enemy and should take care of the olive trees in war. Thus the idea of the long-term oriented development of landscaping achievements we owe the Delphic oracle. Mankind benefited from the oracle in the old days. Let us turn now to the roots of modern foresight.

On the history of modern foresight

The main initial work was performed at the RAND corporation, Santa Monica, in the years following 1948, the pioneers being Kaplan (1950), Helmer (1977), Dalkey et al. (1963, 1969). 'Forecasting', as it was known then, was motivated by Vanevar Bush's book 'Science the endless frontier', advocating the transformation of the US military economy research during World War II (e.g. the Manhattan project) into long-term civilian research and commercial exploitation. The early attempts were also spurred by the amazing scientific successes of the Soviet 'planned economy' (e.g., the hydrogen bomb or the launch of the Sputnik). In the context of forecasting work at RAND, also a new innovation economics developed (including work by Arrow, Winter, Nelson et al.; compare Hounshell, 1996).

Methodological starting points were systems analysis, operations research and comparable procedures. After early successes, many serious misconceptions of what 'forecasting' ought to be arose. In the sixties and early seventies, the mechanical 'prognosis' or 'trend prediction' type of work based on 'linear', i.e. sequential, models ceased to look interesting and the related forecasting activities fell into oblivion. This coincided with the end of the long growth euphoria following the War heralded by the first oil price adjustment; or the 'Limits to Growth' report of the Club of Rome (Meadows et al., 1972). Although the 'linear' models of thought were discarded (e.g. by the project 'Hindsight'; see Isenson, 1967), some science policy communities further supported them for their legitimating power on research spending with no priorities (e.g. the project TRACES by the NSF 1968; compare IIT, 1968).

With the new evolutionary economics coming up with selection procedures and the notion of variety generation by new products, and the sociology of science working on the functions of social systems in science as opposed to technology or the economy emphasising the 'bounds of rationality' and 'negotiating systems', it became clear that there may be a new, different use of forecasting methods. Martin and Irvine (1984) coined the term 'foresight' and pointed to the communication or procedural power of it. The modern perception is that the actions of social systems, in particular science communities, cannot be predicted in terms of 'natural' laws, and that future events in science and technology cannot be determined by extrapolation, but are shaped by these communities and a negotiating system.

However, this present understanding of foresight was available in the literature from the very beginning and, though less-well pronounced than nowadays, may have been found already in one of the earliest papers in the field: 'Policy making rests in part on anticipation of the future (...) and of the consequences of and responses to alternative lines of action. Many policy decisions require

foreknowledge of events which cannot be forecast either by strict causal chains (...) or by stable statistical regularities (...).' Kaplan et al., 1950, p. 93 (emphasis added). Even the forerunner of the term 'foresight' was coined in 1950! 'Verification' or 'falsification' of foresight results are, thus, meaningless ends.

There is constant temptation for foresight to restrict itself to describing the potential supply of scientific and technical solutions and the study of their impact. However, it must do far more than depict the supply factors. The potentials and the risks of technology in the future depend just as much on the pressure of the social, ecological and economic problems expected to arise and make important demands on science and technology. For this reason, any discussion of problems must focus increasingly on factors relating to demand. How one might determine which basic values for innovation activities might be adopted world-wide in the medium and long-term perspectives, and forecast the resulting problems, of course, has no satisfactory empirical answer.

Because of many supply-demand mismatches, initially euphoric expectations of a new technology (mostly on the part of the scientific community) tend to be followed by increasingly cautious developmental phases before the market is finally satisfied. The use or rejection of innovative products often leads to new demands on research and technology, which is why it generally makes sense to speak of 'feedback processes'. Foresight has to incorporate aspects of industrial research and pure research, and consideration must also be given to institutional support. These deliberations also call into question the possible expectation that a technology needs no more than a single action to regulate its impacts. Any hopes of being able to drop the accompanying pure research once the applied objectives have been achieved, will meet with disappointment; tomorrow's science-based technology is shaped continuously through targeted basic research.

Overview of modern approaches to foresight for innovation strategy

Growing competition on the world market and an increasing rate of technological change are forcing economies and organizations to concentrate their research activities on selected areas. In order to identify those technologies which will have the greatest impact on economic competitiveness and social welfare, several new studies on critical technologies have been published in various countries.

During the last years, several such studies have occurred particularly in Europe. Starting with the transformation and restructuring of the Eastern European economies and the unification of Germany, the new political and economic context of Europe made a re-orientation necessary. Parallel to this, national budget restraints brought about new national processes to set priorities in science and technology. The desire to identify those technologies which will have the greatest impact on economic competitiveness and social welfare was expressed from various sides.

This development made new foresight approaches attractive. The term 'foresight' is used in the sense of 'outlook'. This is not the same connotation as 'prediction' which would be closer to 'forecast'. Foresight takes into account that there is no single future. Depending on action or non-action at present, many futures are possible, but only one of them will happen. To select the most desirable future and to make it possible is one of the tasks in technology policy. Foresight is the 'process involved in systematically attempting to look into the longer-term future of science, technology, the economy and society with the aim of identifying the areas of strategic research and

the emerging generic technologies likely to yield the greatest economic and social benefits' (Martin, 1995).

The recent national foresight studies are written with the more or less expressive objective to sort out those technologies which have an impact on economic welfare and competitiveness (and also national defence in the case of the United States). A systematic assessment of the studies shows that they differ considerably in terms of size, disaggregation, methodology and relevance. Technology policy issues and recommendations to master these technologies in the respective firms are rarely spelt out or kept brief. These issues are more likely discussed in other, more general technology policy studies, which commonly lack a detailed list of critical technologies (for a list of critical technology studies see Martin and Irvine, 1989, or Grupp, 1994, p. 381).

Another difficulty in comparing the foresight studies lies in the fact that the definition of 'critical' technologies varies considerably and is not defined precisely in some of the recent approaches. A reconstruction of the selection procedures is not possible but in exceptional cases. Regarding the preferred type of investigation, it is obvious that we have to distinguish two types of approaches. Most of the foresight reports are the results of original scientific work. For some exceptions, no additional data have been collected nor has there been new knowledge found out on critical technologies. These studies represent the structured knowledge of committees and reflect the professionality of the committee members and the amount of time they dedicated to the related discussions and the writing of the report. In most cases, a professional staff group is involved (and paid for) to support the committees. However, in other cases, the foresight report is written from internal sources of government ministries or agencies. Often, the report writing was delegated to personal assistants of the committee members.

With growing interest, the Delphi methodology is applied which includes a comprehensive survey over two rounds with many technological topics included. The Delphi is considered to be highly oriented towards conformity (Woudenberg, 1991) though the huge statistical data base created does not automatically yield evaluations and recommendations. Based on the Delphi data pool holistic assessments seem to be possible, yet, they are not provided automatically from the data. We focus on the Delphi method in later sections of this article.

In all the recent national foresight activities many interesting aspects may be found which are of great importance to European scientist in nutrition research. It is recommended to make use of the published studies in strategic activities. Yet, in some studies there is also lack of rigour in the application of methodologies and there is lack of disaggregation. The formulation of specific policy issues tends to be difficult in cases where large fields of technology, such as biotechnology, are not disaggregated at all but treated as a unity. This makes an exploitation of the results particularly difficult for companies.

Another point relates to the criteria used to assess the selected technologies. Dominant are such criteria like 'economic growth', 'technological competitiveness', 'market size' and, in the United States, 'national defence'. Rarely, and only in the very recent studies, other than economic or security criteria are used additionally like 'welfare', 'quality of life' or 'clean environment'. The most advanced studies in this respect use criteria like 'growth by intelligent technologies'. Key study point in this context is an analysis of a positive impact originating from future technologies (emergence of new industries and products, rejuvenation of existing ones and other favourable ripple effects) as well as a negative impact (erosion of existing industries and products by new and more competitive

ones). The respective studies outline the most important future problems like increasing global interdependence, changing population dynamics and the ageing society, the shortening of working hours (in Japan), improvement to the environment crisis and resource or energy limitations and remedy to the widening social maladies (like drug abuse, terrorism, aids etc.). However, some reports of this type do not clearly state how these criteria are applied when sorting out and evaluating the critical technologies contained in the reports.

In summing up the published recent national foresight activities, it is fair to say that some countries undertook serious and differentiated activities to determine generic critical technologies. The major problem in the studies is the comparability of the methodology used. In some cases, a clear-cut methodology was unavailable, in others the methodological frame is missing or criteria were indicated but not strictly applied. In particular, if criteria to pre-sort and assess the technologies are given, which is not always the case, it remains unclear how the criteria were applied to the critical technologies. Among the sets of criteria, growth and competitiveness issues dominate whereas social or ecological aspects as well as future demand are considered in many, but not all studies.

Synopsis of methods used for foresight

So far foresighting has not devised any clear-cut methodological repertoire of its own. It tends to draw on the respective methods required from neighbour disciplines and forms a suitable mixture of combining them. Here we mention innovation economics, systems analysis, operations research, technology assessment, social and public opinion research. Although no specific, generally binding mathematical algorithms have been developed, certain approaches quite clearly figure more frequently in foresighting than in other disciplines whilst other, more widely used algorithms, on the other hand, occur more rarely.

Figure 1 provides a very condensed overview of foresighting methods. We grouped them into three basic types. The first group we denote as 'cognitive-appreciative methods' and we subdivide these into two classes: large samples versus small samples. Opinion surveys in most cases require large samples in order to avoid biases. If not opinions or normative topics are asked for but facts about the future then the nature of such surveys comes close to what economists term rational expectation. As there are no facts on future events, respondents can only judge on their rational expectation of what might be the case in the future. If such a survey includes feedbacks, that is communication of estimations between the respondents, it is termed a Delphi survey. Delphis were most popular in forecasting exercises of the early days, but also in modern times (see next section).

Some literature reviews distinguish between quantitative and qualitative methods. Qualitative results always occur in the class of cognitive-appreciative methods if small samples are involved. This is typically the case for brainstorming or brainwriting, for expert panels (face-to-face meetings) and individual interviews. 'Intuitive thinking' is also typical for science fiction and the establishment of 'visions'. Brainstorming, for example, is a small group method where a relaxed period of free thinking is used to articulate ideas before more systematic evaluations take place.

The group of statistical and econometric methods we subdivide into the extrapolation class, the econometric class (in the narrow sense) and the decision-making class. Clearly, trend extrapolation is very popular as is the inference from historical analogies. Time series displaying growth or learning curves (or degressive curves) would reflect more advanced extrapolations than simple trends, as at least a rough theory of 'how it goes on' is required. Further we mention diffusion

models or the decomposition of time series (shift share analysis for instance). In the econometric class, which is of course far bigger than indicated in figure 1, especially the models which allow for time lags are useful for foresighting. Linear programming, chaos and fuzzy methods are among the more modern econometric tools for foresight. So-called early warning indicators such as statistics on scientific publications or patent trends which herald market results with a lag of some years can be helpful in short-term forecasting.
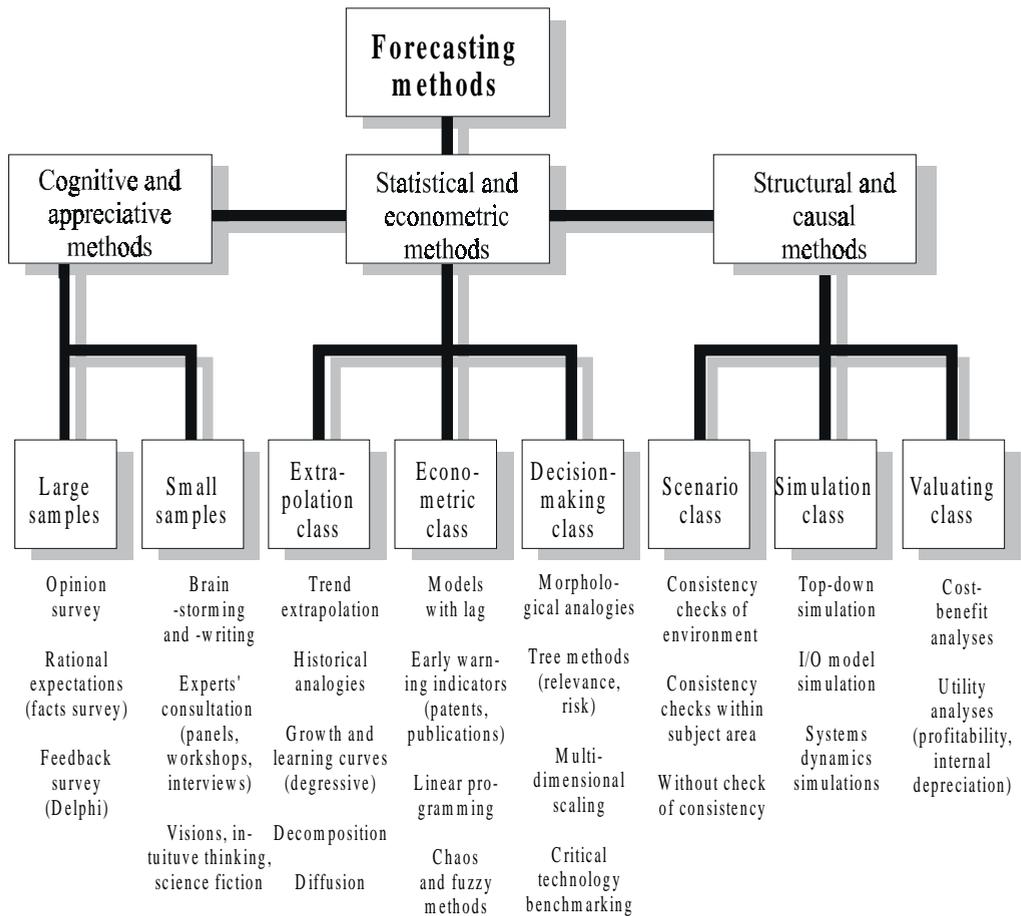
Figure 1: Overview of foresighting methods by types and classes

Patent statistics are a well-known instrument for corporate innovation planning and competitor analysis (see Schmoch and Grupp, 1989). There are two faces to patent indicators: On the one hand development success is documented; on the other hand, economic interest in certain future markets is indicated. Patent indicators are influenced by various factors and do not just reflect output of technology or intellectual property rights. The issue of quality in patent indicators requires a careful separation of the influencing factors. Patent indicators being of good quality for measuring applied research and development output, for example, are not necessarily appropriate to detecting corporate regional strategies (loc. cit.). Less well-established is the use of patent statistics for short-term foresight in technology policy.

A typical short-term foresight problem is how technological productivity relates to future market shares. What one is interested in, is the question whether single countries (or companies) - via specializing in specific high technology fields - can increase their world market shares in high technology areas correspondingly. Thus, if the model of absolute technological competitiveness (Grupp and Münt 1998) applies to all high technology product categories (or technological areas) the same way, we will expect a positive correlation between world market shares and technology intensity.

The analytical use of patent statistics introduced in this introductory section seems to be well suited for broader application in forming innovation strategies in the short term. One can derive some trends in international market power in the next few years from patent analysis. The national technology production seems to be a major component of market success up to now at least for the market-oriented technologies and despite increasing intra-firm flows of knowledge. Patent statistics can be performed in various ways. One has to be very careful in using patent data for foresight purposes. Most national data sets are biased towards the country of origin and cannot be used for this purpose.

Under the umbrella term decision-making we subsume the class of methods such as the morphological analogy, the relevance or risk trees and multi-dimensional mapping methods of all sorts. In recent years, so-called critical or key technology lists have emerged among the 'relevance' methods. The relevance or tree methods consist of identifying technologies by a set of rational criteria which meet certain requirements (importance, critical nature for defence and so forth). Often, a benchmarking analysis is undertaken for comparisons of the entries in the list with one another or with other data sets. Because several criteria are usually applied the relevance identification can become quite complicated and hence may be termed 'tree'. In any case, this class of methods is normative and has its foundations in systems analysis.

The trees can help to arrive at distinct levels of complexity or hierarchy and also a combination of criteria is feasible by cluster analysis or multi-dimensional scaling in order to discern simpler structures. These methods represent an attempt to reduce the analytical diversity of high-dimensional problem areas within a given low dimensional presentation space. This ultimately means that the findings on the future are derived not from statistical inference, but from graphical structures ultimately the detection of hidden structures is the goal.

The third group of methods we term structural-causal methods. Here we mean the scenario class, the class of simulation and the valuating class. Scenarios can be bottom up or top down and they may consist in a quantitative check of consistency in the well-defined subject area or, in addition, in the 'environment' to the area studied. The method consists of organizing information on future possibilities and to write down alternative paths or trajectories. Scenarios will normally be composed of a mixture of quantifiable and non-quantifiable components arranged as alternative logical strings of events. They are particular helpful if combined with other methods, such as panel consultation or Delphi questionnaires. In the simulation class we mention computer simulations of all sorts but also input-output models, systems dynamics simulation and the like. If cost benefit analysis is involved, profitability calculation is aimed at, or utility values are analysed then we should speak of the costing or valuating class within the causal models. If the latter methods are used for foresighting, then always problems of depreciation rates of interests or return have to be solved.

Effectiveness of foresight methods

When looking back to the toolbox in figure 1 over the past 50 years we have to note that the understanding of what foresight is and which methods are considered useful changed. Technology foresight has re-emerged to define a certain class of forecasting approaches for discussion of future prospects and current choices. Foresight does – unlike forecasting – not concern itself with probability predictions of future technologies starting from todays knowledge base. Thus (science and) technology foresight describes a type of combined analysis and communication processes in which informed parties and stakeholders participate in a forward-looking exercise using one or several forecasting methods.

While trend analysis and any sort of statistical and econometric method was well suitable for the older approach of forecasting, in more recent times cognitive and appreciative methods are at the forefront. Comprehensive simulation methods of all sorts were typical for the 1970s with the famous example of the report of the Club of Rome in 1971. Yet, the world models turned out to be too crude and could not resolve specific regions or problems and thus are increasingly replaced by more narrowly defined scenarios. The best way out of the difficulties is the use of multiple perspectives.

It is also more common now than in the past to give more attention to the feedback processes between capability and need which must link up in timely fashion for decision making. While the traditional tools of forecasting, such as trend extrapolation, are appropriate during any stable phase but inherently fail in the chaotic states, the emphasis on communication processes increases our ability to respond capably to any anticipated situation and in particular to effective crisis management. The use of multiple perspectives is particular helpful in this context. Technological foresight has changed in notion and in methods. It will probably require still a much broader approach in the future than ever before.

The assessment of the utility of foresight activities for industrial innovation management is possible if the results of an investigation in Japan on the most effective forecasting methods are considered. A survey of 247 research laboratories mostly in industry were asked during 1989 for views on the degree of application and the effectiveness of technology foresight methods considered or used by these laboratories (NISTEP, IFTECH, 1991). Table 1 displays the results.

Table 1: Technology forecasting methods and their effectiveness

Method	Degree of application (%)	Effectiveness (% of laboratories)
Delphi	22.2	49.7
Trend extrapolation	38.9	37.5
Patent analysis	30.6	57.5
Network method	0.0	21.9
Morphological pattern	22.2	30.3
Relevance trees	13.9	48.9
Technology portfolios	33.3	33.8
Cross impact matrix	2.8	38.1
Scenarios	25.0	46.6
Systems dynamics simulation	0.0	28.0

Most widely used are technology portfolios and trend extrapolation, but these methods do not rank among the most effective ones. Considered as most effective is patent analysis. Yet, the time frame of patent forecasts is limited to about five years. Thus, patent analysis is a very good forecast method but not in the case of medium and long-term forecasting. It is particularly useful on company level. Scenario writing, Delphi and relevance trees rank among the next most effective methods as judged by the Japanese laboratories but they are not widely performed within firms because such methods are complex and expensive. Scenarios need a predetermined frame and setup. It is hard to imagine a comprehensive *and* detailed scenario covering all scientific, technological or social areas. For selected areas, however, scenarios are a recommended forecasting tool.

The modern Delphi method as a tool for strategic negotiation

The Delphi method is considered especially useful for long-range aspects (20-30 years) as expert opinions are the only source of information available, as in the ancient times. The Delphi method is one of those methods developed during the fifties at the RAND Corporation to make better use of the knowledge potential through group interaction. A questionnaire sent to experts more than one time is the medium for group interaction (Martino, 1983). The panel members will usually have widely varying estimates on each question in the beginning of the process and do not always shift their opinion under the influence of the assessments given to them by the other panellists. The main advantage of Delphi is that panel members can shift position without losing face if they see convincing reasons for doing so.

Most recent foresight surveys were undertaken by government agencies. Yet enterprises may also make effective these approaches. One pharmaceutical company in Germany has just concluded its own Delphi investigation on the future of general practitioneers (i.e. physicians in residential areas) and their ability to follow modern trends both in medical technology and in pharmaceuticals assuming an increasing use of information technology in the health care system (Reiss et al., 1995, and Grupp and Reiss, 1997). For a joint European example in the agro-food sector, see the next section.

An important function of modern foresight, in particular the Delphi technique, is moderation between different sectors of the economy, different disciplines in science and different departments in politics and the public. How can one communicate in the best way on the future across these boundaries? We have very well established peer review systems to judge on priorities and on quality within scientific disciplines. Strategic business managers normally know enough in their core business. But we are now in the decade of transdisciplinary matters, the hyphenated technology areas herald the future such as bio-sensors, micro-systems, opto-computers, neuro-informatics, bionics.

How should we proceed with the long-term application-oriented basic research of the hyphenated type (Grupp, 1998)? This is the research where one does not know what will be found out in the laboratory in the next month or year, but it is a research which does not only satisfy scientific curiosity and the enhancement of knowledge. It is a research with a definite long-term economic or social perspective. Let us mention climate research, health research, environmental research and so forth. In days of low budgets many business and policy makers think it is impossible to support each piece of interesting research only for the sake of good quality. One has to discuss the long-term orientation in which we invest our dear money. The public is convinced that science and technology are partly responsible for modern bottlenecks and problems and hence has a right to learn about

priorities in technology and also the opposite, the non-priorities, what is down at the end of the list of priorities.

Consider the situation in which a company or a ministry has to decide which of two research programmes to support, A or B. Programme A is proposed from faculty A and industry A and the peers from discipline A have given their reviews. Programme B in conjunction with industry B originates from faculty B and the peers of discipline B made up their minds. Everybody did her or his best. But how to decide between them? Know the peers each other? Our science and technology system of tomorrow needs, alongside with disciplinary peers, new instruments to mediate between A and B, and here is another function of foresight, across the board.

Most sociologists of science assume that there is a positive relationship between involvement in a research area and assessments of it and that this relationship derives from the tendency of scientists to select problems in areas where there is high pay-off for successful solutions and career. The tendency to overrate fields in which a person works may be termed 'bias'. Not only a tendency towards positive bias for fields in which researchers have been active was found, but also this bias to be stronger in less innovative sub-fields. As market signals fail to be useful for business strategy in the long run and expert assessment is not always objective, Delphi surveys may play a part in science and innovation management.

Two examples from the first German Delphi highlight this dilemma: Specialist experts and thus future knowledge may not be available in some countries. The availability of experts in the case of biotechnology in Germany is mixed. Among the N=73 respondents who are all experts in biotechnology, many did not answer in particular sub-areas (most expressed for tissue and organs). The largest number of specialist experts (i.e. those working in the sub-area) among all experts in Germany is found in molecular biology but not in the sub-area of tissue and organs. An almost perfect correlation was found between the number of experts and their rating of German research performance. In sub-areas where we know more, we are good. In sub-areas where we are not advanced, we know little of the opportunities. In order to explore expert differences in more detail, we present a case study in the Agro-Food field.

A case in study: the Agro-Food Delphi

Although the first Agro-Food products based on modern biotechnology have entered the EU markets, the application of this technology is still intensively discussed in the European Union. Recent opinion polls indicate as well that consumers' acceptance of genetically engineered food and agricultural products is still relatively low, at least in some member states of the EU (e.g. European Commission 1997, Hampel et al. 1997, Grove-White et al. 1997, Gofton et al. 1996, Hamstra 1993, 1994). In contrast, representatives from politics and industry underline the necessity to apply modern biotechnology in the Agro-Food sector, mainly to ensure the competitiveness of the EU agriculture and food industry and for employment reasons.

Against this background, there is a need for a scientific analysis of the future impacts of modern biotechnology in the Agro-Food sector of the EU. Recent studies trying to analyse this issue usually comprise extrapolations of status-quo analyses (e.g. Pezzatti et al. 1996, Henze et al. 1995, OECD 1992, Teuber 1992). What has not been exploited so far in this context are systematic technology forecasting approaches which get comparable information on an international level. Therefore, in this project which was financially supported by the Commission of the EU (DG XII), the impacts of

modern biotechnology on the Agro-Food sector in five member countries of the EU (Germany, Greece, Italy, the Netherlands, Spain) have been analysed with the help of the Delphi methodology. The specific features of this project are the consideration of the scientific and technical development in Agro-Food biotechnology, the development of framework conditions as well as the involvement of different social groups (e.g. farmers, consumers, biotechnology critics), who are not asked in traditional Delphi surveys (see Menrad et al. 1998b).

The project has the following objectives:

- Analysis and foresight of the impacts of biotechnology on the Agro-Food sector in different countries of the European Union,
- Consideration of the scientific and technical development as well as the development of framework conditions for food production and consumption,
- Elaboration of common features and differences between the countries involved,
- Identification of different views of various social groups in selected countries (e.g. farmers, food industry, opponents of biotechnology, consumers),
- Elaboration of recommendations for decision makers in policy, industry and society, allowing anticipation of emerging problems or adaptation of business activities.

The questionnaire containing 71 statements of possible future developments was created in an interactive procedure between the different project teams and specially established national expert committees in the five countries during the first six months of 1996. In total, more than 7,800 experts have been asked in the first round to participate in the Delphi survey. The size of the expert panels range from around 1,200 experts in the Netherlands and Spain to almost 2,400 experts in Germany. The questionnaires of the first round were mailed during autumn 1996, the second round followed in March/April 1997. While in Italy and Spain a relatively low rate of response occurred with around 150 participating experts, 522 experts answered the questionnaire twice in Germany. Around 200 experts filled in the questionnaire of the second round in Greece and the Netherlands.

In a first step the results of the Delphi survey were analysed on a national level. The experts of the Central European countries (Germany, the Netherlands) are more critical than their colleagues from the Mediterranean countries concerning Agro-Food biotechnology, whereas among the latter the Spanish respondents show by far the most positive attitudes (see figure 2). In total, more than half of the statements are appreciated, while less than one fifth are opposed by the experts. The most obvious differences between these two groups of countries emerge concerning the application of modern biotechnology in animal husbandry and animal breeding. The German, Dutch and Greek panels estimate this application field rather critically, while the Spanish and Italian panel members regard this area more positively. Negligible differences in the personal attitude of the experts emerge towards biotechnical approaches which contribute to reduced health problems or to develop products outside the food chain. Moreover, the experts of all countries are in favour of monitoring systems based on modern biotechnology.
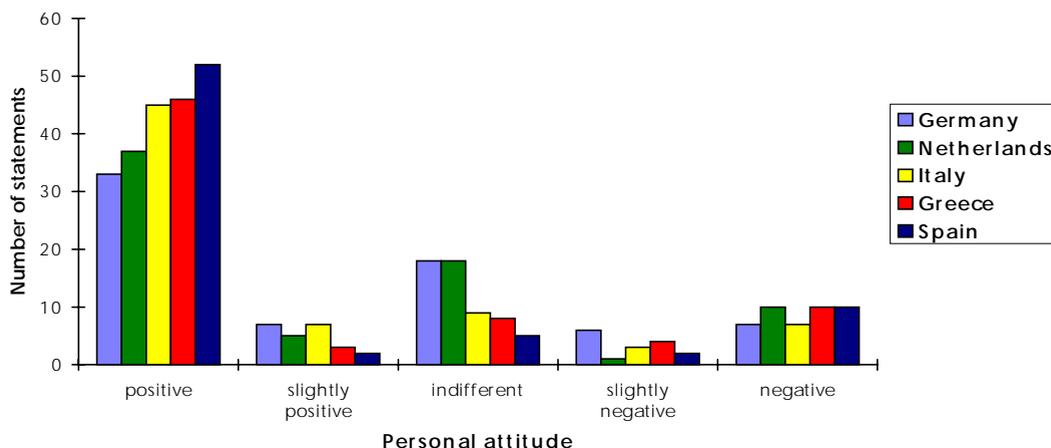
Figure 2: Personal attitude of the experts in the five European countries

Since different expert groups have been included in the survey, it was possible to analyse the answering behaviour of these groups separately. All in all, a different answering behaviour between the expert groups in the Central European and the Mediterranean countries can be discerned. The German and Dutch expert groups show rather polarized answering patterns while the expert groups of the Mediterranean countries mostly indicate a relatively uniform answering behaviour, both concerning personal attitude and time of realization (Menrad et al. 1998b).

The German panel is characterized by the most polarized answering behaviour between the expert groups compared to the other countries. Concerning the assessment of the 'personal attitude' the extreme poles are represented by the experts from industry and research institutions on the one hand and consumers and critics on the other hand. The answering behaviour of farmers is placed between these two poles with clear tendencies towards the consumer/critics cluster (Menrad et al. 1998a). In general, industry and research experts tend to assess the stated developments more positively whereas most of the experts from the farmer, consumer and critics side are more sceptical or reject single developments. The highest differences between the expert groups in Germany are found in statements dealing with the application of enzymes in food industry which are produced or optimized with the help of genetic engineering as well as in the use of genetic engineering approaches in organic farming (see figure 3).

These disparities do not invalidate the Delphi method but point to the well-known fact that different social groups in modern industrialised societies often do not agree on basic moral or political issues. Therefore, Delphi-type investigations also underline challenges of technology policy to determine or 'negotiate' innovation strategy.

Organic farmers in Germany are allowed to integrate specific genetic engineering approaches in their production process (33)
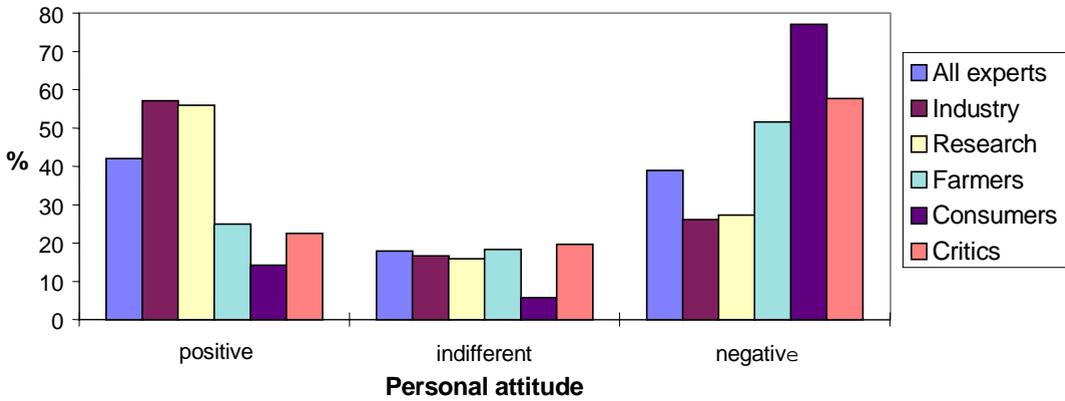
Enzymes optimized by protein engineering are practically used in specific sectors of the food industry (49)
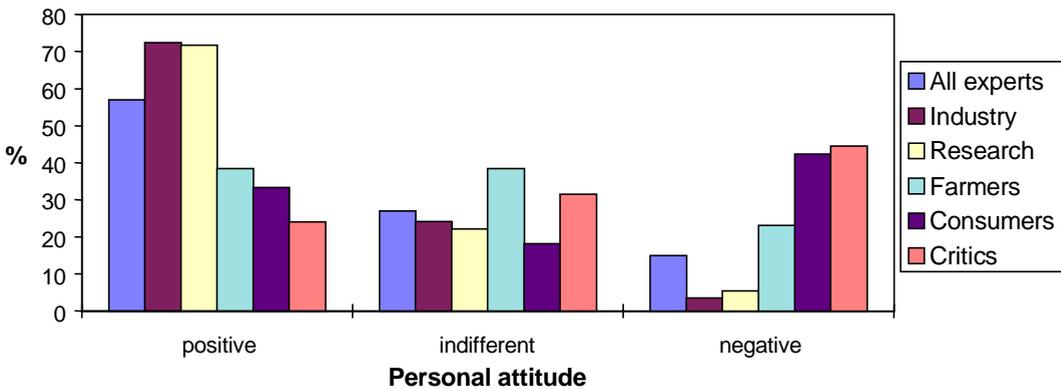
Approximately 90% of the enzymes used in the food processing industry are produced by genetical engineered organisms (50)
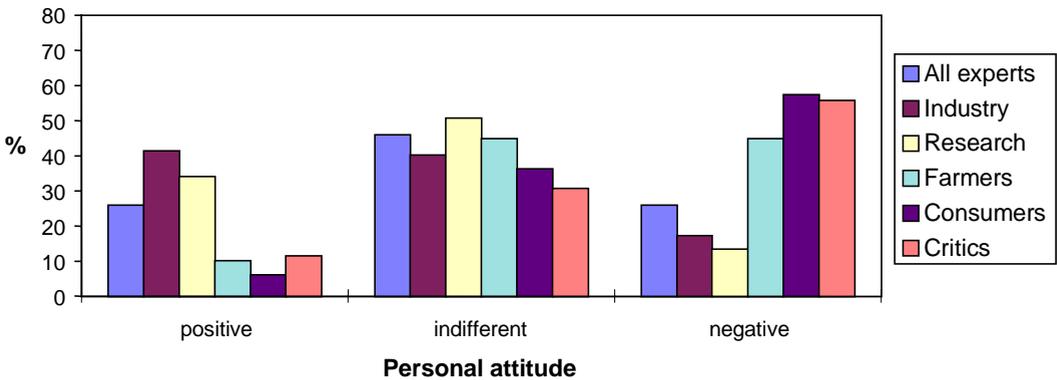
Figure 3: Differences between expert groups in Germany (in brackets the statement no of the original questionnaire)

Challenges of innovation strategy for tomorrow

Contemporary technology policy has moved away from the inappropriate idea that the state can direct technological developments right down to individual national innovations. Equally outmoded is the idea that the state should be satisfied with the role of a subsidiary supporter of research leaving the future control of technology to anonymous market processes. Technology policy for the start of the 21st century requires a middle course, i.e. one in which the state plays an active role as an intermediary between social systems negotiating (companies, associations, interest groups, science, consumers, media, employers' and employees' representatives, etc.). This intermediary role also must take account of the fact that national technology policy is increasingly restricted in its scope, both from above and below. This is because of the activities of the European Union and the efforts of regional bodies such as the Federal Laender in Germany to promote research on a regional basis.

The state's new role as active moderator necessitates a policy process which is coordinated with industry, science and society. However, co-operation does not occur by itself, since too many divergent interests predominate. If there is to be agreement over the possibly selective eligibility for support of technology, dialogues with other social players must be initiated and pursued on a permanent basis. Otherwise, it cannot be expected that lasting co-operation can be achieved or that the platforms to be created for a subject-specific understanding will become more than simply forums for the exchange of information. Don't we need integrated technology foresight to provide the knowledge base for these platforms?

Care has to be taken so that these social negotiations on technological wants should not stray too far from what is reliably known, and wander into the realms of speculation. In view of the typical recursive phases of science-related technological innovations, it can be generally assumed that everything that will dominate technology impacts in ten years' time is already recognisable today. However, strategic planning in enterprises is necessary, aiming towards horizons even further in the future, because new technologies - especially those which will contribute to long sought solutions to problems - must be identified at an early stage.

As far as enterprises are concerned, a considerable improvement of the intramural knowledge base through participation in foresight surveys is reported. There is sporadic evidence that in some companies, during participation in the Delphi, it was felt that too little effort is dedicated towards strategic innovation management and some remedies have been taken. Some companies engaged in own investigations in the direction of an intramural breakdown of the overall national studies towards the special interest of their business areas or establishments, both in the manufacturing and the service sectors. One large chemical company in Germany, especially, started with topics of the Delphi survey, made their own evaluation of the topics and built up a strategy until 2010. In working groups, the information was discussed and distributed. Some smaller-scale comparisons of the business portfolios to the future-oriented areas are also being done in other companies, sometimes assisted by external consultants. These activities are largely confidential.

Several lessons can be learned from the application of foresight methods (Grupp, 1996). Firstly, it is important to note that a foresight activity should not be a single event but should rather become part of a broader strategy which deals with strategic orientation. Secondly, the individual results of a survey should trigger various follow-up activities within the organisation, for example, workshops on selected items. Thirdly, going through the process of a foresight survey itself is a very valuable undertaking, since great numbers of experts are motivated to think critically about future scenarios favoured or rejected by their peer colleagues. Fourthly, for the company, the benefits of a foresight

survey should not only be seen as gains in information and reputation among its clients, but also extended to the internal situation: the strategies for dealing with challenges of the future must become broad company issues which are to be discussed and supported by many employees, thereby contributing to an increase of in-house motivation and identification.

From the social point of view, the direction to be taken in the future may be derived from the increasing demands made on technological development in terms of minimal use of resources, elimination of emissions, recycling economy and sustainable development. These demands require the creation of the new framework conditions, especially those of a non-technical nature, such as legal regulations. Equally important as such ecological problems is the sociopolitical dimension - in particular the unemployment problem. From the point of view of technology policy, we need a form of technological development which encourages wide-ranging participation by employees in various sectors, and firms of varying size, which leads to an open market with no specific centralised structure.

It is in the nature of long-term foresight that it is burdened with a high degree of uncertainty how the decision-making groups will behave; it is not unusual for wishful thinking, arising from the most diverse motives, to be presented as a probable future event. Taking the long term view, the motivating power of guiding visions is helpful in that it releases social energies and the willingness to undertake concerted action. Long-term lead projects in technology can produce lasting motivation and unite powers which can work towards problem-solving requirements recognisable in the long term, and also produce successes along the way (through desirable multiplier effects).

Lead projects in technology which represent outline solutions to large, global, economic, social and ecological problems, and especially the visionary view of technological development and the challenges now facing us, throw up other, more radical questions of technology policy than those set out here for the time being. It was not the aim of this brief essay to give the questions more concrete form. However, it has been possible to indicate that innovation strategy, technology assessment and foresight can themselves provide the key to far-reaching changes in future policy. The technology policy of tomorrow must be in place to shape policy in the long run.

In Germany, generally, there is a public tendency to be critical about new technology, often without going into any detail. After some foresight studies were published - rich in presenting visions of detailed trends in science and technology - several 'second thought' articles concerning the public understanding of technology by science journalists were published. The message in these articles is basically that dogmatic scepticism against new technology as such should be replaced by public reservations against *certain* technologies. A *technology-specific* public debate on the future of the so-called 'science and technology nation' need to be triggered off. From these observations one is tempted to conclude that the assessment and foresight processes have a lasting and direct impact on society as it affects our notions of future technology. By reflecting future opportunities and impacts of technology, we reflect our procedures to get there.

We are not fully convinced that our present society has already made optimal use of foresight for innovation strategy. There is great potential if we look forward. We are aware of the fact that some people are very sceptical and see no progress in technology and society. For these, foresight is both costly and irrelevant. However, many problems can be solved so that we are sure that foresight has a role to play in formulating innovation strategy which consists in uttering answers - and reformulating - complicated questions thrown up at the interface of technology, industry and society.

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Co-ordination of food / nutrition oriented research in Japan

T. Kobayashi

Dept. of Bio-Eng., Soka Univ., Hachiouji-shi, Japan (192-8577)

1 Past and present circumstances of Japan

A historical record of 'Average Life Time' (Fig. 1), i. e. the expecting years of life for just born babies [1,2] and an evaluation of the development of food supplies gives reason to think, that the observed changes are partly due to the improvement of the food supplies, mainly by an increase in the content of proteins and some essential amino-acids, such as *Lysine, Hystidine* in case of Japanese dishes. Of course, we cannot forget the contributions of the medical advancements, but the basic improvement of physiological situations, derived from the balanced food supply, especially to the young generation and pregnant women, have been approved to effect directly on the 'Average Life Time'.
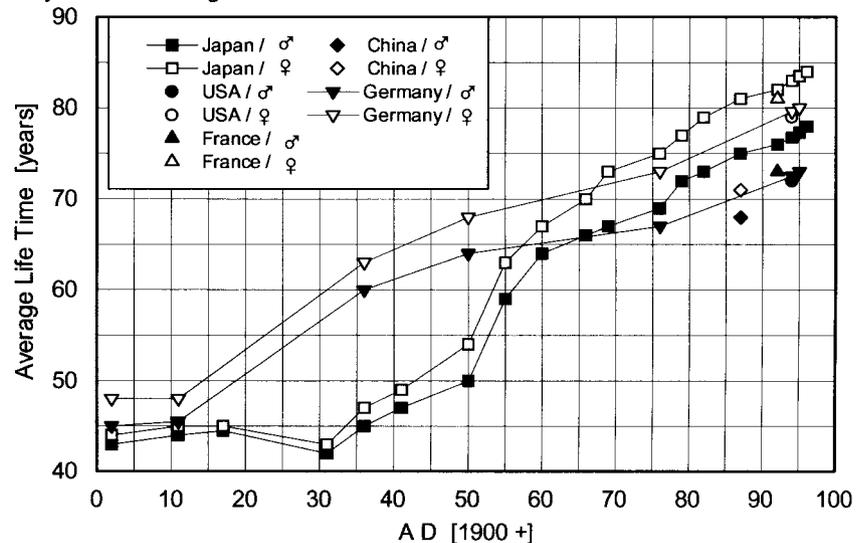
Fig. 1: Change of 'Average Life Time' in different countries

But now in Japan, we meet other problems related to nutrition. Such as:

(1)   New contamination by both micro-organisms and some chemicals, e.g. *O-157, Clostridium,* ... and *Endocrine Disrupting Chemicals (EDC),* e.g. *Dioxin, Bisphenol-A,* ... .

(2)   Increasing numbers of 'invisible patients' with diseases mostly caused by nutritional habits, e.g. *Diabetic, Hypertension, Alzheimer type dementia,* ... .

(3)   Appropriate food supply to the aged generation. A generation which has very different life habits and physiological situations, especially those who live 'alone' in local area.

(4)   Rapid disappearance of home cooking due to the increase of working women, and the effects to nutritional imbalanced foods, such as less *Calcium* intake than before.

Based on the present problems, many spreading efforts are now going on in Japan, some of the representative research directions or topics are explained below.

2 Research for food safety: 'Foods with information'

2.1 Rapid and easy inspection methods

Commercial supplies of cooked and semi-cooked food including frozen food, are at the moment very prospering in Japan and the amount of market is estimated to be more than 230.000 Million $, which is approximately 6 % of GNP in Japan. Large scale processing of these foods requires a very responsible attitude in order to avoid any contamination by micro-organisms or foreign chemicals. So, all relevant substances, i.e. raw materials, additives, packaging materials, even washing water, and operating conditions are monitored very attentively. Systematic procedures of rapid inspections, including new sampling techniques and inspection procedures, are applied even in small-scale factories. One of the obstacles to face is that for special micro-organisms the judgement requires time, at least a couple of hours. While using the fluorescent detection the sensitivity has been much improved, and the on-line inspection is now applicable.

The number of samples from day to day processing is increasing from year to year, and the total expenses to these inspections including labour costs, become enormous. Consequently one aim of the present research is to decrease the cost of inspections without lowering the level of the reliability. One part of the research efforts results in technical improvement of the monitoring process, other results, concerning the material flow are based on the huge amount of past records on safety related aspects and their reliability, which is however very different for each individual material in question.

2.2 Automatic manufacturing methods

Historically commercial food processing has been taking place in different mostly small scale in backyards and small factories operated by family workers or experienced amateurs. Competitive wholesalers and chained retailers, however, supported by the increasing demand of processed food, have eliminated these small-scale operations. The present introduction of HACCP system has accelerated the rapid change of industrial structure of Japan. An other factor, which also supported changes, were the cost of labour, which have been rising in the past years. A standard labour costs now 6-8 $/hour. These trends in the Japanese food industries are forcing the mechanisation of processing by educated specialists.

Mechanization and robotization of food processing requires of course high installation costs. But considering the continuous and effective long term operation of the equipment and the reliability of products including lower micro-organism contamination compared to the contamination caused by workers, it is justified that the application of modern processing is steadily expanding to middle and large scale processors.

The present technology is mainly focusing on sensing devices, such as CCD-camera and image analyses, near infrared spectroscope, electrochemical detector, ... An example of robotization is the automated poultry leg deboning robot [3], which has the ability of deboning of 900 legs/hr. Adjusting to the different sizes of poultry legs the system cuts at the right point and this achieves not only a high yield of meat, but also a high speed processing. Moreover, it is possible to apply a minimum labour to contact the goods, so contamination by micro-organisms is kept as low as possible.

2.3 Consistent flow of data from farmer's field to consumer

HACCP, which is now discussed and examined with many different technical groups, with public institutes including universities and with industry, it is pointing to the fundamental technical problem. Huge industrial records concerning Critical Control Points (CCP) have to be transferred to interested groups outside of companies, sometimes even to overseas. If not transferred along with the goods, the detailed adjustments of processing conditions, which are based on variety of raw materials and resources, are not successful. Some of the recent developments which are of social relevance, e.g. genetically engineered cereals and organic vegetables (less application of pesticides), require this data transfer in order to be correctly estimated. The appropriate data transfer however faces considerable technical problems:

(1) The practical methods of data transfer are not yet established. Definitions and e.g. analytical methods vary from country to country, and the detailed ways of measurements are also different. We can expect however that these problems are to be solved by FAO/WHO Codex committees.

(2) The reliability of data has to be objectively verified from time to time by authorities. Also responsibilities have to be established.

(3) The technical transfer of data from generation to application, by paper or electromagnetic records as interface media, requires standardization in any respect. And so on.

In summary the transfer of raw materials and food from the agricultural fields to the showcase of retailers, through distribution channels and processors, has to be accompanied by detailed data on processing conditions. Food safety means that reliable data are available, whether the data are visible or not.

3 Research for better food quality: New trends of 'Value Added Materials'

3.1 Exploitation of functional foods

Since 1980 the third function of food, namely physiological effects apart of the effects of nutrients and vitamins/minerals, have been moved into focus, especially for the exploitation of value added food materials. In 1991 the first functional foods (40-50 items) were introduced in the Japanese market. The composition of these foods is aimed to achieve specific physiological effects. The products have to be approved as 'Authorised Healthy Food of Japan' by a Committee of the Japanese Ministry of Health and Welfare. Including all of the related materials, so called functional food in Japan had a market of ca. 5,000 Million $ in 1997.

The practical research work and the product development for functional foods have been performed by public institutions including universities, many commercial institutions and through the co-operation of those institutions. An example for an officially recognised group is the Association for New Food Creation [4], which is financially supported up to 50% by Ministry of Agriculture, Fisheries and Food. This association is focusing on the utilization of carbohydrates. Some examples of the research are on *Cyclodextrins, Glycolipids, Anti-allergies, Anti-tooth decay, ...* . The members of the association have the obligation to co-operate and to publish the final results.

The Mitsubishi General Institute has surveyed the present market potential and the potential to realize new materials through the analyses of questionnaires completed by Japanese authorities. The results [5] are shown in Fig. 2.

■	Bowel Control
□	Cholesterol Management
●	Anti-Tooth Decay
○	Anti-High Blood Pressure
▲	Anti-Diabetes
△	Anti-Platelet Coagulation
▼	Anti-Congenital Metabolite
▽	Management of Nutrient Absorption
◆	Insensitive to Allergens
◇	Lipidperoxid Management
+	Management of Blood Formation
×	Activation of Immunity
✳	Management of Intake Function
▥	Activation of Lymph System
◍	Anti-Tumor
⬥	Control of Central Nerves System
⬦	Control of Peripheral Nerves System

Fig. 2: Market scale and realizability of 'functional food'

3.2 Evaluation of taste characteristics

Simply cooked rice is still the most important food in Japan, though the amount of consumption has been decreasing to a half of its peak time, namely in 1937 (135 kg/year• person). In 1996 the Japanese consumption was on average 67 kg/year. The japonica type rice has different varieties and a lot of public efforts have been devoted to research on rice properties and on rice breeding. All of new developed varieties have been sensually tested for better acceptance before the background of analyses of the general taste. The results of the scientific analyses are that the major elements of sensory impression come from the texture in the oral cavity, derived from the contents of protein and Amylopectin, especially at the cold state of cooked rice. The direct chemical effects to tongue, such as flavour or aroma, are much less important than the physical texture. Some commercial apparatuses, which measures the taste of raw rice by near - infrared spectroscopy, even with a single rice grain which is under breeding, have been developed and supplied.

The work on the sensory properties of rice has also revealed that chewing characteristics in the individual oral cavity are very significant for the evaluation of the quality of foods and allow to distinguish individual physical specificities. An attempt has been made to estimate the tongue movement through the electromyographic activity (EMG) and through the multiple-point sheet sensor [6,7,8]. The results of this analysis are mostly focusing on the perception of fine materials and on the physical characteristics of cooked rice and crackers. The detailed analyses of these experiments demonstrates that the amount of loading, the supply of moisture to tongue (cohesiveness, adhesiveness) and the following swallowing action are closely related to the chewing activity.

4 Easy evaluation of individual food intake: Clearing the 'Food Habit'

The rapid increase of the aged generation, elder than 65-years, and the amount of social expenses for medical treatment, i.e. about 200,000 Million $ and increasing 3-5 %/year, are indicating new requirements for technical development. In addition statistics show the invisible (latent) diabetes patients in Japan are already more than 10 % of total population, which means that more than 13 Million of Japanese have to control their daily food intake. Usually a card system is used to record the details of daily food and drink consumption. Data for longer time periods are however inaccurate because of problems with the recording procedures.

Despite these difficulties quantitative and easy measurement of individual food intake is strongly required. Once those records are available in accumulated form, a lot of new and valuable information is expected on the clinical and physiological situation of certain population segments also with respect to their ethnological background. In practice it is thought that digital cameras and telephones are able to overcome the present problems. Photographs, taken at the eating table during daily meals are analysed by a computer system with the help of trained dieticians. From this an easy and rapid evaluation of each intake with respect to the clinical and the physiological situation of each patient is expected. The development of such new technical systems has just been started by a group of specialists [9]. The local application will offer new business opportunities for dieticians. The image plane is shown in Fig. 3.
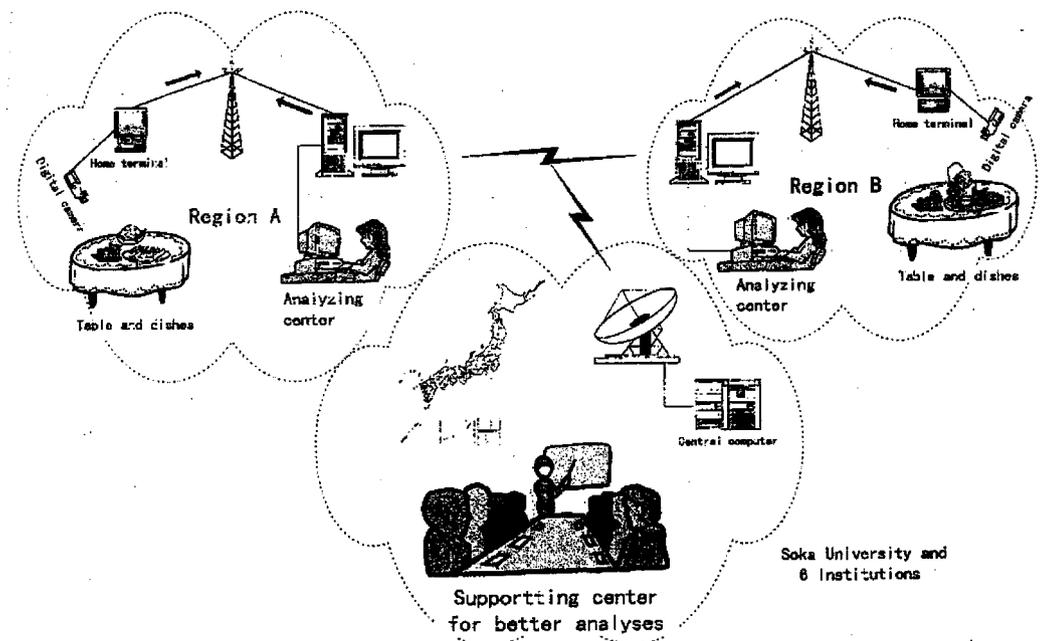
Fig. 3: Evaluation of individual food habit: New method of qualitative and quantitative analyses of dishes

The practical procedure meets the following difficulties, which have to be solved:

(1) Shooting of natural photographs of individual food tables and dishes is difficult. The major problem is the technical adjustment to the natural light situation and a further problem is the protection of privacy.

(2) The table of Standard Food Contents of Japan is composed for raw food materials. The tables of Standard Contents for prepared dishes are not available for the time being.

(3) The qualitative identification and quantitative evaluation of each dish is carried out semi-automatically by computers. The help of trained dieticians to correct and evaluate the information has to be minimized.

(4) The patients, whose daily food intake is continuously observed by digital cameras, will feel some mental restriction. Elimination of these feelings and obtainment of better photographs for analyses are the requirements for long time application.

(5) With photographic recording system it is not possible to obtain the data of invisible food contents, such as salt, additives, ..., which are not observed through the photographs. To overcome the problem it is now proposed that locally a dietician for each patient will be charged. He has to meet them and to discuss with them in certain intervals their intake habits.

(6) A co-operation with medical doctors, who have been taking care of the patients, has to be established. Significant data for clinical treatment have to be given to the doctors and those responsible for intake evaluation a co-operative way.

(7) Once the precise records of individual food habits and their physiological impact have been accumulated, methods for their physiological classification have to be established. Also predictions of individual health situations, i.e. invisible diseases, should be possible.

The technical developments are now taking place. It will take a couple of years to overcome those problems and to find good solutions, which we need, for making good progress.

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3rd Karlsruhe Nutrition Symposium: European Research towards Safer and Better Food
Review and Transfer Congress, Congress Centre, Karlsruhe, Germany
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3rd Karlsruhe Nutrition Symposium: European Research towards Safer and Better Food
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Congress Facts

Congress Facts

1 Aims and scope of the event

As the 4th EU framework programme expired in late 1998, it seemed advisable to review and discuss its outcomes also with a view to the 5th framework programme beginning in spring of 1999. Upon the initiative of the Federal Research Centre for Nutrition (FRCN), a congress was organized on some special segments covered by the FAIR programmes to discuss the important fields food safety, nutrition and novel technologies in particular. The programme was supplemented by a review workshop on meat organized jointly with the FLAIR-FLOW network, and by papers dealing with organization and structure of the 5th framework programme.

The scientific programme was planned and implemented as a series of lectures plus supplementing poster exhibition. To organize the lectures, project leaders of the working fields mentioned above were asked to submit proposals out of which the programme was built up in cooperation with GD XII of the European Commission. Proposals which could not be presented as lectures because of the limited time available were included into the poster programme for which, in addition, a Europe-wide call had been published. The poster programme thus contained also papers not related to the FAIR projects. In this way it should be demonstrated that EU programmes are not to be understood as exclusive circles. This was important especially for those parts of the event informing about the 5th framework programme.

As a satellite event a one-day series of lectures on the significance of phytochemicals had been organized by the DGE (German Society for Nutrition). Following the Congress, a 'brokerage event' was held by the Bureau for International Research and Technology Cooperation (BIT), Vienna, in which 120 groups participated.

2 Organization and implementation

The Congress was planned and organized by the FRCN, Institute of Process Engineering, from funds of the European Commission and the Federal Ministry of Food, Agriculture and Forestry. Co-sponsors were EFFoST and *Verein der Freunde des kältetechnischen Instituts zu Karlsruhe e.V.* which sponsored the social programme from donations.

First promotional measures were taken about 15 months before the Congress by direct contact to FAIR working groups. Several announcements appeared in the EFFoST calendar. BIT and DGE supported the dissemination of information about the Congress by a systematic combination of information material. Most important, however, was the personal information of European scientists working in the fields concerned. Address files were obtained from the EU and EFFoST.

3 Scientific programme

The lecture programme comprised 52 papers, 18 of which were presented in plenary sessions. The most important subjects were consumer behaviour and transfer strategies, followed by nutrition, food safety and process engineering. The meat workshop was a one-day event (see figure 1). Of about 300 posters totally, one third dealt with food processing and nutritional problems, followed by food safety, meat, consumer questions and transfer strategies (see figure 2).
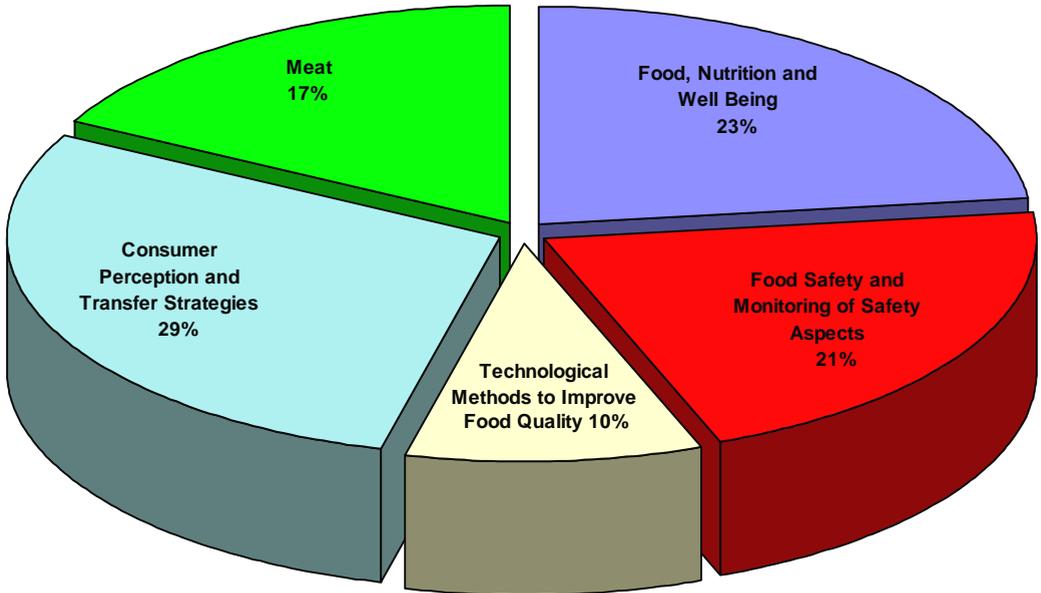
Fig. 1: Lectures subdivided according to subjects (52 lectures totally)
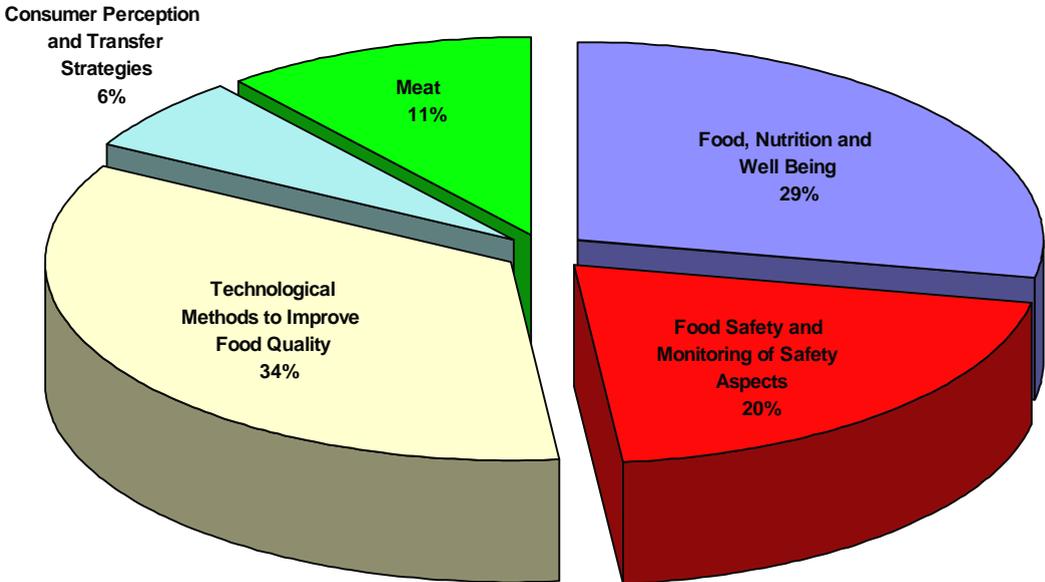
Fig. 2: Scientific posters subdivided according to subjects (293 posters totally)

Independently from the supply of lectures and posters, the participant's interest concentrated on nutritional questions; food safety, food processing and meat were of secondary interest; consumer problems and transfer strategies were nearly neglected (see figure 3). All contributions to the Congress - as far as manuscripts were available - were published in this 3-part congress proceedings volume.
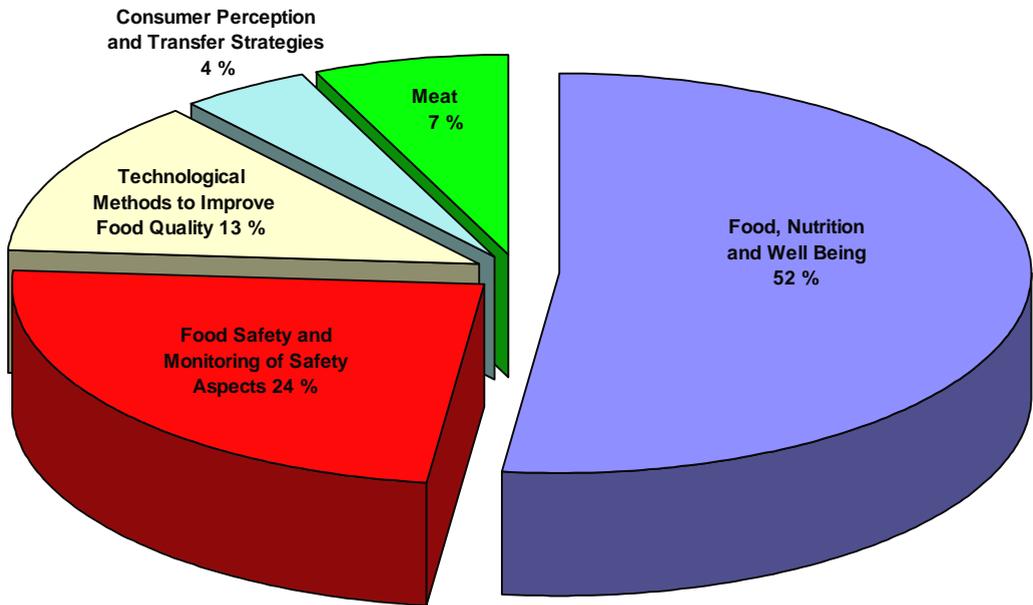
Fig. 3: Participants' fields of interest (subdivided according to subject, in %)

4 Participants

604 participants had registered; besides these, advanced students and post-graduates of the Universities Karlsruhe and Hohenheim participated non-registered. Participants in the DGE event, too, were granted free admission. The number of participants thus totalled about 700. About 1000 persons were present at the opening reception.

Registered participants came primarily from Germany (about 50 %); of the European countries, most came from Great Britain. Many scientists were from partner institutes of the FRCN in Poland, Japan and Korea (see figure 4). The majority of participants belonged to scientific institutions, one eighth to industry, a small group only were delegates of consumer organizations (see figure 5).

Despite the large number of participants, certain areas of interest and certain countries were not represented as it was anticipated. So, for example, it was expected that more representants of consumer organisations would use the opportunity for comprehensive first-hand information - however, these groups lack definitely in funds to attend such scientific meetings. Another group which was expected to be stronger represented was the industrial sector for utilizing results in the meat area, obviously two well-established meetings in a timely neighbourhood prevented interest groups to attend the Karlsruhe Conference. Similarly, attendance of French scientists was reduced because of the Paris Food Fair (GIA) held at the same time as the Karlsruhe Congress.
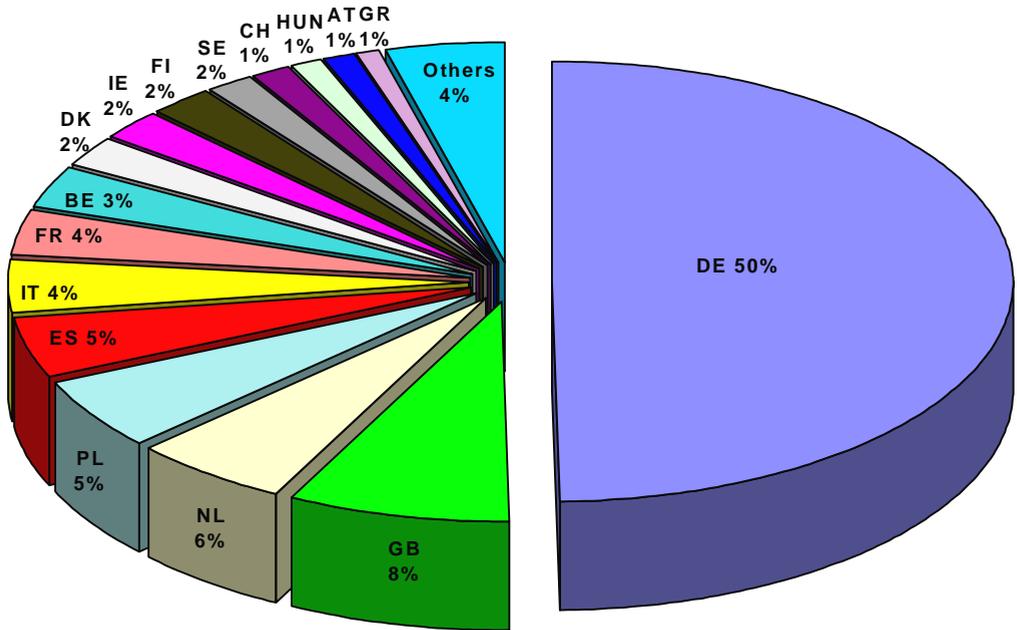
Fig. 4: Nationalities (in %) (604 participants totally)
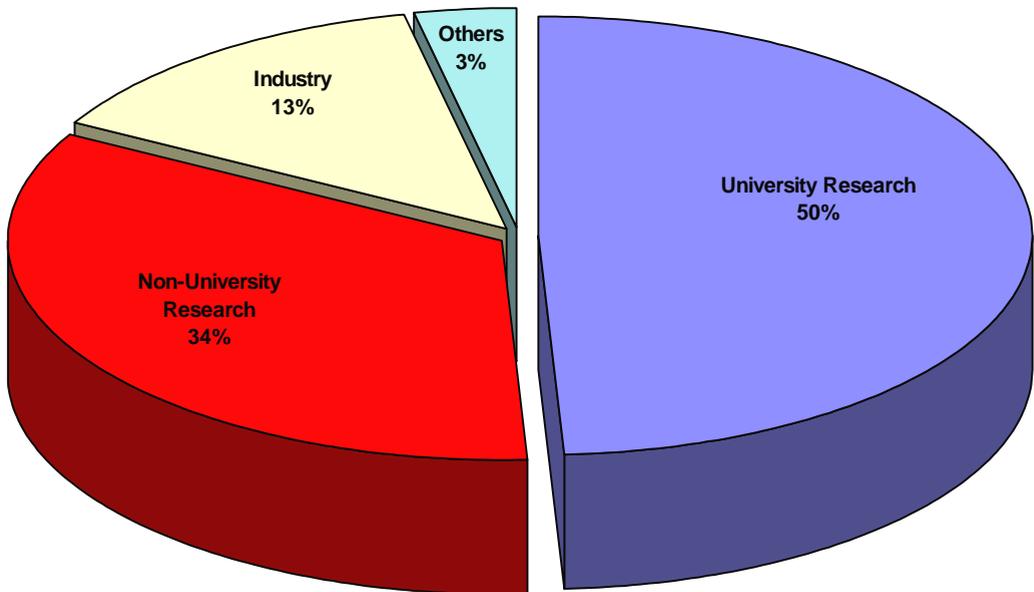
Fig. 5: Grouping of participants according to research institutions

5 Market Place

To prepare partnerships for projects of the 5th framework programme, interested institutions were given a chance of presenting themselves at a marketplace. 106 institutions took the chance (see figure 6). As far as nationalities are concerned, attendants of the market place were comparable to

those participating in the brokerage event (see figure 7). It is worth mentioning that one fifth of the participants in the brokerage event came from industry (see figure 8).
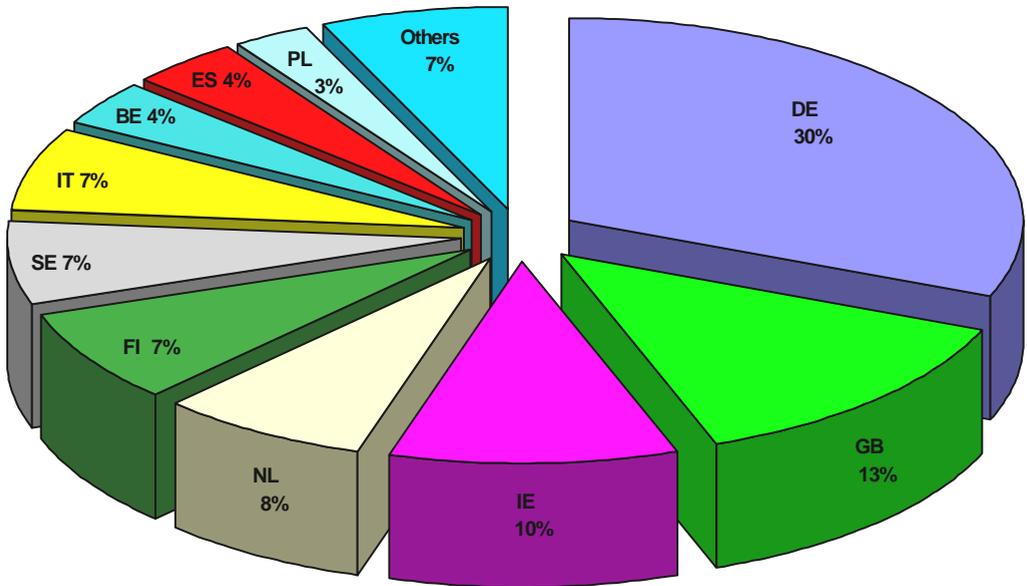
Fig 6:   Nationalities of participants presenting posters
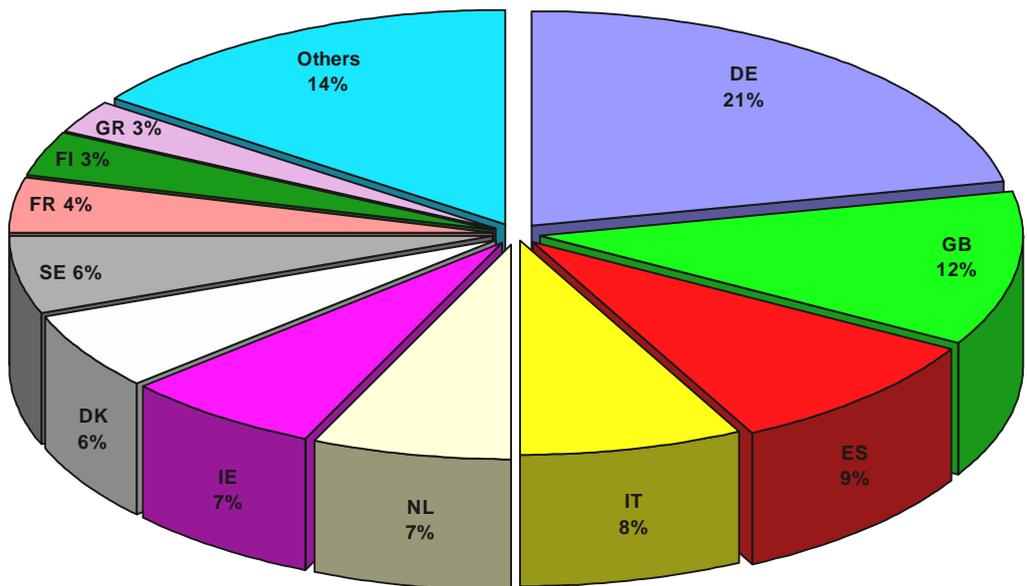
Fig. 7: Nationalities of participants in the brokerage event
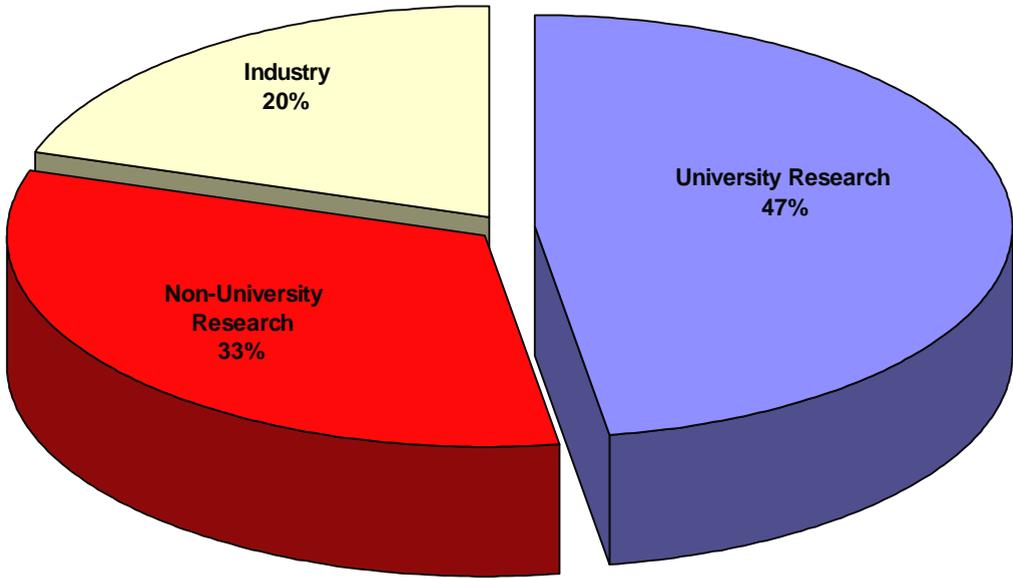
Fig. 8: Grouping of participants in the brokerage event according to research institutions

3rd Karlsruhe Nutrition Symposium: European Research towards Safer and Better Food
Review and Transfer Congress, Congress Centre, Karlsruhe, Germany
October 18-20, 1998

List of Participants

Abee, Tjakko
Wageningen Agricultural University, Dep. of Food
Technology and Nutritional Science
Bomenweg 2
NL-6703 HD Wageningen

Abilgaard, Christina
The Research Council of Norway
Box 2700, St. Hanshaugen
N-0131 Oslo
christina.abildgard@forskningsradet.no

Adam, R.
Bundesforschungsanstalt für Ernährung, IHT
Engesserstr. 20
D-76131 Karlsruhe

Adlercreutz, Herman
University of Helsinki, Department of Clinical
Chemistry, PL 60
Mannerheimintie 97
FIN-00014 Helsinki
Herman.Adlercreutz@helsinki.fi

Ahvenainen, Juha
VTT Biotechnology and Food Research
P.O. Box 1500
FIN-02044 VTT
juha.ahvenainen@vtt.fi

Alcocer, Marcos
Institute of Food Research, Norwich Research Park
Colney
GB-Norwich NR4 7UA, UK

Aldamiz-Echebarria, Paloma
GAIKER
Pargue Technologico, Edificio 202
E-48170 Zamudio
aldamiz@gaiker.es

Allen, Paul
TEAGASC National Food Centre, Dunsinea,
Castleknock
IRL-Dublin 15

Alt, Axel
University Pharmakognosie u. Anal. Phytochemie
Postfach 15 11 50
D-66041 Saarbrücken
pb13hbaa@rz.uni-sb.de

Ames, Jenny
The University of Reading, Dept. of Food Science
and Technology
Whiteknights
GB-Reading RG6 6AP
j.m.ames@afnovell.reading.ac.uk

Anderer, Ralf
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
ui19@rz.uni-karlsruhe.de

Andrada, Carlos
Universidad Nacional de Catamarca
Esquiu 612
ARG-4700 Catamarca
salud@catam.unca.edu.ar

Andrieu, Julien
Université Lyon 1-ESCPE Lyon, Laboratoire
d´Automatique et de Genie des Procedes
43 Boulevard du 11 Novembre 1918
F-69622 Villeurbanne cedex
andrieu@lagep.univ-lyon1.fr

Anese, Monica
Instituto die Produzioni e Preparazioni Alimintari, Univ.
of BARI-seat of FOGGIA
Via Napoli 25
I-71100 Foggia

Arnoldi, Anna
University of Milan - DISMA
via Celoria 2
I-20133 Milano
Anna.Arnoldi@unimi.it

Atkinson, Hilary
BIBRA International
Woodmansterne Road, Carshalton
GB-Surrey SM5 4DS
HATKINSON@bibra.co.uk

Augenstein, Martina
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Azpiroz, Almudena
GAIKER
Pargue Technologico, Edificio 202
E-48170 Zamudio
azpiroz@gaiker.es

Babski, Ryszard
Warsaw Agricultural University, Depart. Food
Engineering
ul. Nowoursynowska 166
PL-Warsaw 02 - 766

Baek, In-Girl
Justus-Liebig-University
Suedanlage 6
D-35390 Giessen
In-Girl.Baek@ernaehrung.uni-giessen.de

Bähner, Monika
Universität Karlsruhe, Zoologisches Institut,
Lehrstuhl I
Kornblumenstr. 13, Postfach 69 80
D-76128 Karlsruhe

Balfoort, Arnold J.
Suikerstichting Nederland
Amsterdamsestraatweg 39A
NL-3744 MA Baarn
Suikerstichting.nl@wxs.nl

Barat, José Manuel
Food Technology Department
Camino de Vera s/n
E-46022 Valencia
jmbarat@tal.upv.es

Barc, Marie-Claude
Department of Microbiologie, Faculte de Pharmacie
Rue J.B. Clement
F-92296 Chatenay-Malbry Cedex
marie-claude.barc@cep.u-psud.fr

Bardowski, Jacek
IBB. Polish Academy of Sciences
Pawinskiego 5a
PL-02-106 Warsaw
jacek@ibb.waw.pl

Bartels, Paul
Agrotechnical Research Institute (ATO-DLO)
P.O.Box 17
NL-6700 AA Wageningen
p.v.bartels@ato.dlo.nl

Barth, Christian A.
Deutsches Institut für Ernährungsforschung(DIFE)
Arthur - Scheunert - Allee 114 -116
D-14558 Bergholz-Rehbrücke

Bauer, Bernhard
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
bernhard.bauer@bfe.uni-karlsruhe.de

Bäumer, Dietrich
Fakultät für Biologie, Universität Bielefeld
D-33615 Bielefeld
dietrich.bauemer@Biologie.Uni-Bielefeld.de

Becker, Biserka
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
biserka.becker@bfe.uni-karlsruhe.de

Becker, Hans
University Pharmakognosie u. Anal. Phytochemie
Postfach 15 11 50
D-66041 Saarbrücken
pb13tib@rz.uni-sb.de

Becker, Tilman
Universität Hohenheim, Institut für Agrarpolitik und
Landwirtschaftliche Marktlehre
D-70593 Stuttgart
tbecker1@uni-hohenheim.de

Behrend, Olaf
Universität Karlsruhe, LVT
Kaiserstr. 12
D-76128 Karlsruhe
olaf.behrend@ciw.uni-karlsruhe.de

Behsnilian, Diana
Bundesforschungsanstalt für Ernährung, IVT
Engesserstr. 20
D-76131 Karlsruhe
diana.behsnilian@bfe.uni-karlsruhe.de

Bek, Verena
Frauenhofer Institut Physikalische Meßtechnik,
Heidenhofstrasse 8
D-79110 Freiburg
bek@ipm.fhg.de

Bellara, Sanjay
University of Birmingham, School of Chemical
Engineering
Edgbaston
GB-Birmingham B15 2TT
s.r.bellara@bham.ac.uk

Benner, Marco
Wageningen Agricultural University, Division of
Integrated Food Technology
P.O. Box 8129
NL-6700 EV Wageningen
marco.benner@ift.fdsci.wau.nl

Bennis, Frederik
TU Delft
41 Dijkmanzoet
NL-4007 XE Tiel
frederikchrbennis@yahoo.com

Bentrop, Joachim
Universität Karlsruhe, Zoologisches Institut 1
Kornblumenstr. 13
D-76128 Karlsruhe
dc02@rz.uni-karlsruhe.de

Berg, H.E.
TNO, Nutrition and Food Research Institute
P.O. Box 360
NL-3700 AJ Zeist
h.berg@voeding.tno.nl

Berghäuser, Jürgen
Wirtschaftliche Vereinigung Zucker e.V.
Am Hofgarten 8
D-53113 Bonn

Berk, A.
Institut für Tierernährung der FAL
Bundesallee 50
D-38116 Braunschweig

Betsche, Thomas
Bundesanstalt für Getreide-, Kartoffel-, und
Fettforschung (BAGFK)
Postfach 1354
D-32703 Detmold
Betsche.BAGKF@online.de

Biacs, Peter A.
KÉKI, Central Food Research Institute
P.O. Box 393
H-1537 Budapest
h9740bia@elle.hu

Biekman,
ATO-DLO
P.O. Box 17
NL-6700 AA Wageningen

Birch, Gordon
University of Reading, Dept. of Food Science and
Technology
Whiteknights / P.O. Box 226
GB-Reading RG6 6 AD

Bitsch, Irmgard
Universität Gießen, Institut für
Ernährungswissenschaften
Wilhelmstr.20
D-35392 Gießen
irmgard.m.bitsch@ernaehrung.uni.giessen.de

Bitsch, Roland
Universität Jena, Inst. für Ernährung und Umwelt
Dornburger Str.25/27
D-07743 Jena
b6biro@uni.jena.de

Björkroth, Johanna
Bundesforschungsanstalt für Ernährung
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Blanchfield, J R
J Ralph Blanchfield Consultancy
17 Arabia Close, Chingford
GB-London E4 7DU
jralphb@easynet.co.uk

Blatt, Eva
Bundesministerium für Ernährung, Land-, wirtschaft u.
Forsten, Ref. 222
Postfach 14 02 70
D-53107 Bonn

Blaut, Michael
Dept. of Gastro-intestinal Microbiology
Arthur-Scheunert-Allee 114-116
D-14558 Bergholz-Rehbrücke
blaut@www.dife.de

Blivet, Dominique
Centre National d'Etudes Vétérinaires et Alimentaire,
Unité d'Hygiene et Qualité des
Prd. Avic. et Porc.
Beaucemaine - BP 53
F-22440 Ploufragan
d.blivet@ploufragan.cneva.fr

Blockhaus, Mark
Bundesforschungsanstalt für Ernährung
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Blüthgen, A.
Bundesanstalt für Milchforschung, Institut für Hygiene
Postfach 6069
D-24121 Kiel

Bochereau, Laurent
European Commission, Directorate-General XII
SDME 8/12 Rue de la Loi 200
B-1049 Brussel

Bockelmann, W.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Bode, Ch.
Universität Hohenheim, Ernährungsphysiologie
Garbenstr. 28
D-70599 Stuttgart

Boelskov, Marjon
EuroCenter danish Agency for Trade and Industry
Raadhuspladsen 14
DK-1550 Copenhagen V
mab@schultz.dk

Böhme, T.
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Bollmann, J.
Bundesministerium für Ernährung, Land-, wirtschaft u.
Forsten, Ref. 222
Postfach 14 02 70
D-53107 Bonn

Borch, Elisabeth
Swedish Meat Research Institute
P.O. Box 504
S-244 24 Kävlinge
elisabeth.borch@kl.scan.se

Borch, Kristian
Riseo National Laboratory, SYS-110
P.O. Box 49
DK- 4000 Roskilde
kristian.borch@risoe.dk

Borsch, Christian
Institut für Ernährungswissenschaft
Wilhelmstr. 20
D-35392 Giessen

Boureau, Hèléne
Department of Microbiologie, Faculte de Pharmacie
Rue J.B. Clement
F-92296 Chatenay-Malbry Cedex

Bourlioux, Pierre
Universite Paris Sud, Dept. de Microbiologie -
Immunologie
Rue J.B. Clement
F-92296 Chantenay-Malabry Cedex

Bourquin, Dieter
Dr.-Sartoris-Str. 45 a
D-67435 Neustadt

Bover-Cid, Sara
Department of Nutrition and Food Sciences, Faculty
of Pharmacy, University of
Av. Joan XXIII s/n
E-08028 Barcelona
bover@farmacia.far.ub.es

Bozzi, Silvia
APRE
Via Flaminia 43
I-Rom
bozzi@apre.it

Brack, Günter
Bundesanstalt für Getreide-, Kartoffel- und
Fettforschung
Schützenberg 12
D-32756 Detmold

Brandt, Kirsten
Danish Institute of Agricultural Sciences
PO Box 102
DK-5792 Aarslev
kirsten.brandt@agrsci.dk

Braun, Anette
Deutsche Gesellschaft für Ernährung e.V., DGE
Im Vogelsang 40
D-60488 Frankfurt am Main

Bräutigam, Martin
Universität Hohenheim
Garbenstr. 28
D-70599 Stuttgart
martinhb@uni-hohenheim.de

Breithaupt, Dietmar
Universität Hohenheim, Institut für Lebensmittelchemie
Garbenstr. 28
D-70599 Stuttgart
breithau@uni-hohenheim.de

Bresch, Horst
Bundesforschungsanstalt für Ernährung, IHT
Engesserstr. 20
D-76131 Karlsruhe

Breslin, Liam
Commission of the EU (DG-XII-E-2,8/12), Agro
Industrial Research
SDME 8/12 Rue de la Loi 200
B-1049 Brussels

Brian, Marcus
Am Oberberg 11
D-61476 Kronberg
lassebrian@aol.com

Brooker, Brian
Institute of Food Research
Earley Gate, Whiteknights Road
GB-Reading RG6 6BZ
brian.brooker@bbsrc.ac.uk

Brösel, Sabine
Universität Karlsruhe, Institut für
Lebensmittelverfahrenstechnik
Kaiserstr. 12
D-76128 Karlsruhe
sabine.broesel@ciw.uni-karlsruhe.de

Brown, Wendy
Institute of Food Research,, Reading Laboratory
Earley Gate, Whiteknights Road
GB-READING RG6 6BZ
wendy.brown@bbsrc.ac.uk

Brückner, Hans
Universität Gießen, Institute of Nutritional Sciences
Südanlage 6
D-35390 Gießen
hans.brueckner@ernaehrung.uni-giessen.de

Brüggemann, Jörg
BAGFK, Institut für Biochemie von Getreide und
Kartoffeln
Postfach 1354
D-32703 Detmold
betsche.bagkf@t-online.de

Brühl, L.
Bundesanstalt für Getreide-, Kartoffel-, und
Fettforschung (BAGFK)
Piusallee 76
D-48147 Münster
aitzetm@uni-muenster.de

Bruin, Solke
Unilever Research
P.O. Box 114
NL-3130 AC Vlaardingen
solke.bruin@unilever.com

Brümmer, J.-M.
Bundesanstalt für Getreide-, Kartoffel-, und
Fettforschung (BAGFK)
Postfach 1354
D-32703 Detmold
bagkf@t-online.de

Bruns, Karl - Ingo
Landwirtschaftliche Rentenbank
D-Frankfurt am Main

Bub, Achim
Bundesforschungsanstalt für Ernährung, IEP
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Buckenhüskes, H.
Gewürzmüller GmbH
Klagenfurter Str. 1-3
D-70444 Stuttgart

Bulteau, Stephane
Department of Microbiologie, Faculte de Pharmacie
Rue J.B. Clement
F-92296 Chatenay-Malabry Cedex

Butz, Peter
Bundesforschungsanstalt für Ernährung, ICB
Engesserstr.20
D-76131 Karlsruhe
Peter.Butz@bfe.uni-karlsruhe.de

Cabo, Marta López
Marine Research Institute (CSIC)
Eduardo Cabello, 6
E-36208 Vigo
marta@iim.csic.es

Cano, M. Pilar
Instituto del Frio, CSIC, Ciudad Universitaria
E-28040 Madrid
pcano@if.csic.es

Carlin, Frederic
INRA,, Station de Techn.des Prodiuts Vegetaux
Domaine St. Paul, site Agroparc
F-84914 Avignon
carlin@avignon.inra.fr

Cayuela, Chantal
CIRDC, Groupe Sante-Nutrition
15, Avenue Galilee
F-92350 Le Plessis-Robinson
ccayuela@danone.com

Christensen, Torben Bo Toft
The Danish Agricultural Council
Axeltorv 3
DK-1609 Copenhagen V
tbc@landbrug.dk

Chung, Sook
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Clemens, Evelin
Johanisstr.76
D-53225 Bonn

Coffey, Aidan
Teagasc
Moorepark, Fermoy
IRL-Cork
acoffey@moorepark.teagasc.ie

Collignon, Anne
Department of Microbiologie, Faculte de Pharmacie
rue J.B. Clement
F-92296 Chatenay-Malbry Cedex
anne.collignon@cep.u-psud.fr

Cox, Lynton
Central Science Laboratory
Sand Hutton
GB-York, YO411L2
l.cox@csi.gov.uk

Cresci, Alberto
Dipartamento di Scienze Igienistiche, Universita degli
Studi di Camerino
Viale E.Betti 3
I-62032 Camerino
cresci@camserv.unicam.it

Crews, Helen
CSL Food Science Laboratory, Norwich Research
Park
Colney
GB-Norwich, Norfolk NR4 7UQ
n.crews@csl.gov.uk

Cuperus, F. Petrus
Agrotechnological Research Institut
Bornsesteeg 59 P.O. Box 17
NL-6700 AA Wageningen

Czeczko, Renata
Department of Chemistry, Agricultural Academy
Akademicka str. 15
PL-20-950 Lublin
renata@hermes.umcs.lublin.pl

Czyz, Beata
Technical University of Gdansk, Department of Food
Preservation
ul. Narutowiczka 11/12
PL-80-952 Gdansk
jagoda@altis.chem.pg.gda.pl

Dalla Rosa, Marco
Università di Udine, Dipartimento di Scienze degli
Alimenti
Via Marangoni 97
I-33100 UDINE
marco.dallarosa@dsa.uniud.it

Damkjær, Søren
SanaCare
Fruebjergvej 3
DK-2100 Copenhagen
damkjaer@sanacare.dk

Danak, Agata
Warsaw Agricultural University, Depart. of Food
Engineering
ul. Nowoursynowska 166
PL-02-787 Warszawa
tzy_kiimps@alpha.sggw.waw.pl

Daniel, Hannelore
Universität Gießen, Institut für
Ernährungswissenschaft
Wilhelmstraße 20
D-35392 Gießen

Daniewski, Marek
Institute of Food and Nutrition
61/63 Powsinska Street
PL-02-903 Warszawa
daniewski@izz.waw.pl

Davies, Laurence
The University of Birmingham, School of Chemical
Engineering
Edgbaston
GB-Birmingham B15 2TT
l.j.davies@bham.ac.uk

Day, Brian Philip
Dept. of Process and Product Development,
Campden & Chorleywood Food Res. Assoc.
Stadion Road
GB-GL55 6LD Chipping Campden

de Jong, Peter
Netherlands Institute for Dairy Research, NIZO Food
Research
P.O. Box 20
NL-6710 BA Ede
pdejong@nizo.nl

de Vrese, M.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Dejmek, Petr
Lund University, Food Engineering
P.O. Box 124
S-221 00 Lund
petr.dejmek@livstek.lth.se

Delincée, Henry
Bundesforschungsanstalt für Ernährung, IEP
Haid-und-Neu-Str.9
D-76131 Karlsruhe

Diaz, Georgina
Lonquimay 581
Chile La Reina - Santiago

Dickau, Torsten
Leiter Referat Gemeinschaftsverpflegung
Deutsche Gesellschaft für Ernährung e.V., DGE
Im Vogelsang 40
D-60488 Frankfurt am Main

Diehl, J.F.
Wildbader Str.6
D-76228 Karlsruhe

Dimitriou, Triantafillia
ETH-Zürich (Labor für Humanernährung)
Seestr. 72
CH-8803 Zürich
triantafillia.dimitriou@ilw.agrl.ethz.ch

Ditchev, Stefan
Bulgarian Higher Inst. of Food &, Flavour Industries
26 Maritza Blvd
BUL-4002 Plovdiv
ditchev@plov.omega.bg

Dolara, Piero
Department of Pharmacology, University of Florence
Viale Morgagni 65
I-50134 Florence
dolara@stat.ds.unifi.it

Dolatowska, Ilona
Warsaw Agricultural University, Depart. Food
Engineering
ul. Nowoursynowska 166
PL-Warsaw 02 - 766

Doman, Monika
Dept. of Food Technology, Agricultural University
Skromna 8
PL-20-950 Lublin
monik@ibbrain.ibb.waw.pl

Donelli, Gianfranco
Instituto Superiore di Sanita
Viale Regina Elena 299
I-00161 Rom
donelli@ul.net,iss.it

Dörnenburg, Heike
TU Berlin
D-Berlin

Dossena, Arnaldo
University of Parma
Viale delle Scienze
I-43100 Parma
dossena@ipruniv.cce.unipr.it

Dunajski, Edward
Technical University of Gdansk, Department of Food
Preservation
ul. Narutowiczka 11/12
PL-80-952 Gdansk

Eber, Matthias
Bundesanstalt für Fleischforschung BAFF, Institut für
Technologie
E.-C.-Baumann-Straße 20
D-95326 Kulmbach
baff@compuserve.com

Egli, Ines
Laboratory for Human Nutrition ETH Zürich
Seestrasse 72
CH-8803 Rüschlikon
ines.egli@ilw.agrl.ethz.ch

Ehlermann, Dieter
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Eichner, Karl
Universität Münster, Institut für Lebensmittelchemie
Corrensstr. 45
D-48149 Münster
eichner@uni-muenster.de

Eidenmüller, Ullrich
Stadt Karlsruhe
Rathaus, Marktplatz
D-76133 Karlsruhe

Eikmanns, Bernhard
Universität Ulm, Abt. für Angewandte Mikrobiologie
D-89069 Ulm
bernhard.eikmanns@biologie.uni-ulm.de

Eltoukhy, Nabil
Bundesforschungsanstalt für Ernährung
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Ender, Barbara
Landesuntersuchungsanstalt
D-Leipzig

Endress, Hans-Ulrich
Herbstreith & Fox KG Neuenbürg
Kniebisstr. 13
D-75303 Neuenbürg

Engfer, Meike
Justus-Liebig-Universität Gießen, Institute of
Nutritional Sciences, Biochemistry Unit
Wilhelmstr. 20
D-35952 Gießen
meike.b.engfer@ernaehrung.uni-giessen.de

Enneking, Ulrich
Forschungs- und Studienzentr. für
Veredelungswirtschaft
Driverstraße 22
D-49377 Vechta
uenneki@fosvwe.uni-vechta.de

Erbe, Thorsten
Justus-Liebig-Universität Giessen
Südanlage 6
D-35390 Giessen
Thorsten.Erbe@ernaehrung.uni-giessen.de

Erbersdobler, Helmut
Institut fhr Humanernährung
Düsternbrooker Weg 17
D-24109 Kiel

Eriksson, Caj
Diffchamb AB, Sweden
Storakersgatan 4
S-254 54 Helsingborg
caj.e@helsingborg.mail.telia.com

Erle, Ulrich
Universität Karlsruhe, Institut für
Lebensmittelverfahrenstechnik
Kaiserstr. 12
D-76128 Karlsruhe
ulrich.erle@ciw.uni-karlsruhe.de

Fabiani, Roberto
Dept. Cell Molec. Biol. UNIVERSITY OF PERUGIA
Via del Giochetto
I- 06126 PERUGIA
fabirob@krenet.it

Fanfani, Roberto
University of Bologna
via Belle Arti 41
I-40126 Bologna
fanfani@stat.unibo.it

Farkas, József
University of Horticulture and Food Industry
Menesi ut 45
H-1118 Budapest
huto@hoya.kee.hu

Faulks, Richard
Institut of Food Research Norwich Laboratory,
Norwich Research Park, Colney
GB-Norwich NR 4 7UA U.K.
Richard.Faulks@bbsrc.ac.uk

Fedele, Francoise
Novartis Nutrition Research AG
CH-3176 Neuenegg
francoise.fedele@nutrition.novartis.com

Fenselau, Susanne
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Fenwick, Roger
Royal Society of Chemistry, AFRC Institute of Food
Research, Colney
GB-Norwich NR4 7UA
roger.fenwick@bbsrc.ac.uk

Fernández Garcia, Avelina
Bundesforschungsanstalt für Ernährung, ICB
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
gast3.icb@bfe.uni-karlsruhe.de

Fernandez-Monge, Mercedes
AZTI
Txatxarramendi Irla Z/G
E-48395 Sukarrieta (Bizkaia)

Ferrando, Montserrat
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Ferro-Luzzi, A.
Instituto Nazionale Della Nutrizione
Via Ardeatina 546
I-00178 Roma

Fetlinski, Andrzej
Biolacta-Texel Spolka z o.o.
Al. Warszawska 111
PL-10-701 Olsztyn
biolacta-texel.@etos.olsziyn.pl

Fischer, Klaus
Bundesanstalt für Fleischforschung BAFF, Institut für
Fleischerzeugung und Vermarktung
E.-C.-Baumann-Straße 20
D-95326 Kulmbach
baff@compuserve.com

Fischer, Siglinde
BioLinx GmbH
Steinlestr. 6
D-60596 Frankfurt
sfischer@biolinx.de

Fito, Pedro
Universidad Politécnica de Valencia, Food Technology
Department
Camino de Vera s/n
E-46007 Valencia
pfito@tal.upv.es oder jmbarat@tal.upv.es

Fletcher, John
Unilever Research Laboratory, Colworth House
Sharnbrook
GB-BEDFORD MK44 1LQ
john.fletcher@unilever.com

Fogher, Corrado
Plantechno Srl
Via Ticino 32
I-26041 Casalmaggiore
fogher@pc.unicatt.it

Fons, Michel
I.N.R.A.
UEPSD - Bat 440 - CRJ - INRA
F-78352 Cedex Jouy-en-Josas
fons@biotec.jouy.inra.fr

Francis, Gillian
University of Limerick, Food Science Research Centre,
Life Sciences Dept.
University of Limerick
IRL-Limerick Ireland
jillian.francis@ul.ie

Franke, K.
DIL, Verfahrenstechnik/Lebensmitteltechnologie
Prof.-von-Klitzing-Str. 7
D-49610 Quakenbrück

Fretzdorff, Barbara
BAGFK, Institut für Biochemie von Getreide und
Kartoffeln
Postfach 1354
D-32703 Detmold
bagkf@t-online.de

Frfr. von Cramm, Dagmar
Freie Journalistin
D-Freiburg

Friis, Alan
Department of Biotechnology, Technical University
of Denmark
DTU, Building 221
Dk-2800 Lyngby
af@ibt.dtu.dk

Führ, Arndt
Qualicon Europe LTD.
Eichstraße 6a
D-67434 Neustadt
arnst.fuer@t-online.de

Fürst, Peter
Universität Hohenheim, Inst. für Biolog. Chemie u.
Ernährungswissenschaften
Garbenstraße 30
D-70599 Stuttgart
langb140@rs1.rz.uni-hohenheim.de

Gaiser, Markus
Department of Food Engineering, University College
Cork (UCC)
IRL-Cork
m.gaiser@ucc.ie

Gaitis, Fragiskos
ETAT S.A:, Greek Focal Point
80 Vouliagmenis Ave.
GR-11743 Athens
etatinfo@hol.gr

Gänsbauer, Heike
Central Laboratories Friedrichsdorf, of the Numico
Company
Bahnstr. 14-30
D-61381 Friedrichsdorf

Gareis, Manfred
Bundesanstalt für Fleischforschung BAFF, Institut für
Mikrobiologie und Toxikologie
E.-C.-Baumann-Straße 20
D-95326 Kulmbach
baff@compuserve.com

Gärtner, Uta
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
uta.gaertner@bfe.uni-karlsruhe.de

Gaßmann, Berthold
Deutsches Institut für Ernährungsforschung, DIfE
Jean-Paul-Str. 12
D-4558 Bergholz-Rehbrücke

Gaukel, Volker
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
volker.gaukel@bfe.uni-karlsruhe.de

Gebhardt, Erich
IGV Institut für Getreideverarbeitung GmbH
Arthur-Scheunert-Allee 40/41
D-14558 Bergholz-Rehbrücke
igv-regrow@geb1.ins-coin.de

Geis, Arnold
Bundesanstalt für Milchforschung, Institut für
Mikrobiologie
Hermann-Weigmann-Straße 1
D-24103 Kiel

Geisen, Rolf
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
rolf.geisen@bfe.uni-karlsruhe.de

Gerhards, Christian
National Starch & Chemical
Friedenstr. 10
D-76 133 Karlsruhe
christian.gerhards@nstarch.com

Gerhäuser, Clarissa
Deutsches Krebsforschungszentrum
Im Neuenheimer Feld 280
D-69120 Heidelberg
c.gerhauser@dkfz-heidelberg.de

Gibis, Monika
Universität Hohenheim, Institut für Lebensmitteltechn.,
Fach. Fleischtechn.
Garbenstr. 25
D-70599 Stuttgart
gibis@uni-hohenheim.de

Gierschner, Karlheinz
Universität Hohenheim
Äußere Lochern 10
D-72501 Gammertingen

Gnauck, Guenter
Bestfoods Europe Euro R & D Center, Nutrition and
Safety
Knorrstr. 1
D-74074 Heilbronn

Goenaga, Xabier
European Commission, Directorate-General XII
SDME 8/12 Rue de la Loi 200
B-1049 Brussel
Xabier.Goenaga-Beldarrain@dg12.cec.be

Goli, Thierry
CIRAD
F-34032 Montpellier
thierry.goli@cirad.fr

Gómez Telletxea, Alberto
Departamento de Marketing, AZTI - Fisheries and
Food Technologycal Institute
Isla de Txatxarramendi s/n
E-48395 SUKARRIETA (Bizkaia)
agomez@sg.azti.es

Gonzalez, Remedios
Universitat de Valencia
A. Blasco Ibañez 21
E-46010 Valencia
gonzalrb@uv.es

Gonzalez Martinez, Chelo
Polytechnic University
P.O. Box 22012
E-46071 Valencia
cgonza@tal.upv.es

Goodacre, Christina
Ministry of Agriculture, Fisheries and Food (MAFF),
Room 641, St. Christopher House
80-112 Southwark Street
GB-London SE1 0UD
c.goodacre@aftd.maff.gov.uk

Gormley, T.R.
Teagasc, National Food Centre
Dunsinea Castleknock
IRL-Dublin 15
p.moriarty@nfc.teagasc.ie

Gorris, L.G.M.
Unilever Research Vlaardingen
Oliver van Noortlaan 120
NL-3133 AT Vlaardingen

Graßhoff, A.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel
grsshoff@bafm.de

Gries, Arnold
Diabetes Forschungsinstitut, Heinrich Heine
Universität
D-Düsseldorf

Grunert, Klaus G.
MAPP-Centre
Haslegaardsvej 10
DK-8210 Aarhus V
klaus.grunert@mar.hha.dk

Grünewald-Funk, Dorle
aid - Verbraucherdienst
Konstantinstr. 124
D-53179 Bonn

Grupe, Helga
Monsanto GmbH
Immermannstr. 3
D-40210 Düsseldorf
helga.grupe@monsanto.com

Grupp, Hariolf
Fraunhofer-Institut für Systemtechnik, und
Innovationsforschung
Breslauer Str. 48
D-76139 Karlsruhe

Güell, Carme
University Rovira i Virgili
Ramon y Cajal 70
E-43005 Tarragona
cguell@etse.urv.es

Guerzoni, M. Elisabetta
University di Bologna, Dipart. di Protezione e
Valorizzazione Agroalimentare
Via Giacomo 13
I-40126 Bologna

Guillard, Anne-Sophie
CTSCCV, R&D
7, av. Gal de Gaulle
F-94700 Maisons-Alfort
guillard@vet-alfort.fr

Gusko, Michael
VK Mühlen Food Service GmbH
Trettaustrasse 49
D-21107 Hamburg
Michael.Gusko@t-online.de

Haase, N. U.
Bundesanstalt für Getreide-, Kartoffel-, und
Fettforschung (BAGFK)
Postfach 1354
D-32703 Detmold
bagkf@t-online.de

Haberer, Petra
Bundesforschungsanstalt für Ernährung, IHT
FZK, Bau 325
D-76344 Eggenstein-Leopoldshafen
petra.haberer@bfe.fzk.de

Haendler, Hans
IMC GmbH, Industrial & Management Consultant
Hochstr. 64
B-4700 Eupen

Hagedoorn, Leo-Frans
European Commission GD III
B-1049 Brussels
leo-frans.hagedoorn@dg3.cec.be

Hahn, G.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Hald, Thorsten
DANSKE SLAGTERIER
Axeltorv 3
DK-1609 Copenhagen V
toh@ds-data.dk

Hammer, P.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Hankus, Maria
Warsaw Agricultural University, Dept. of Food
Engineering
ul. Nowoursynowska 166
PL-02-787 Warszawa
hankus@alpha.sggw.waw.pl

Hanley, A. Bryan
CSL Food Science Laboratory, Norwich Research
Park, Colney
GB-Norwich, Norfolk NR4 7UQ
bhanley@csl.gov.uk

Hansen, Bruno
Europäische Kommission, FTE - Maßnahmen
Biowissenschaften und Technologie
Rue Montoyer 75
B-1040 Brüssel

Hapke, Hans-Jürgen
Institut für Pharmakologie und Toxikologie,
Hochschule Hannover
D-Hannover

Harrington, Maria
Faculty of Food Science and Technology, University
College
IRL-Cork
fcis@ucc.il

Hartwig,
Universität Karlsruhe, Institut für Lebensmittelchemie
D-76131 Karlsruhe

Hasch, Markus
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
hasch@bfe.uni-karlsruhe.de

Hedinspottir, Ragnheidur
Federation of Icelandic Industries
P.O. Box 1450
ICE-121 Reykjavik
ragnheidur@si.is

Heil, Monika
Nestlé Deutschland
Lyonerstr. 23
D-60523 Frankfurt
monika.heil@de.nestle.com

Heilgeist, M.
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Heisterüber, Dominik
Franz Zentis GmbH
Jülicher Straße 177
D-52070 Aachen
Heisterueber@t-online.de

Hendrickx, M.
Catholic University of Leuven, Laboratory of Food
Technology
Kardinaal Mercierlaan 92
B-3001 Heverlee
Marc.Hendrickx@Agr.KULeuven.ac.be

Herlihy, Denis
Waterford Food Unit 14,The Friary
Main Street
IRL-Dungarvan Waterford

Hernandez-Herrero, Manuela
Universitat Autonoma de Barcelona, Higiene e
inspección de los alimentos
Facultad de Veterinaria
E-08193 Bellaterra
IVHI2@cc.aub.es

Herrera, Juan
Instituto Investigaciones Marinas (CSIC)
Eduardo Cabello, 6
E-36208 Vigo
juan@iim.csic.es

Herrewegh, Arnold
NIZO Food Research
P.O. Box 20
NL-6710 BA Ede
herrewegh@nizo.nl

Hirvi, Timo
National Veterinary and Food Research Institute
P.O. Box 368
FIN-00231 Helsinki
timo.hirvi@eela.fi

Hoffmann, Norbert
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
hoffmann@bfe.uni-karlsruhe.de

Hoffmann, W.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Hofmann, Klaus
Bundesanstalt für Fleischforschung BAFF, Institut für
Chemie und Physik
E.-C.-Baumann-Straße 20
D-95326 Kulmbach

Holm, Finn
FoodGroup Denmark
Gustav Wieds Vej 10
DK-8000 Aarhus C
fh@foodgroup.sp-aarhus.dk

Holst, Birgit
Technical Univ. Berlin
Königin-Luise-Str. 22
D-14195 Berlin
hobi1531@mailszrz.zrz.tu-berlin.de

Holzapfel, W.H.
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Honikel, Karl-Otto
Bundesanstalt für Fleischforschung, Institut für
Chemie und Physik
E.-C.- Bamann-Straße 20
D-95326 Kulmbach
kohonikel.baff@t-online.de

Honkanen-Buzalski, Tuula
National Veterinary and Food Research Institute
P.O. Box 368
FIN-00231 Helsinki
tuula.honkanen-buzalski@eela.fi

Hopia, Anu
University of Helsinki
PO Box 27
FIN-00014 Helsinki
anu.hopia@helsinki.fi

Hornig, Dietrich
F. Hoffmann - Le Roche Ltd.
CH-4070 Basel

Hortynski, Jerzy
Akademia Rolnicza
ul. Akademicka 13
PL-20 950 Lublin

Huber, Harry
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Incze, Kalman
Hungarian Meat Research Institute
Gubacsi út 6/b.
H-1097 Budapest

Isengard, Heinz- Dieter
Universität Hohenheim, Institut für
Lebensmitteltechnologie
Garbenstraße 25
D-70593 Stuttgart
isengard@uni-hohenheim.de

Jacobs, E.
Institut für Lebensmittelchemie, Universität Karlsruhe
Kaiserstraße 12
D-76128 Karlsruhe

Jagla, A.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Jähde, Constance
Bundesanstalt für Getreide-, Kartoffel-, und
Fettforschung (BAGFK)
Postfach 1354
D-32703 Detmold
bagkf@t-online.de

Jahn, Carsten
Universität Hohenheim, Institut für Lebensmittelchemie
Garbenstr. 28
D-70599 Stuttgart

Jahreis, Gerhard
Institut für Ernährung und Umwelt, Friedrich Schiller
Universität
D-Jena

James, S.
University of Bristol, Food Refrigeration and Process
Eng. Research Centre
Churchill Building
GB-BS18 7 DY Langford-Bristol
frperc-bris@bristol.ac.uk

Jamieson, Linda
EA Technology Ltd.
GB-Capenhurst Chester CH1 6ES
lij@eatl.co.uk

Janse, Lisette
Eureka Office, Project officer at the Eureka Office
Grote-Marktstr. 43
Postbus 30 732
NL-2500 GS Den Haag
l.janse@minez.nl

Jansen, G.J.
Department of Medical Microbiology, University of Groningen
Hanzeplein 1, entrance 21, 2nd floor
NL-9700 RB Groningen
g.j.jansen@med.rug.nl

Jany, Klaus-Dieter
Bundesforschungsanstalt für Ernährung
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Jaworski, Andreas
Inst. Nut. Sci., Dept. Food Sci., JLU Giessen
Südanlage 6
D-35390 Giessen
Andreas.J.Jaworski@ernaehrung.uni-giessen.de

Jirikovsky, Sabine
Chair f. Brewery Installations, and Food Packaging Technology
Weihenstephaner Steig 22
D-85350 Freising-Weihenstephan
Sabine.Jirikovsky@bl.tum.de

Johansson, Maria
Swedish Meat Research Institute
P.O. Box 504
S-244 24 Kävlinge
maria.johansson@ki.scan.se

Jones-Celik, Patricia
Bundesforschungsanstalt für Ernährung, IEP
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Jongen, Wim
Wageningen Research Center, Agricultural University
Bomenweg 2
NL-6703 HD Wageningen
wim.jongen@algemeen.glf.wau.nl

Jost, Gerda
Milupa GmbH & Co KG
Bahnstr. 14-30
D-61381 Friedrichsdorf

Kadlec, Pavel
Institute of Chemical Technology
Technicka 3
CZ-16628 Prague 6
pavel.kadlec@vscht.cz

Kalisch, Christoph
Bundesforschungsanstalt für Ernährung, INF
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Kaminski, Eugeniusz
Engineering Agricultural University of Wroclaw, Institute of Agricultural
ul. Chelmonskiego 37/41
PL- 51-630 Wroclaw
kaminski@imr.ar.wroc.pl

Karg, Georg
TU Weihenstephan, Lehrstuhl für Wirtschaftslehre des Haushalts
Weihenstephaner Steig 17
D-85350 Freising-Weihenstephan

Karjalainen, Tuomo
Univ. Paris-Sud, Fac Pharmacy, Dept Microbiology
5, rue JB Clément
F-92296 Châtenay-Malabry Cedex
tuomo.karjalainen@cep.u-psud.fr

Karl, Andrea
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Karl, Horst
Bundesforschungsanstalt für Fischerei, Institut für Biochemie und Technologie
Palmaille 9
D-22767 Hamburg
karl.ibt@bfa-fisch.de

Käse, Mario
Universität Bremen
Leobener Str. / UFT
D-28359 Bremen
mk@uni-bremen.de

Kasper, Heinrich
Med. Universitätsklinik
Josef-Schmeisler-Str. 2
D-97080 Würzburg

Kast, Ute
Lidl Fleischwerk GmbH & Co KG, Qualitätssicherung
Grundäckerstraße 20
D-74078 Neckarsulm

Katakis, Ioanis
University Rovira I Virgili, Escola Tecn. Superior d´Enginyeria Quinyca
Carretera de Salok s/n
E-43006 Tarragona
ikatakis@etseq.urv.es

Katona, Eva
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Katzek, Jens A.
TH Darmstadt, Institut für Biochemie
Petersenstr. 22
D-64287 Darmstadt
J.Katzek@kws.de

Kearney, Mary
Unilever Research Vlaardingen
P.O. Box 114
NL-3130 Vlaardingen

Keim, Susanne
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Kennedy, Christopher
University of Leeds, Proctor Dept. of Food Science
GB-Leeds LS2 9JT
C.J.Kennedy@food.leeds.ac.uk

Kerry, John Francis
Food Technology Dept., University College Cork
Cork, Ireland
uccdft@indigo.ie

Kiely, Barry
National Food Biotechnology Centre, University
College Cork
Western Road
IRL-Cork
NFBC@.ucc.ie

Kiiyukia, Ciira
Jomo Kenyatta University of Agriculture
P.O. Box 62000
Nairobi, Kenya
fsptjku@arcc.or.ke

Kimmich, Reinhard
Hochschule für Technik und Wirtschaft
Anton-Günther-Str.51
D-72488 Sigmaringen

Kinkelin, Thomas
Schafft Fleischwerke Ansbach
Eyber Str. 81
D-91522 Ansbach
thomas.kinkelin@unilever.com

Kirschbaum, Jochen
Justus-Liebig University of Giessen
Südanlage 6
D-35390 Giessen
Jochen.Kirschbaum@ernaehrung.uni-giessen.de

Kiss, I.F.
University of Horticulture and Food Industry, Dept. of
Refrigeration and Livestock Products Technology
Menesi Üt 45
H-1118 Budapest

Klein, Michael
European Commission, Directorate-General XII
Rue de la Loi/Westraat 200
B-1049 Brüssel
michael.klein@dg12.cec.be

Kloos, Kurt
GNT
Kackert-Str.22
D-52072 Aachen

Kluza, Franciszek
Agricultural University of Lublin, Department of
Refrigeration & Drying
Ul.Doswiadczalna 44
PL-20236 Lublin

Knasmüller, Siegfried
Universität Wien, Institut für Tumorbiologie-
Krebsforschung
Borschkegasse 8a
A-1090 Vienna
siegfried.knassmuller@univie.ac.at

Knoblauch, Rainer
LUFA Augustenberg
Neßlerstraße 23
D-76227 Karlsruhe

Knorr, Dietrich
TU Berlin, Lebensmitteltechnologie
Königin-Luise-Str. 22
D-14195 Berlin
foodtech@mailszrz.zrz.tu-berlin.de

Knörr, Michael
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Knowles, Michael E.
CocaCola Greater Europe
1424, Chaussee de Mons
B-1070 Brussels

Koaze, H.
Japan International Cooperation Agency, JKUAT
Project
P.O. Box 62000
Nairobi, Kenya
fsptjku@arcc.or.ke

Kobayashi, Toshio
Faculty of Eng., Soka Univ.,, 1-236, Tanki-machi,
192-8577,Hachiouji-shi,Tokyo
Japan
<tkobayas@t.soka.ac.jp>/<toskoba@ibm.net>

Kofoth, Christina
Bundesanstalt für Fleischforschung BAFF, Institut für
Mikrobiologie und Toxikologie
E.-C.-Baumann-Straße 20
D-95326 Kulmbach
100440.72@compuserve.com

Kolanowski, Wojciech
Warsaw Agricultural University - SGGW, Faculty of
Human Nutr.
ul. Nowoursynowska 166
PL-02-760 Warsaw
kolanowski@alpha.sggw.waw.pl

Koller, Wolf-Dietrich
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

König,
Geschäftsstelle für Internationale Agrarforschung,
c/o ZADI
Villichgasse 17
D-53177 Bonn

Körmendy, Imre
University of Horticulture and Food Industry, Dept. of
canning technology
Ménesi u. 45.
H-1118 Budapest
MESZAROSL@HOYA.KEE.HU

Korn, L.
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Korteniemi, Veli-Markku
Aromtech Ltd.
P.O. Box 221
FIN-95401 Tornio
korteniemi@lapinnova.fi

Kostaropoulos, A.E.
E. Venizelou Str. 50
GR-15237 Filothei - Athen
ak@auadec.aua.ariadne-t.gr

Kotlowski, Roman
Technical University of Gdansk, Department of Food
Preservation
ul. Gabriela Narutowicza 11/12
PL-80-952 Gdansk
romank@altis.chem.pg.gda.pl

Kottke, V.
Universität Hohenheim, Inst. f.
Lebensmitteltechnologie (150)
Garbenstraße 25
D-70593 Stuttgart

Koufokotsiou, Lina
MEVGAL S.A.
Koufalia
GR-57100 Thessaloniki
lina@mevgal.gr

Kramkowski, Ryszard
Engineering Agricultural University of Wroclaw,
Institute of Agricultural
ul. Chelmonskiego 37/41
PL- 51-630 Wroclaw
kaminski@imr.ar.wroc.pl

Kristanti, Indah
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
indah.kristanti@bfe.uni-karlsruhe.de

Kröckel, Lothar
Bundesanstalt für Fleischforschung BAFF, Institut für
Mikrobiologie und Toxikologie
E.-C.-Baumann-Straße 20
D-95326 Kulmbach

Kübel, Jörg
Bundesforschungsanstalt für Ernährung, ICB
Engesserstr. 20
D-76131 Karlsruhe
Peter.Butz@bfe.uni-karlsruhe.de

Kühne, Ditmar
Bundesanstalt für Fleischforschung BAFF, Institut für
Technologie
E.-C.-Baumann-Straße 20
D-95326 Kulmbach
institut_T.baff@t-online.de

Kulling, S.E.
Institut für Lebensmittelchemie, Universität Karlsruhe
Kaiserstraße 12
D-76128 Karlsruhe

Kunz, Benno
Universität Bonn, Inst. of Food Techn.
Römerstr. 164
D-53117 Bonn
b.kunz@uni-bonn.de

Kurz, Sybille
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Lack, Wilhelm Klaus
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
wlack@young-world.com

Laguerre, Onrawee
Cemagref
BP. 44
F-92163 Antony cedex
onrawee.laguerre@cemagref.fr

Laue, Chr.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Le Rohellec, Annabelle
EFFoST
P.O. Box 17
NL-6700 AA Wageningen
effost@ato.dlo.nl

Leach, Steve
CAMR, Porton Down
Salisbury, Wilts
GB-SP4 0JG, Salisbury
steve.leach@camr.org.uk

Leao, Jean-David
T.P.A. sa, Lot Bersol
9 Av de la Madeleine
F-33170 Gradignan
Tpa@wanadoo.fr

Leaper, Su
TQMS Dept., Food Research Association, Campden
& Chorleywood
Chipping Campden
GB-Gloucestershire, GL55 6 LD
s_leaper@campden.co.uk

Lehmann, L.
Institut für Lebensmittelchemie, Universität Karlsruhe
Kaiserstraße 12
D-76128 Karlsruhe

Leist, N.
LUFA Augustenberg
Neßlerstraße 23
D-76227 Karlsruhe

Lelieveld, Huub
Unilever Research Laboratory
P.O. Box 114
NL-3130 AC Vlaardingen
Huub.lelieveld@unilever.com

Lenart, Andrzej
Warsaw Agricultural University, Department of Food
Engineering
ul. Nowoursynowska 166
PL-02-787 Warsaw
A.Lenart@SGGW.WAW.PL

Lenz, Dagmar
Deutsche Gesellschaft für Ernährung e.V., DGE
Im Vogelsang 40
D-60488 Frankfurt am Main

Leonhäuser, Ingrid-Ute
Institut für Ernährungswissenschaften, Justus Liebig
Universität Gießen
D-Gießen

Leschik-Bonnet, Eva
Leiterin Referat Wissenschaft
Deutsche Gesellschaft für Ernährung e.V., DGE
Im Vogelsang 40
D-60488 Frankfurt am Main

Leslie, J.
Unilever Research Laboratory, Colworth House
Sharnbrook
GB-Bedford MK44 1LQ
jim.leslie@inilever.com

Leuschner, Renata
Unilever Research Colworth
Sharnbrook
GB-Bedford MK44 1LQ
Renata.Leuschner@unilever.com

Lewicki, Piotr
Warsaw Agricultural University, Depart. Food
Engineering
ul. Nowoursynowska 166
PL-Warsaw 02 - 766
lewicki@alpha.sggw.waw.pl

Lichtenthaler, H. K.
Universität Karlsruhe (TH), Botanik II
(Pflanzenphysiologie)
Kaiserstraße 12
D-76128 Karlsruhe

Lick, Sonja
Bundesanstalt für Milchforschung, Institut für
Mikrobiologie
Hermann-Weigmann-Str. 1
D-24103 Kiel
lick@batm.de

Liegibel, Ute
Friedrich-Schiller-Universität Jena, Institut für
Ernährung und Umwelt
Dornburger Str. 25
D-07743 Jena
b8liut@rz.uni-jena.de

Lindhauer, Meinolf G.
Bundesanstalt für Getreide-, Kartoffel-, und
Fettforschung (BAGFK)
Postfach 1354 Institut 1
D-32703 Detmold
post.bagkf@t-online.de

Lindner, Annette
Bundesforschungsanstalt für Ernährung, IEP
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Lindsay, David
Institute of Food Research, Norwich Laboratory,
Colney
3, Patcham Grange
GB-Brighton BNI 8 UR
dlindsay@mcmail.com

Lopez, Francisco
University Rovira I Virgili, Faculty Of Enology
Ramon Y Cajal 70
E-43005 Tarragona
flopez@etseq.urv.es

Lorenzen, P.Chr.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Lubecki, Irmhilde
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Luchetti, A.
European Commission
B- Brüssel

Lücke, Friedrich-Karl
Fachhochschule, University of Applied Sciences
Marquardstr. 35
D-36039 Fulda
friedrich-karl.luecke@he.fh-fulda.de

Lückemeyer, M.
Bundesministerium für Ernährung,, Landwirtschaft
und Forsten (BML)
Postfach 14 02 70
D-53107 Bonn

Luttermann-Semmer, E.
Deutsche Gesellschaft für Ernährung
Im Vogelsgesang 40
D-60488 Frankfurt

Maciunska, Jadwiga
Technical University of Gdansk
ul. Narutowicza 11/12
PL-80-952 Gdansk
jagoda@chem.pg.gda.pl

Manneck, Iris
Merck Produkte-, Vertriebsgesellschaft & Co.
Rößlerstraße 96
D-64293 Darmstadt
(daniela.graf@merck.de)

Marazuela, Dolores
Laboratory for Human Nutrition ETH Zürich
Seestrase, 72
CH-8803 Rüschlikon
dolores.marazuela@ilw.agrl.ethz.ch

Marmolle, Frank
CNRS
1 rue Blaise Pascal, BP 296
F-67000 Strasbourg
lpcb4@chimie.u-strasbg.fr

Martens, Toon
ALMA University Restaurants
E. Van Evenstraat 2C
B-3000 Leuven
toon.martens@alma.kuleuven.ac.be

Martin, D.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Martinez, Antonio
CSIC, Centro de Edafologia y Biologia
Apartado de correos 73
E-46100 Burjassot, Valencia
conaml@iata.csic.es

Martinez-Monzó, Javier
Polytechnical University
P.O. Box: 22012
E-46071 Valencia
xmartine@tal.upv.es

Martínez-Navarrete, Nuria
Politechnical University
P.O. Box: 22012
E-46071 Valencia
nmartin@tal.upv.es

Marx, Stefan
Technische Hochschule Aachen, Lehrstuhl für
Biotechnologie, Sammelbau Biologie
Worringer Weg 1
D-52056 Aachen
s.marx@biotec.rwth-aachen.de

Mastrantonio, Paola
Instituto Superiore di Sanita
Viale Regina Elena 299
I-00161 Rom
pmastran@net.iss.it

Materska, Malgorzata
Department of Chemistry, Agricultural Academy
Akademicka str. 15
PL-20-950 Lublin
mpalka@ursus.ar.lublin.pl

Mathieu, Christine
SSTC
RUE DE LA SCIENCE 8
B-1000 BRUXELLES
math@belspo.be

Matraszek, Renate
University of Agriculture, Department of Plant
Physiology
Akademicka Street 15
PL-20-950 Lublin

Matteuzzi, Diego
University of Bologna, Departm. of Pharmaceutical
Sciences
Via Belmeloro 6
I-40126 Bologna
matt@alma.unibo.it

Matthäus, Bertrand
Bundesanstalt für Getreide-, Kartoffel-, und
Fettforschung (BAGFK)
Postfach 1705
D-48006 Münster
aitzetm@uni-muenster.de

Matthies, Kirsten
Verbraucherzentrale Baden-Württemberg e.V.
Paulinenstr. 47
D-70178 Stuttgart
info@verbraucherzentrale.de

Mattila-Sandholm, Tiina
VTT, Biotechnology and Food Research
Tietotie 2, P.O. Box 1501
FIN-02044 VTT
Tiina.Mattila-Sandholm@vtt.fi

Mayer-Miebach, Esther
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Mazur, Witold
University of Helsinki and Folkhälsan Research
Center
Mannerheimintie 97
FIN-00280 Helsinki
witold.mazur@helsinki.fi

Mc Farlane, Maria
AgrEvo UK Ltd.
Chesterford Park
GB-Essex CB10 1XL

Medina, Luis M.
Dept. de Bromat. y Tec. de los Alim., Universidad de
Cordoba
Campus Universitario Rabanales
E-14071 Cordoba
al1mecal@lucano.uco.es

Mehret, Christa
Bundesforschungsanstalt für Ernährung, MBZ
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Menrad, Klaus
Fraunhofer-Institut für Systemtechnik, und
Innovationsforschung
Breslauer Str. 48
D-76139 Karlsruhe
me@isi.fhg.de

Mercier, Christiane
Groupe Danone
7 Rue de Teheran
F-75381 Paris Cedex 08
cmercier@groupe.danone.com

Mészáros, László
Univ. of Horticulture and Food Industry
Ménesi ut 45.
H-1118 Budapest
MESZAROSL@HOYA.KEE.HU

Metzler, Manfred
Institut für Lebensmittelchemie, Universität Karlsruhe
Kaiserstraße 12
D-76128 Karlsruhe
Manfred.Metzler@chemie.uni-karlsruhe.de

Meuser, Friedrich
TU Berlin -Inst. f. Lebensmitteltechn., Fachgebiet
Getreidetechnologie
Seestr. 11
D-13353 Berlin

Meyer, Ulrich J.
Institut für Chemo- und Biosensorik
Mendelstr. 7
D-48149 Münster
meyerul@uni-muenster.de

Michel-Drees, Angelika
Arbeitsgemeinschaft der Verbraucherverbände e.V.
Heilsbachstr. 20
D-53123 Bonn

Midtvedt, Tore
Karolinska Institute, Laboratory of Medical Microbial
Ecology
Doktors Ringen 4A
S-171 77 Stockholm
tore.midtvedt@cmb.ki.se

Miertsch, Heike
TU Dresden, Institute for Food- and Bio-Engineering
Mommsenstr.13
D-01062 Dresden
miertsch@mvll00.mw.tu-dresden.de

Mikos-Bielak, Maria
Department of Chemistry, Agricultural Academy
Akademicka str. 15
PL-20-950 Lublin
mbmaria@ursus.ar.lublin.pl

Millikan, Mary
School of Life Sciences and Technology, Viktoria
University of Technology
P.O. Box 14428
AUS - Melbourne Victoria 8001
Mary.Millikan@vut.edu.au

Min, Sang Gi
Kon-Kuk University
93-1, Mojin-dong, KwangJin-Ku
Seoul, Korea
minsg@kkucc.konkuk.ac.kr

Montini, Anna
The University of Bologna, Dept. of Statistics
"P.Fortunati"
via Belle Arti, 41
I-40100 Bologna
montini@stat.unibo.it

Moreto, Miguel
University of Barcelona, Dept. Fisiologia de
Farmacia
Av. joan XXIII s/u
E-08028 Barcelona
mmoreto@farmacia.far.ub.es

Moritzen, Peter
Kreismedizinaldirektor, Gesundheitsamt
D-Itzehoe

Morozzi, Guido
University of Perugia, Dept. Cell Molec. Biol.
Via del Giochetto
I- 06126 Perugia
fabirob@krenet.it

Moscicki, Leszek
Dept. of Food Process Engineering, Agricultural
University in Lublin
Doswiadczalna 44
PL-20-236 Lublin

Moser, Jörg
Vitaminkraut GmbH
Stubalpenstraße 4
A-8720 Knittelfeld
office@sauerkraut.at

Muhs, Hans-J.
Forst- und Holzwirtschaft, Institut für Forstgenetik
Sieker Landstr. 2
D-22927 Großhandsdorf

Mukherjee, Kumar D.
Institute for Biochemistry and Technology of Lipids,
BAGKF
Piusallee 68
D-48147 Münster
ibtfett@uni-muenster.de

Mulder, R.W.A.W.
DLO - Institute of Animal Science and Health
c/o P.O. Box 65
NL-8200 AB Lelystad
R.W.A.W.Mulder@id.dlo.nl

Mulet, Antonio
Universidad Politecnica de Valencia, Dept. Food
Technology
Camino de Vera S/N
E-46071 Valencia
amulet@tal.upv.es

Müller, Kerstin
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Müller, Klaus
Frauenhofer-Institut Verfahrenstechnik und
Verpackung
Giggenhauser Straße 35
D-85 354 Freising
kom@iw.fhg.de

Münch, S.
Bundesanstalt für Fleischforschung BAFF, Institut für
Chemie und Physik
E.-C.-Baumann-Straße 20
D-95326 Kulmbach
baff@compuserve.com

Münzing, K.
Bundesanstalt für Getreide-, Kartoffel-, und
Fettforschung (BAGFK)
Postfach 1354
D-32703 Detmold
bagkf@t-online.de

Muskat, Erich
Chemiedirektor a.D.
D-Asslar

Neale, Roger John
University of Nottingham, Division of Nutritional
Biochemistry
Sutton Bonington Campus
GB-Loughborough, LE12 5RD
roger.neale@nottingham.ac.uk

Neff, Stefan
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Nesvadba, Paul
The Robert Gordon University, School of Applied Sciences
St Andrew Street
GB-Aberdeen, AB25 1HG
p.nesvadba@rgu.ac.uk

Netzel, Michael
Institut für Ernährungswissenschaft
Wilhelmstr. 20
D-35392 Giessen
michael.e.netzel@ernaehrung.uni-giessen.de

Neuer, Gabriele
Bestfoods Europe Euro R & D
Knorrstraße 1
D-74074 Heilbronn

Neve, Horst
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel
neve@bafm.de

Nguyen-the, C.
INRA, Station de Technologie
Domaine St-Paul, Site Agro Parc
F-84914 Avignon Cedex 9
nguyenth@avignon.inra.fr

Ni Eidhin, Deirdre
University of Limerick, Food Science Research Centre, Life Sciences Dept.
University of Limerick
IRL-Limerick , Ireland
deirdre.nieidhin@ul.ie

Nicolai, Bart
KU Leuven, Dept. of Agro-Engineering and Economies
Kardinaal Mercielaan 92
B-3001 Heverlee

Norin, Elisabeth
Karolinska Institute
Doktorsringen 4a
S-17177 Stockholm
elisabeth.norin@cmb.ki.se

Noteborn, Hubert P.J.M.
RIKILT-DLO, State Institute for Quality Control of Agricultural Products
P.O.Box 230
NL-6700 AE Wageningen
h.p.j.m.noteborn@rikilt.dlo.nl

Nowak, Dorota
Warsaw Agricultural University, Dept. of Food Engineering
ul. Nowoursynowska 166
PL-02-787 Warszawa
nowak@sggw.waw.pl

Nychas, George
Agric. Univ. Athens, Dept. of Agric. Industries-Lab.
Iera Odos 75
GR-11855 Athens
gjn@auadec.-aua-ariadne-t.gr

O'Connor, Jacqueline
Northern Ireland Centre for Diet and Health (NICHE), School of Biomedical Sciences
University of Ulster (UUC)
Coleraine BT52 1SA, Northern Ireland
jm.oconnor@ulst.ac.uk

O´Beirne, David
University of Limerick, Food Science Research Centre, Life Sciences Dept., University of Limerick
IRL-Limerick
David.OBeirne@ul.ie

Oberritter, Helmut
Deutsche Gesellschaft für Ernährung e.V., DGE
Wissenschaftlicher Leiter
Im Vogelsang 40
D-60488 Frankfurt am Main

Ohlsson, Thomas
SIK
Box 5401
S-40229 Göteborg
to@sik.se

Olsson, Pär
SIK
Box 5401
S-402 29 Göteborg

Oltersdorf, U.
Bundesforschungsanstalt für Ernährung, IÖS
Garbenstr.13
D-70599 Stuttgart
bfe@uni-hohenheim.de

Onyango, Christine
Jomo Kenyatta University of Agr.
P.O. Box 62000
Nairobi, Kenya
fsptjku@arcc.or.ke

Opitz, Barbara
Universität Karlsruhe, Institut für Lebensmittelverfahrenstechnik
Kaiserstr. 12
D-76128 Karlsruhe
barbara.opitz@ciw.uni-karlsruhe.de

Orpianesi, Carla
Dipartimento di Scienze Igienistiche, Universita degli Studi di Camerino
Viale E.Betti 3
I-62032 Camerino
cresci@camserv.unicam.it

Ortolani, Claudio
Dpt. di Allergia e Immunologia, Clinica-Divisione
Bizzozzero
Piazza Ospedale Maggiore 3
I-20162 Milano
ortolani@stnet.net

Ostermann, Albrecht E.
SIG Combitec Forschung und Entwicklung GmbH
Grüner Weg 19
D- Erkelenz

Otto, Marion
Schumann & Sohn GmbH
Degenfeldstraße 4
D-76131 Karlsruhe

Palacha, Zbigniew
Warsaw Agricultural University, Depart. of Food
Engineering
ul. Nowoursynowska 166
PL-02-787 Warszawa
palacha@alpha.sggw.waw.pl

Palenzona, Domenico
Universita' degli Studi di Bologna, Dept. of
Evolutionary Biology / Laboratory of
Via Selmi 2
I-40126 Bologna
paledom@kaiser.alma.unibo.it

Park, Kwan Hwa
Seoul National University, Research Center for New
Bio-Materials
for New Bio-Materials in Agriculture
441-744 Suwon, Korea
parkkh@plaza.snu.ac.kr

Pastoriza, Laura
Marine Research Institute (CSIC), Instituto de
Investigaciones Marinas
Eduardo Cabello 6
E-36208 VIGO
laura@nautilus.iim.csic.es

Patel, Pradip
Leatherhead Food RA, Rapid Methods
Randall Roads
GB-Surrey KT 22 7 RY
ppatel@lfra.co.uk

Paulsen, R.
Universität Karlsruhe, Zoologisches Institut,
Lehrstuhl I
Kornblumenstr. 13, Postfach 69 80
D-76128 Karlsruhe

Paulus, Klaus O.
Sensor Marketing + Research
Oberstr. 11 a
D-64297 Darmstadt

Pazir, Fikret
Ege University Engineering Faculty, Food Engineering
Department
TUR-35100 Bornova-Izmir
pazir@textile.ege.edu.tr

Pérez-Villarreal, Begona
AZTI- Fisherie and Food Technological Institute
Txatxarramendi ugartea z/g
E-48395 Sukarrieta
bego@ta.azti.es

Persin, Christoph
Yakult Deutschland GmbH
Zeppelinstraße 9
D-50667 Köln

Petrohilou, Ionna
ETAT S.A:, Greek Focal Point
80 Vouliagmenis Ave.
GR-11743 Athens
etatinfo@hol.gr

Petry,
Bundesministerium für Gesundheit
D-Bonn

Pfaff, Eberhard
Bundesforschungsanstalt für Viruskrankheiten der Tiere
Paul-Ehrlich-Straße 28
D-72076 Tübingen

Pfau, Cornelie
Bundesforschungsanstalt für Ernährung, IÖS
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Pfeiffer, E.
Institut für Lebensmittelchemie, Universität Karlsruhe
Kaiserstraße 12
D-76128 Karlsruhe

Pichner, Rohtraud
Bundesanstalt für Fleischforschung, Institut für
Mikrobiologie und Toxikologie
E.-C.- Baumann-Str. 20
D-95326 Kulmbach
100440.72@compuserve.com

Piironen, Vieno
University of Helsinki, Dept. Applied Chemistry and
Microbiology
P.O. Box 27
FIN-00014 Helsinki
Vieno.Piironen@helsinki.fi

Pool-Zobel, Beatrice
Universität Jena, Ernährung und Umwelt
Dornburger Str.25/27
D-07743 Jena
b8pobe@rz.uni-jena.de

Pour Nikfardjam, Martin
Forschungsanstalt, Fachgebiet Weinanalytik u.
Getränkeforschung
Rüdesheimer Str. 28
D-65366 Geisenheim

Prasch, Albert
Langnese - Iglo GmbH
Lüther Weg 50
D-31515 Wunstorf
albert.prasch@langnese-iglo.de

Praznik, Werner
Institute for Agrobiotechnology
Konrad Lorenz Str. 20
A-3430 Tulln
praznik@ifa-tulln.ac.at

Precht, D.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Pudel, Volker
Ernährungspsychologische Forschungsstelle,
Universität Göttingen
D-Göttingen

Pugh, Stephen
Ministry of Agriculture Fisheries & Food, Rm 663 St.
Christopher House
Southwack Street
GB-London SE 1 OUP
s.pugh@fvsd.maff.gov.uk

Purkarthofer, Erich
Büro für Intern. Forschungs- u.
Technologiekooperation, BIT
Wiedner Hauptstr. 76
A-1040 Vienna
purkarthofer@bitserver.bit.ac.at

Quisthoudt-Rowohl, Godelieve
European Parliament
Goschenstr. 80
D-31134 Hildesheim
gquisthoudt@europarl.eu.int

Ramesh, M.N.
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Range, Peter
Landesanstalt für Pflanzenbau Forchheim
D-76287 Rheinstetten

Rathjen, Axel
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Rathmann, Kersten
Markant Wasgau Fleisch & Wurst GmbH
Rheinstr. 27
D-66955 Pirmasens

Raunhardt, Otto
F. Hoffmann-La Roche AG, VML, B. 241/815
Postfach
CH-4070 Basel
otto.raunhardt@roche.com

Rechkemmer, Gerhard
Bundesforschungsanstalt für Ernährung, IEP
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Reig, Milagro
Inst. de agroquimica y tecnologia de alimentos (CSIC),
IATA-Lab. carne
Apt. 73
E-46100 Burjassot, Valencia
carmrr@iata.csic.es

Reimerdes, Ernst H.
Nestec LTD, Technology Transfer
Avenue Nestlé 55
CH-1800 Vevey

Renerre, Michel
INRA, Station Nationale de la Recherches sur la Viande
Theix
F-63122 Saint-Genes-Champanelle

Resch, Katja
Bundesforschungsanstalt für Ernährung, ICB
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Reynolds, Catherine
Institute of Food Research, Norwich Research Park
Colney
GB-Norwich NR4 7UA
catherine.reynolds@bbsrc.ac.uk

Rhoades, Jonathan
South Bank University, School of Applied Science
103 Borough Road
GB-London SE1 0AA
rhoadejr@sbu.ac.uk

Richardson, Philip
CCFRA, Campden & Chorleywood Food Res. Assoc.
Stadion Road
GB-GL55 6LD Chipping Campden
richardson@campden.co.uk

Richmond, Peter
Department of Physics
Trinity College
IRL-Dublin 2
richmond@sys.uea.ac.uk

Rissler, Katharina
Gene-Scan gmbH
Bötzinger Str. 29a
D-79111 Freiburg
info@genescan.com

Risvik, Einar
Matforsk,, Norwegian Food Research Institute
Osloveien 1
N-1430-AS
liv.bakke@matforsk.no

Ritter, Wolfgang
Geschäftsstelle für Internationale Agrarforschung,
c/o ZADI
Villichgasse 17
D-53177 Bonn
ritter@zadi.de

Roberts, T.A.
59 Edenham Crescent
GB-Reading RG1 6HU

Rodbotten, Rune
MATFORSK
Osloveien 1
N-1430 Ås
rune.rodbotten@matfors.no

Rodis, Panayotis
Dept of Food Sciences & Technology, Agicultural
University of Athens
Irea Odos 75, Votanikos
GR-118 55 Athens
prodis@auadec.aua.gr

Rodrigo, Miguel
CSIC, Agrochemistry & Food Research Institute
Apartado de Correos 73
P.O. Box 73
E-46100 Burjassot, Valencia
mrodigo@iata.csic.es

Röger, Barbara
Institut für Gemüse- und Zierpflanzenbau,
Großbeeren/ Erfurt e.V.
Theodor-Echtermeyer-Weg
D-14 979 Großbeeren

Roller, Sibel
South Bank University, School of Applied Science
103 Borough Road
GB-London SE1 0AA
rollers@sbu.ac.uk

Romond, Marie-Bénédicte
Université de Lille 2
rue Laguesse BP 83
F-59006 Lille
mromond@phare.univ-lille2.fr

Roos, N.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Rosenbauer, Heiko
Bundesanstalt für Fleischforschung BAFF, Institut für
Chemie und Physik
E.-C.-Baumann-Straße 20
D-95326 Kulmbach

Rössner, Martin
Bäuerliche Erzeugergemeinschaft Schwäbisch Hall
Hallerstr. 20
D-74549 Wolpertshausen

Rottka, Helmut
Institut für Pharmazie, Freie Universität Berlin
D-Berlin

Rowland, Ian
University of Ulster, Northern Ireland Centre for Diet
and Health
NIRL-Coleraine, BT52 1SA
I.Rowland@ulst.ac.uk

Rudzinska, Barbara
Department of Chemistry, Agricultural Academy
Akademicka str. 15
PL-20-950 Lublin
barbara@ursus.ar.lublin.pl

Ryhänen, Eeva-Liisa
Agricultural Research Centre of Finland
FIN-31600 Jokioinen
eeva.liisa.ryhanen@mtt.fi

Sagoo, Satnam
South Bank University, School of Applied Science
103 Borough Road
GB-London SE1 0AA
Sagooska@sbu.ac.uk

Sandström, Brittmarie
Royal Veterinary&Agricultural Univers., Research
Departm. of Human Nutrition
Rolighedsvej 30
DK-1958 Frederiksberg C
BrittMarie.Sandstroem@fhe.kvl.dk

Saupe, Sybille
Bundesforschungsanstalt für Ernährung, INF
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Schäfer, Heike
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Scherer, Reinhold
FH Fulda, FB Lebensmitteltechnologie
Marquardstr. 35
D-36039 Fulda

Schick, R.
Langnese - IGLO
Aeckern 1
D-48734 Reken

Schick, R.
Bundeswehrkrankenhaus
D-Ulm

Schillinger, U.
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Schillo, Markus
Universität Karlsruhe, Zoologisches Institut 1
Kornblumenstr. 13
D-76128 Karlsruhe
dc02@rz.uni-karlsruhe.de

Schindler, H.-J.
Hirschstraße 105
D-76131 Karlsruhe

Schlemmer, U.
Bundesforschungsanstalt für Ernährung, Institut für
Ernährungsphysiologie
Engesserstr. 20
D-76131 Karlsruhe

Schmidt, Karsten
University of Karlsruhe, Institut für MVM
Kaiserstr. 12
D-76128 Karlsruhe
Karsten.Schmidt@ciw.uni-karlsruhe.de

Schmitt, Joachim J.
Milupa GmbH & Co. KG
Bahnstr. 14-30
D-61381 Friedrichsdorf

Schoch, Alexander
Universität Hohenheim, Institute of Food Technology
Garbenstr. 25
D-70599 Stuttgart
schoch@uni-hohenheim.de

Schöch, Gerhard
Direktor des Forschungsinstituts für Kinderernährung
D-Dortmund

Schöffler, Joachim
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Scholz-Ahrens, Katharina E.
Bundesanstalt für Milchforschung, Institut
fürPhysiologie und Biochemie der Ernährung
Hermann-Weigmann-Str. 1
D-24103 Kiel
scholz-ahrens@bafm.de

Schonhof, Ilona
Institut für Gemüse- und Zierpflanzenbau,
Großbeeren/ Erfurt e.V.
Theodor-Echtermeyer-Weg
D-14 979 Großbeeren
igzer@t-online.de

Schrezenmeir, J.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Schröder, Volker
Universität Karlsruhe, Institut für
Lebensmittelverfahrenstechnik
Kaiserstr. 12
D-76128 Karlsruhe
volker.schroeder@ciw.uni-karlsruhe.de

Schubert, Helmar
University of Karlsruhe
Kaiserstr. 90
D-76128 Karlsruhe
Helmar.Schubert@ciw.uni-karlsruhe.de

Schulz, Simone
Universität Karlsruhe, Zoologisches Institut, Lehrstuhl I
Kornblumenstr. 13, Postfach 69 80
D-76128 Karlsruhe

Schwack, Wolfgang
Universität Hohenheim, Institut für Lebensmittelchemie
Garbenstrasse 28
D-70599 Stuttgart
wschwacxk@uni-hohenheim.de

Schwägele, Fredi
Bundesanstalt für Fleischforschung, Institut für Chemie
und Physik
E.-C.- Baumann-Str. 20
D-95326 Kulmbach
baff@compuserve.com

Schweigert, Florian J.
Institut für Ernährungswissenschaft
Arthur-Scheunert-Allee 114-116
D-14558 Bergholz-Rehbrücke
fjschwei@www.dife.de

Schwertfeger, Marcus
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel
schwertfeger@bafm.de

Sereno, Alberto M.C.
University of Porto, Faculty of Engineering, Dept.
Chemical Engineering
Rua Bragas
P-4099 Porto / Codex
sereno@fe.up.pt

Sgorbati, Barbara
University Dep. Scienze e Tecnologie, Microbiology
Via Filippo Re 6
I-40126 Bologna

Sharp, Richard
CAMR, Porton Down
Salisbury, Wilts
GB-SP4 0JG, Salisbury
richard.sharp@camr.org.uk

Shemer, Michael
Tivall (1993) LTD., Kibbutz Lochamei Hagetaot
Il-M.P. Galil Maaravi 25220
Haifa, Israel
shemer@tivall.co.il

Sibakov, Mervi
Technology Development Centre, Finland
P.O. Box 69
FIN-00101 Helsinki
mervi.sibakov@tekes.fi

Sijtsema, Siet
LEI - DLO
P.O. Box 29703
NL-2502 LS, The Hague
s.j.sijtsema@lei.dlo.nl

Sirin, Cemile
University of Bielefeld, Department of Biology
Section of Plant morphology W5-108
D-33615 Bielefeld

Sjöberg, Anna-Maija
VTT, Biotechnology and Food Research
P.O. Box 1501
FIN-020HH VTT, Espoo
anna-maija.sjoberg@vtt.fi

Sjöholm, Ingegerd
Food Engineering Dep., Lund University
P.O. Box 124
S-221 00 Lund
Ingegerd.Sjöholm@livstek.lth.se

Skibsted, Leif
The Royal Veterinary and Agricultural University,
Dept. of Dairy + Food Science
Thorvaldsensvej 40
DK-1871 Frederiksberg C
Leif.Skibsted@mli.kvl.dk

Skjöldebrand, Christina
SIK-The Swedish Institute for food and biotechnology
AB, Box 5401
S-402 29 Göteborg, Sweden
cs@sik.se

Smid, Eddy J.
Agrotechnological Research Institute, ATO-DLO
P.O.Box 17
NL-6700 AA Wageningen
E.J.Smid@ATO.DLO.NL

Smith, Tony
Department of Agriculture and Food, Agriculture
House
IRL-Dublin 2
tony.smith@daff.irlgov.ie

Soerflaten, Egil
European Commission, DG 12
Rue de la Loi/Westraat 200
B-1049 Brussel

Sölter, Karl-Heinz
Verwaltungsleiter der DGE
D-Frankfurt am Main

Somogyi, Zoltán
OMFB,Governmental R.
Szervita tér 8.
H-1052 Budapest
zoltan.somogyi@omfb.x400gw.itb.hu

Sontag, Gerhard
Universität Wien, Insitut für Analytische Chemie
Waehringerstr. 38
A-1090 Wien
gerhard.sontag@univie.ac.at

Southon, Susan
Institute of Food Research, Norwich Laboratory
Norwich Research Park, Colney
GB-Norwich NR4 7UA
sue.southon@bbsrc.ac.uk

Spamer-Riether, Petra
WDR - Fernsehen
Kopfelweg 45/1
D-69118 Heidelberg

Spieß, C. Katharina
Prognos AG-Basel,Berlin
Dove Str.
D-Berlin

Spieß, Markus
Universität Heidelberg
D-69121 Heidelberg

Spieß, W.E.L.
Bundesforschungsanstalt f. Ernährung, Institut für
Verfahrenstechnik
Engesserstrasse 20
D-76131 Karlsruhe 1

Spring, José. A.
16 Spencer Court
Spencer Road
GB-London, SW 20 0QW, UK
jspring.a0106755@infotrade.co.uk

Stacey, Julia
MAPP-Aarhus, School of Business
Haslegaardsvej 10
DK-8210 Aarhus V
julia.stacey@mar.hha.dk

Steenkamp, J.B.
KUL, Dept. of Marketing
Naamsestr. 69
B-3000 Leuven
Jan-Benedict.steenkamp@econ.kuleuven.ac.be

Stefansson, Gudmundur
Icelandic Fisheries Laboratories
P.O. Box 1405, Skulagata 4
ICE-121 Reykjavik
gst@rfisk.is

Stegeman, Dick
ATO-DLO
P.O. Box 17
NL-6700 AA Wageningen
d.stegeman@ato.dlo.nl

Stehle, Peter
Universität Bonn, Institut für
Ernährungswissenschaft
Edenicher Allee 11-13
D-53115 Bonn

Stein, Matthias
Unilever Research, Beverages Innovations,
Colworth House
Sharnbrook
GB-Bedford MK44 1LQ
matthias.stein@unilever.com

Stengel, Eckehard
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Stenzel, Wolf-Ruediger
Free University of Berlin, Inst. for Food Hygiene
Koenigsweg 69
D-14163 Berlin
gklein@zedat.fu-berlin.de

Stijnen, Daniella
EFFoST
P.O. Box 17
NL-6700 AA Wageningen
effost@ato.dlo.nl

Stockmeyer, Bernhard
FML Weihenstephan, Institut f. BWL
Weihenstephaner Berg 1
D-85350 Freising
stockmeyer@bwl.blm.tu-muenchen.de

Stolp, Willem
Wageningen Agricultural University, Department of
Food Science
P.O. Box 8129
NL-6700 Ev- Wageningen

Storck, T.
Bundesforschungsanstalt für Ernährung, INF
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Storcksdiek, Stefan
Bundesforschungsanstalt für Ernährung
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
stefan.storcksdiek@ernaehrung.uni-giessen.de

Straub, Joachim
Universität Hohenheim, Institut für
Lebensmitteltechnologie
Garbenstr. 28
D-70599 Stuttgart
jostraub@uni.hohenheim.de

Stute, Rolf W.
Bestfoods Europe
Knorrstraße 1
D-74074 Heilbronn

Suhren, G.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Swiader, Anna
University of Agriculture, Department of Plant
Physiology
Akademicka Street 15
PL-20-950 Lublin

Symons, Hugh
VITSAB
35 Knights Park
GB-Kingston-upon-Thames Surrey KT1 2QH
samjum@aol.com

Szwajgier, Dominik
Department of Food Technology, Agricultural
University Lublin
Skromna 8
PL- 20 740 Lublin
dzwtr@ursus.ar.lublin.pl

Tauer, Andrea
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Tauscher, B.
Bundesforschungsanstalt für Ernährung, ICB
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Tavman, Ismail
Ege University, Food Engineering Department
TUR-35100 Bornova Izmir

Tavman, Sebnem Demir
Ege University, Food Engineering Department
TUR-35100 Bornova Izmir
tavman@textil.ege.edu.tr

Tevini, Dirk
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
dirk.tevini@uni-karlsruhe.de

Tevini, M.
Universität Karlsruhe, Botanik II (Pflanzenphysiologie)
Kaiserstraße 12
D-76128 Karlsruhe
Manfred.Tevini@bio-geo.uni-karlsruhe.de

Thielecke, Klaus
Zuckerinstitut e.V.
Langer Kamp 5
D-38106 Braunschweig

Thorkelsson, Gudjon
Icelandic Fisheries Laboratories
P.O. Box 1405, Skulagata 4
ICE-121 Reykjavik
gudjont@rfisk.is

Thuault, Dominique
ADRIA
20 Avenue de la Plage des Gueux
F-29196 Quimper
dominique.thuault@adria.fm.fr

Toldrá, Fidel
CSIC, Instituto de Agroquimica y Tecnologia de
Alimentos
Apt. 73
E-46100 Burjassot
ftoldra@iata.csic.es

Tornberg, Eva
Swedish Meat Research Institute
P.O.Box 504
S-24424 Kaevlinge
eva.tornberg@ki.scan.se

Torreggiani, Danila
I.V.T.P.A.
Via Venezian 26
I-20133 Milano
torreggiani.ivtpa@interbusiness.it

Torringa, Erik
ATO-DLO, Bornsesteeg 59
NL-Wageningen 6700 AA
h.m.torringa@ato.dlo.nl

Trägardh, Christian
Lund University, Food Engineering
P.O. Box 124
S-221 00 Lund
christian.tragardh@livstek.lth.se

Traill, W. Bruce
University of Reading, Dept. of Agricultural Food
Economics
P.O. Box 237
GB-Reading RG6 6AR
wbtraill@infonet.tufts.edu

Trargonsky
Rektor Akademii Rolniczej
ul. Akademicka 13
PL-20-950 Lublin, Polen

Tröndle, Sigrid
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Troy, Declan
National Food Centre, TEAGASC, Dunsinea,
Castleknock
IRL-Dublin 15
D.Troy@NFC.TEAGASC.IE

Tucker, Gary
CCFRA, Campden & Chorleywood Food Res. Assoc.
Stadion Road
GB-GL55 6LD Chipping Campden
tucker@campden.co.uk

Van Amelsvoort, Johan
Unilever Research
P.O. Box 114
NL- 3130 Al Vlaardingen
hans-van.amelsvoort@unilever.com

Van Amerongen, Aart
ATO-DLO, Agrotechnological Research Institute
P.O. Box 17
NL-6700 AA Wageningen / Netherlands
A.vanAmerongen@ATO.DLO.NL

van Betteray, Klemens
CSB-System AG
An Fürthenrode
D-52511 Geilenkirchen
betteray@csab.de

van den Einde, R. M.
Wageningen Agricultural University
Bomenweg 2
NL-6703 HD Wageningen
rene.vandeneinde@94.student.wau.nl

Van den Heuvel, Ellen
TNO Nutrition and Food Research
P.O.Box 360
NL-3700 AJ Zeist
vandenHeuvel@voeding.tno.nl

van der Bent, Arie
Agrotechnological Research Institute
P.O. Box 17
NL-6700 AA Wageningen
A.vanderBent@ATO.DLO.NL

van Klink, Hans I.
COSUN
Zuilenstraat 100
NL-4814 ND Breda
Hans-van.Klink@cosun.com

Van Loo, Jan
Orafti
Aandorenstraat 1
B-3300 Tienen
jvl@rafti.eunet.be

Verachtert, Barend
European Commission, DG 12
Rue de la Loi/Westraat 200
B-1049 Brussel
barend.verachtert@dg12.cec.be

Verboeket-van de Venne, Wilhelmine
Maastricht University, Department of Human
Biology, NUTRIM
P.O. Box 616
NL-6200 MD Maastricht
W.Verboeket@HB.Unimaas.nl

Vetter, Joachim
Projektträger BEO, Forschungszentrum Jülich
Leo-Brandt-Str.
D-52425 Jülich
j.vetter@fz-juelich.de

von Rohrscheidt, Christian
ALFATEC Pharma GmbH
Im Neuenheimer Feld 519
D-69120 Heidelberg
cvr-consult@t-online.de

von Stetten, Felix
Forschungszentrum für Milch und Lebensmittel, TU-
München-Weihenstephan
Vöttingerstraße 45
D-85350 Freising
felix.v.stetten@lrz.tu-muenchen.de

Vormann, Jürgen
Protina Arzneimittel
Adalperostr. 30
D-85737 Ismaning
vormann.juergen@protina.de

Walz, Elke
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Watzl, Bernhard
Bundesforschungsanstalt für Ernährung
Engesserstr. 20
D-76131 Karlsruhe
watzl@bfe.fzk.de

Weber, Beate
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Weber, Gilbert
Hoffmann-La Roche Ltd, Vitamins and Fine Chemicals
Division
Dept. VM2, Bldg. 241/925
CH-4070 Basel
gilbert.weber@roche.com

Weber, Nikolaus
BAGKF, Institute for Biochemistry and Technology of
Lipids
Piusallee 68
D-48147 Münster
ibtfett@uni-muenster.de

Weber, Ralph
Institute for Environmentally Compatible, Process
Technology
Im Stadtwald 47
D-66111 Saarbrücken
a.faehnrich@rz.uni-sb.de

Wehrstein, Andreas
Monsanto Services International
Immermannstr. 3
D-40210 Düsseldorf

Weisbrodt, Thomas
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe
tweisbrodt@aol.com

Weisser, Horst
TU München, Brauereianlagen und Lebensmittel-
Verpackungstechnik
Weihenstephaner Steig 22
D-85350 Freising-Weihenstephan
horst.weisser@bl.tum.de

Wenzel, Uwe
Justus-Liebig-Universität Gießen, Institute of
Nutritional Sciences, Biochemistry Unit
Wilhelmstr.20
D-35 392 Gießen
Uwe.Wenzel@ernaehrung.uni-giessen.de

Werlein, Hans-Dieter
Universität Hannover, Inst. f.
Lebensmittelwissenschaft
Wunstorfer Str. 14
D-30453 Hannover
hd.werlein@mbox.lw.uni-hannover.de

Wesolowski, Marian
Rektor Akademii Rolniczej
ul. Akademicka 13
PL-20-950 Lublin, Polen

Westensee, John
Mapp. Centre for market surveillance, The Aarhus
school of business
Haslegaardsues 10
DK-8210 Aarhus
john.westensee@mar.hha.dk

Weyen, Jens
Saaten-Union Resistenzlabor GmbH
Hovedisser Str. 92
D-33818 Leopoldshoehe
weyen@sulab.kunden.de

Wiebe, Chr.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Wijaya, Agus
Bundesforschungsanstalt für Ernährung, IHT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Wild, D.
Bundesanstalt für Fleischforschung BAFF, Institut für
Mikrobiologie und Toxikologie
E.-C.-Baumann-Straße 20
D-95326 Kulmbach

Wild, Helen. G.
IFST, 5 Cambridge Court
210 Shepherds Bush Road
GB-London W6 7NJ
ifst@easynet.co.uk

Wilkinson, Clare
Agrotechnological Research Institute, ATO-DLO
P.O. Box 17
NL-6700 AN Wageningen
e.c.wilkinson@ato.dlo.nl

Willhöft, Corinna
Bundesforschungsanstalt für Ernährung, IÖS
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Williams, Graham
Aplin & Barrett Ltd, Technical Department
15 North Street
GB-Beaminster,Dorset DT8 3DZ

Wirtanen, Gun
Senior Research Scientist, VTT Biotechnology and
Food Res.
P.O. Box 1501
FIN-02044 VTT Espoo
gun.wirtanen@vtt.fi

Wolf, W.
Bundesforschungsanstalt für Ernährung, IVT
Haid-und-Neu-Str. 9
D-76131 Karlsruhe

Wolff, J.
BAGFK, Institut für Biochemie von Getreide und
Kartoffeln
Postfach 1354
D-32703 Detmold
bagkf@t-online.de

Wolfram, Günter
Präsident der DGE, Institut für
Ernährungswissenschaften TU München
Alte Akademie 10
D-85350 Freising-Weihenstephan

Wollny, Mathis
Universität Karlsruhe, Institut für
Lebensmittelverfahrenstechnik
Kaiserstr. 12
D-76128 Karlsruhe
mathis.wollny@ciw.uni-karlsruhe.de

Wolter, Frank P.
Norddeutsche Pflanzenzucht, Universität Hamburg
Ohnhorststraße 18
D-22609 Hamburg
wolter@botanik.uni-hamburg.de

Wolters, I.
Wageningen Agricultural University
Bomenweg 2
NL-6703 HD Wageningen
(rene.vandeneinde@94.student.wau.nl)

Zahner, Peter
Nestlé R&D Center, Lebensmittelforschung GmbH
Weiding
Postfach 334
D-84445 Mühldorf

Zeleny, Reinhard
IFA Tulln, Analytikzentrum
Konrad Lorenz Strasse 20
A-3430 Tulln
zeleny@ifa-tulln.oc.at

Zenz, K.I.
Bundesanstalt für Milchforschung
Postfach 6069
D-24121 Kiel

Ziesenitz, Susanne
Suedzucker AG Mannheim-Ochsenfurt
Marktbreiter Str. 74
D-97199 Ochsenfurt
Dr.Ziesenitz.suedzucker@t.online.de

Zorn, Holger
Universität Hohenheim, Institut für
Lebensmittelchemie
Garbenstr. 28
D-70599 Stuttgart

Zunft, H.-J. F.
DIFE
Arthur-Scheunert-Allee 114-116
D-14338 Bergholz-Rehbrücke
zunft@www.dife.de