2002

Institute of Food Research
Norwich Research Park
Colney
Norwich NR4 7UA, UK

Telephone: +44 1603 255000
Fax: +44 1603 507723
E-mail: ifr.communications@bbsrc.ac.uk
(for general enquiries)
Website: www.ifr.bbsrc.ac.uk

Director: Professor Alastair Robertson

The Institute of Food Research is located four miles west of Norwich on the Norwich Research Park, adjacent to the John Innes Centre and Sainsbury Laboratory, the Norfolk and Norwich University NHS Trust's new Hospital, and five minutes walk from the University of East Anglia campus.

'IFR in 2002' Editor:
Catherine Reynolds BSc, MIPR

Photography and illustrations:
IFR Communications unless stated

Design and layout:
Cobalt id

Print:
Norwich Colour Ltd

ISBN 0 7084 0637 8

ISSN 1352 7495

IFR in 2002

focused on food

2002

The UK's only integrated basic science provider focused on food, sponsored by the Biotechnology and Biological Sciences Research Council

Our mission is:

- To be a recognised world leader in our chosen areas of science (Food Safety; Diet and Health; Food Materials and Ingredients)
- To exploit and/or apply the output of our research for the benefit of our stakeholders

In this Report we highlight progress in 2001 and look forward to the future.

Contents

our vision and progress

focused on food

"I want to create a new winners circle from the science laboratory to the production line ..... making our country an incubator for innovation ....."
The Rt. Hon. Gordon Brown M.P., Chancellor of the Exchequer, 2001

Alastair Robertson (IFR) signs a Memorandum of Understanding between IFR and the USA Agricultural Research Service, witnessed by Jim Lindsay (ARS National Programme Leader Food Safety), March 2002

Introduction

IFR is a significant contributor to the national science base and arguably underpins the most sensitive area of public interest and concern today. The Institute also supports a particularly important UK industry which, post factory gate, has a turnover in excess of £56 billion, representing 7.6% of the UK's Gross Domestic Product.

Our science and enterprise strategy

The purpose of our science strategy is to provide a fundamental scientific understanding of food and its interaction with the human organism. We examine what constitutes hazard, seeking to reduce and eliminate it; define optimal nutrition and aim to ensure dietary delivery of it; and identify what constitutes satisfaction through eating, seeking to incorporate this into consideration of nutrition and food production. Our choice of themes and programmes, introduced in 'IFR in 2002' on pages 6 and 7 (food safety), 14 and 15 (diet and health) and on 22 to 23 (food materials and ingredients) links overall strategy with research objectives.

The building of a commercial infrastructure within the Institute has been a particular priority for IFR. The Food and Health Network, launched in July 2001 to integrate the food industry with outward-facing, problem-driven research, has proved successful with 20 companies participating across each of the clusters. The Network is providing an important mechanism for ensuring contact between IFR and its commercial stakeholder base, with the growing potential of attracting research with industrial companies through one-to-one relationships, group projects and, importantly, LINK programmes (see pages 31 to 32).

Both science and enterprise strategies are well developed to map onto the needs of our stakeholders, and to build closer and better relationships with them.

A successful assessment

During 2001 we underwent a detailed Institute Assessment Exercise (IAE), examining the quality of our science, the management and care of PhD students, our Knowledge Transfer arrangements, and our forward plans *via* a Visiting Group of eminent academics and business people. Feedback from the IAE has given us confidence that we have in place a sound base from which to further develop the international quality of our research over the next reporting period.

The IAE has demonstrated that, in parallel with the intense activity that has been required to consolidate two laboratory sites, both geographically and scientifically, IFR scientists have also significantly increased the quantity and quality of their scientific output over the reporting period 1996-2001. In addition, the Institute has sustained its role in supporting its stakeholders through a combination of fundamental and applied research and information provision.

The foundations laid during this period have provided stability on which to build future plans, new strategies and a culture of continuous change. Both the Knowledge Transfer Panel and the Visiting Group have acknowledged that, in consolidating and restructuring, IFR has introduced important new skills, established good supporting infrastructures and management structures, and developed effective strategies which are now providing the basis for internationally competitive food research, responsive to the needs of the Institute's stakeholders.

It was particularly gratifying to see both the referees and the Visiting Group commenting on the significant proportion of IFR's current science judged to be of high quality and relevance, internationally competitive and, in some cases, unique.

Fostering interactions

The IFR message is being spread across the Norwich Research Park by the contribution of its scientists to Park-wide symposia and meetings such as 'Thinking outside the box' to foster multidisciplinary research approaches, and 'Microbes in Norwich' which encourages collaboration particularly for younger researchers. IFR has benefited from these interactions by the generation of several new research collaborations. Staff publish, and travel widely, to communicate IFR science and scientific opinion (see pages 35-36) and at national and international level we have been involved in a number of initiatives.

We brought together European Directors of Food Research organisations with European Commission staff last year to start discussing issues that will be important in the Framework VI programme; discussions are on-going within 'Food*force*', and a number of new partnerships are being developed in areas such as food allergy.

We are founder members of the SAFE Consortium of European food institutes with an international reputation for their food safety research (with INRA in France, VTT in Finland, Wageningen Research Centre and TNO in The Netherlands, and ISPA in Italy). SAFE operates independently of industrial and governmental interests and is primarily designed to support the EC and the new European Food Safety Authority with independent information. Our developing collaboration with scientists in the USA Agricultural Research Service Food Safety programme has already led to their involvement in a new IFR-led EU contract to study growth in single cells. We are delighted to have signed a formal Memorandum of Understanding with ARS.

People

The Institute's future relies on the quality of its people and career training and investment is critical to achieving our aims (see page 34).

At senior management level, I want to acknowledge the support of members of the Governing Body and the work of members of the Science Executive – Professor Mike Gasson, Professor Sue Fairweather-Tait, Dr Reg Wilson and Dr Margaret Robins. Following our successful IAE, I am particularly pleased that we have been able to confirm the appointments of Sue Fairweather-Tait and Reg Wilson as heads of Nutrition and Consumer Science, and Food Materials Science Divisions, respectively. Margaret Robins is leading on the vitally important issue of quality assurance, of which more later.

There have been some senior staff moves, and we welcome the new opportunities for collaboration which follow now that Professor Gary Williamson has joined Nestlé, in Switzerland. Dr Jenya Vulfson is working in the USA. Peter Whitfield, Head of Finance, and Ian Lester, Business Manager have also left IFR; Peter has been succeeded by John Kingsmill FCCA who has substantial experience in both industry and charity sectors. Our incoming Enterprise Manager, Sue Southon, has built an international scientific reputation for her work at IFR. Her understanding of the Institute, its research, and its stakeholders will be a valuable asset.

Among new starters in science, Dr Marjon Bennik undertook postdoctoral research in molecular microbiology at Harvard University, and then worked on food-borne bacterial pathogens at ATO and the Wageningen Centre of Food Sciences in The Netherlands. At IFR, her work involves molecular microbiology of *Clostridium botulinum.* Dr Sean Hanniffy has joined from the University of Cambridge with expertise in vaccine discovery; he will be working with lactic acid bacteria as delivery systems for vaccine and therapeutic products.

Dr Marjon Bennik working in the Cl. botulinum *containment laboratory at IFR*

'IFR3' - formerly the British Sugar Technical Centre, purchased by BBSRC in January 2002. This building and parts of IFR2 are available for rental and several organisations are already on site.

Site and infrastructure

Redevelopment of the site and infrastructure has continued apace, with the addition of the former British Sugar Technology Centre (some 30,000 sq. ft of laboratories and offices) to the portfolio of buildings. The library has been moved to its new site and our Finance team is now accommodated in IFR1. During 2002/3 we plan to link our two main buildings with a covered walkway and move the visitor entrance, so that our lecture theatre can become a more attractive conference venue.

The need for the storage of high volumes of data is increasing and pervasive across the research areas of the Institute. We will be upgrading our network storage facilities, part-funded by the Office of Science and Technology. In 2002/3, we will be installing two terabytes of storage, supported by an automated tape library and major upgrades to our local area network. In 2003/4, the system will be expanded to five terabytes.

Quality assurance

IFR is in the process of setting up a formal Institute-wide Quality Assurance (QA) system, having made a commitment two years ago to putting in place a system which mirrored the key components of ISO9001. Although primarily a basic research organisation, we recognised that the area in which IFR operates is highly sensitive and often publicly visible. In addition we considered that the basic laboratory systems and procedures expected in analytical laboratories shared much common ground with those underpinning the reliability of research findings. Though we employ highly competent scientists and publish our work in peer-reviewed journals we did not think that this substituted for demonstrable methodological consistency and precision *via* a formalised QA system.

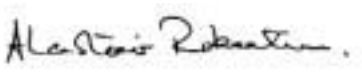
In parallel with the requirements for QA in our scientific work, we are re-examining our management procedures and decision-making processes. This is an essential aspect of Quality Management, as identified in ISO9001.

Business risk management

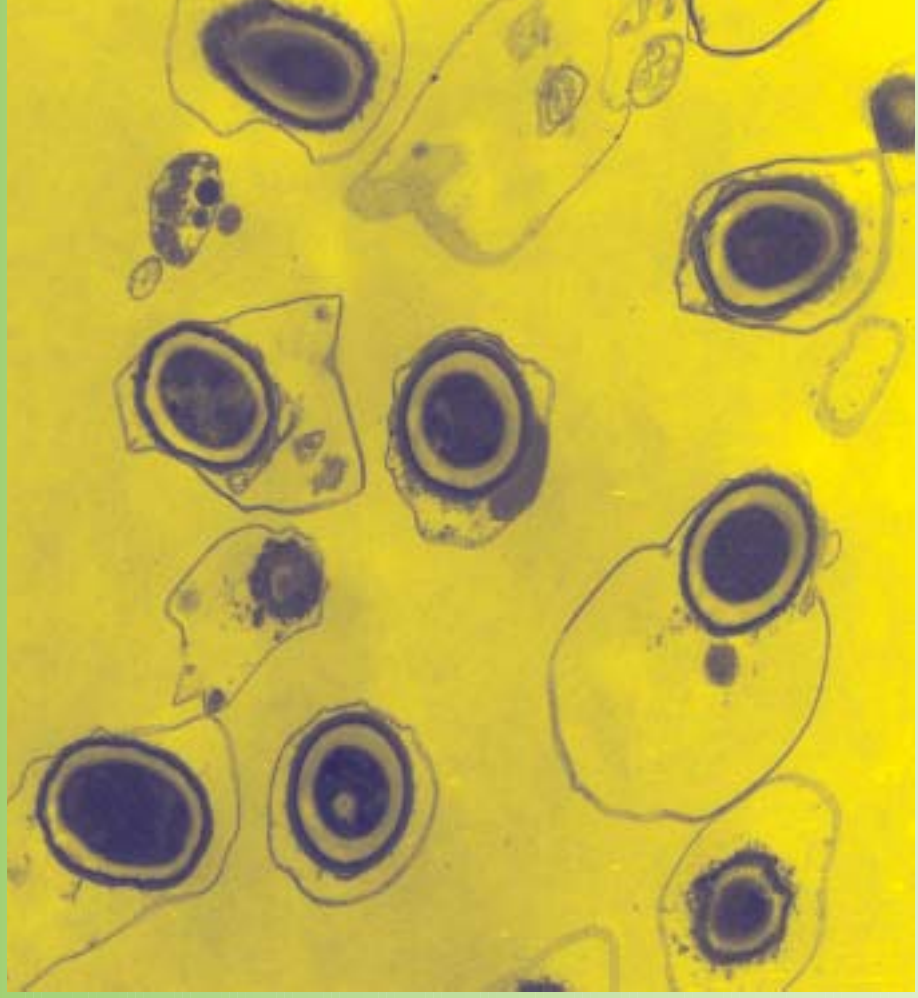
As part of the 'Statement of Recommended Practices' (SORP 2000) reporting processes for charities, our Governing Body (as Directors of IFR) have delegated to members of the senior management team the responsibility of ensuring that all the key business risks facing us are identified and understood, and measures are in place to mitigate them. In many respects this is the formalising of good housekeeping, but the review process has been useful as it has led us to revisit our processes for science quality control, reputation management, disaster recovery etc.; we are confident that the Governing Body will continue to find our processes for risk management satisfactory.

And finally...

The Institute is at the beginning of an era of new growth as a provider of independent science for public policy goals, in supporting the competitive advantage of a major UK industry and in underpinning science-based advice for the public good. The next 4-5 years will be crucial in meeting these aims and reliable, stable funding to underpin existing and new skills will be essential in maintaining our strategy and attaining new goals.



Alastair Robertson
Director
April 2002



Germinating spores of Clostridium botulinum, *the food-poisoning organism that causes botulism, a rare but severe disease (Transmission Electron Micrograph by Mary Parker, IFR)*

Food Safety Theme Leader

Professor Mike Gasson

food safety

Theme overview

Food safety is important to all areas of our stakeholder base including government, consumers and industry. Our approach to food safety involves a 'whole food chain' strategy and the current emphasis addresses the problem of microbial food poisoning. Bacterial food poisoning across the food chain is of major topical importance. *Salmonella* remains a major concern, *E. coli* is of increased significance and *Campylobacter* appears to be an ever-growing problem. These species have prominence in our portfolio of research but we maintain flexibility so as to respond to emerging microbial problems of the future.

GM food is of importance to consumers, regulators and industry. Scientific underpinning of regulation is a vital activity that is well represented in the research programme.

Involvement of social scientists and consumer science approaches are increasingly important to our work. This facilitates understanding the public response to new technologies and their uptake of advice intended to ensure consumer safety.

Our objective is:

- **Preventing microbiological food poisoning and ensuring the safety of novel foods**

May 2002

food safety

from a food chain perspective

Bacterial pathogenesis and response to environmental stress
The core of our research on bacterial food poisoning is a fundamental programme that addresses the biology of key bacterial pathogens. This work involves a molecular genetics approach and takes full advantage of our state-of-the-art skills in the exploitation of whole genome sequence data. We seek to understand how these bacteria cause disease and how their gene expression changes in response to environmental stress.

In taking this core activity forward, we have placed the emphasis on two related and interactive aspects: the basis of bacterial virulence and the response to environmental change, including stress.

Predicting the behaviour of bacterial food-borne pathogens
Our fundamental approach is complemented by work that aims to understand and predict food-borne pathogen behaviour in food. This includes investigating the effect of food structure on microbial growth and the development and application of techniques for predictive modelling and quantitative microbial risk assessment.

We address the safety of minimally heat-processed foods, which are of growing significance in the marketplace. *Clostridium botulinum* is of especial relevance in these foods and we have specialist facilities for the study of this dangerous pathogen. Our established expertise in the physiology of *Cl. botulinum* is complemented by a newer molecular biological approach that will take advantage of its soon-to-be-complete genome sequence.

Commensal bacteria in biocontrol and fermentation
We study commensal bacteria that naturally inhabit the gastro-intestinal tracts of food animals and man, as well as those involved in food-fermentation, itself a traditional process of food preservation that has evolved into a major industry. This work provides a proactive dimension to food safety research in that it is concerned with biological approaches to the control and eradication of food-borne pathogens. We intend to develop new approaches to pathogen control both in food and in raw material production. Our work combines investigation of antimicrobials, vaccine delivery and competitive exclusion with the longer-term objective of eliminating human pathogens from the food chain.

Gene expression and metabolism in plants and fungi
We study gene expression and cellular metabolism in higher plants and fungi and aim to understand aspects of the relationship between gene expression, protein production and cellular metabolism. This is fundamental to biotechnology and central to the rational safety evaluation of GM plants and microorganisms. Current work includes a large research effort on substantial equivalence, the comparative approach that is used universally to guide the safety evaluation of GM foods and food constituents. We exploit our expertise in metabolic profiling, proteomics and DNA arrays to investigate the use of new molecular profiling approaches that may improve safety assessment procedures for novel foods.

Work on filamentous fungi focuses on their physiological responses that lead to changed lipid synthesis and protein secretion.

Perceptions, attitudes and behaviour
We are interested in the social science dimensions of food safety issues related to microbial pathogens and novel food. We investigate public risk perceptions associated with different food hazards, develop effective communication about food risks and refine the risk management process.

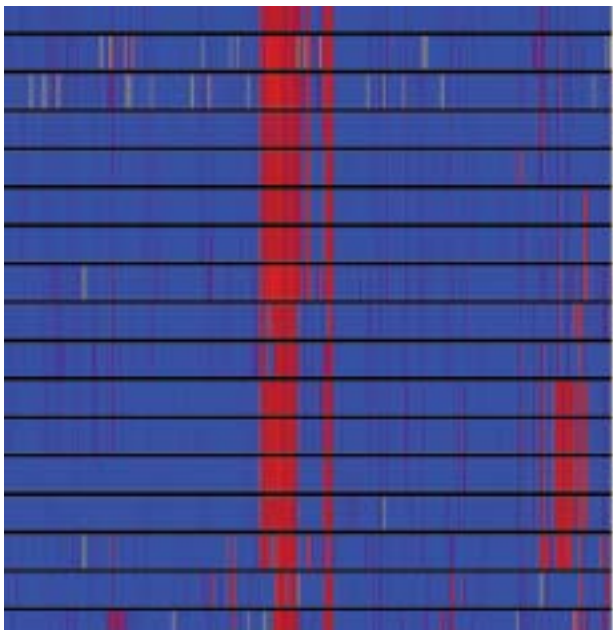
An area of increased importance is developing ways to evaluate effective public consultation practices associated with food risk policy.

keeping our food safe

answers to frequently asked questions

As consumers, we all want to be confident that the food we buy will be good for us, and not make us ill. The Government sets the policy for food safety, and our science helps industry and Government understand how to achieve it. Scientific research can't be done in a vacuum and we need input from society about what's important to consumers.

Most food has microbes in it, or associated with it – some are friendly, others are dangerous; we are interested in both. There is also a lot of debate about the pros and cons of genetic modification; our role here is to find out whether anything unexpected happens when food ingredients are modified, so that Government can assess the safety issues.



Are the bacteria in your food dangerous? This image shows the presence (blue) or absence (red) of 3000 genes in 26 types of E. coli. *At IFR we have pioneered a new approach, still in the research stage, called 'Genomic Indexing' that will be used to predict which ones cause food-poisoning (see page 9).*

? Why do you work on food-poisoning organisms?

The number of *reported* cases of food poisoning has been increasing over the last 20 years, and a 2001 Food Standards Agency 'Consumer Attitudes to Food' survey, published in February, 2002 states that 12% of UK consumers - 5.5 million people - said they had food poisoning in the previous year. More needs to be done to understand how these microbes behave, and our fundamental work should lead to new methods of control and eradication.

? Why do you work on *Salmonella, Campylobacter, Escherichia coli* and *Clostridium botulinum?*

Because they are the most significant causes of bacterial food-borne illness, in terms of numbers of cases and severity of the resulting disease. All are responsible for serious health problems and high cost to the UK economy in lost output and reduced consumer confidence.

? Where are the bacteria coming from?

They appear at all stages of the food chain and often don't make animals ill. We need a farm-to-fork approach to study where they arise, so as to control them most effectively.

? How safe can we expect food to be?

Like all things in life, food can't be 100% safe. There is always a risk, but we can minimise the risk and make food safer. Understanding what people think about risk and uncertainty is an important part of communicating messages about safe food handling, storage and preparation at all stages in the food chain.

functional genomics of food-borne pathogens

investigating how genes work

Further reading

Clements, M., Ericsson, S., Tezcan-Merdol, D., Hinton, J. C. D. & Rhen, M. (2001) Virulence gene regulation in *Salmonella enterica.* Annals of Medicine **33** 178-185

Goldberg, M. D., Johnson, M., Hinton, J. C. D. & Williams, P. H. (2001) Role of the nucleoid-associated protein Fis in the regulation of virulence properties of enteropathogenic *Escherichia coli.* Molecular Microbiology **41** 549-559

Hautefort, I. & Hinton, J. C. D. (2002) Molecular methods for monitoring bacterial gene expression during infection. Methods in Cellular Biology **31** 56-91

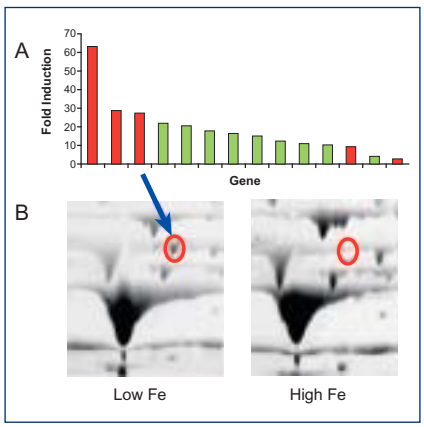
Thompson, A. S., Lucchini, S. & Hinton, J. C. D. (2001) It's easy to build your own Microarrayer! Trends in Microbiology **9** 154-156

Wells, J. M. & Bennik, M. (2002) Impact of genomics on food-borne pathogens. Nutrition Research Reviews (in press)

We have a particular interest in the types of stresses encountered by bacteria during food production and during infection, but this research will also be important in understanding the bacterial factors and mechanisms involved in survival in extra-intestinal environments such as faecal matter, water, bedding and food. Mechanisms of adaptation and survival are critical for host-to-host spread on the farm and greater knowledge in this area might lead to the development of improved practical strategies to control infection. Additionally, in EU-funded research we aim to exploit two-component systems as a new class of targets for the development of novel pharmacological interventions to control infectious disease and combat microbial drug resistance. New genome information, and functional genomic tools, are helping us gain a better understanding of the effects of environmental stress on gene expression in *Campylobacter, E. coli* and *Salmonella.* DNA microarray methods for genetic indexing have been used to investigate genetic diversity among strains of *E. coli* and *Campylobacter jejuni.*

Campylobacter research

Here, functional genomics tools and signature-tagged mutagenesis are being used to identify cellular processes involved in survival in complex environments, as well as the adaptation and response to environmental stress. This research involves a number of collaborative studies on specific stresses including oxidative, iron, nitric oxide, pH and global stress response. IFR microarray and proteomic data on genes induced under low-iron conditions are shown - iron is recognised as a limiting factor for bacterial growth, especially in animal and human hosts. We are currently investigating the properties of a large plasmid isolated from a clinical isolate of *C. jejuni* including antibiotic resistance, conjugation and its role in virulence.



Panel A. Microarray expression profiling reveals several Campylobacter *genes induced by low iron stress. Genes indicated in red were previously shown to be regulated by the ferric regulator protein that is found in many different bacteria*

Panel B. Section of a 2D protein gel showing iron regulated expression of a putative siderophore receptor (CfrA)

Genomic indexing of *E. coli*

We have been using DNA microarrays to investigate the relatedness of pathogenic *E. coli* isolates in one of the first research projects to benefit from the IFR Microarray facility. In collaboration with Professor Martin Woodward (Veterinary Laboratories Agency), the presence and absence of 4262 genes was determined in 26 *E. coli* strains isolated from humans, animals and the environment. We have discovered that pathogenic *E. coli* strains are lacking between 5 and 20% of the genes carried by the *E. coli* K12 laboratory strain. We have identified a novel 22Kb chromosomal deletion carried by all the pathogenic *E. coli* isolates studied, which could provide an important new diagnostic marker. In addition, we have identified a number of catabolic genes that are missing from the genome of *E. coli* pathogens, and may have significant effects on virulence. Our findings may lead to new approaches for classifying *E. coli.*

Further information

www.ifr.bbsrc.ac.uk/Safety/Microarrays/

Contact

Jerry Wells[1] or Jay Hinton[2]
(Programme leaders)
Campylobacter[1] *E. coli*[2]

mathematical modelling tools for food microbiology

tools to underpin safe food production

Increasingly, foods are being sold which have received minimal preservation treatments. We are developing new mathematical modelling tools to help ensure the continued safe development of these foods. IFR is internationally known for fundamental work on modelling, and translating this knowledge into software programs and advice to assist industry.

Modelling bacterial growth curves

In the last decade a new approach to mathematical modelling of bacterial growth in food has been developed at IFR. Since the model enabled prediction under dynamic conditions, such as fluctuating temperatures, it was called the *D-model* but it is now internationally known as the *Baranyi-model,* after the IFR mathematician who created it. More than 300 references have been made to the new model in the literature, and it has attracted international collaborative projects and external funding.

Two user-friendly software packages were also developed to help fellow scientists apply the model to their data - *DMFit* and *MicroFit* are freely available on the web at the request of sponsors.

Relational databases of microbial responses to food environment

IFR and the Agricultural Research Service of the United States Department of Agriculture (ARS/USDA) are developing a ***com***mon data***base*** *(ComBase)* of microbial responses to food environment. The joint database will include microbiology data generated in the ARS/USDA Pathogen Modelling Programme, and also publicly available data from IFR, partner institutes and from the literature. The database contains user-friendly navigation programs whose development was funded by the UK Food Standards Agency. The FSA have also supported this project by making available datasets compiled from the literature.

ComBase is expected to be available by the end of 2002, with the potential of becoming an invaluable source to help and harmonise the work of risk assessors world-wide.

Stochastic modelling

Bacterial lag time can be very variable, particularly under non-optimal conditions. Our aim is to develop and then validate a new stochastic approach to model the lag time of single cells. Characterisation of the variability of the lag times of individual cells will give improved prediction of bacterial growth, helping to ensure food safety. A three-year-long, €1.5M project (BACANOVA) has recently started, coordinated by IFR, to explore the potential of the new approach.

From functional genomics to prediction

In the future we hope to apply our state-of-the-art research in proteomics and genomics in order to gain improved understanding, and consequently prediction, of microbial responses to food environments.

Further reading

Baranyi, J. & Pin, C. (2001) A parallel study on modelling bacterial growth and survival curves. Journal of Theoretical Biology **210** 327-336

Further information

DMFit and *MicroFit* are at www.ifr.bbsrc.ac.uk/websites

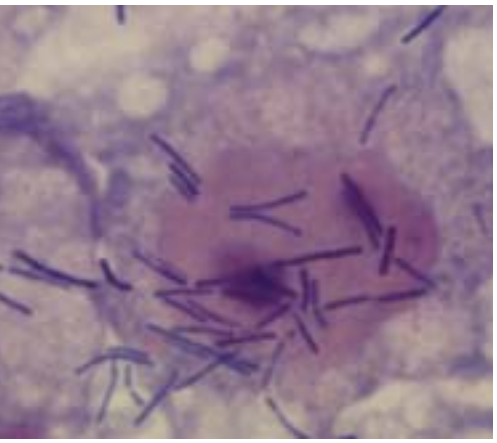
Contact

Mike Peck (Programme leader)

The opening screen and a query-generated output form of ComBase

commensal bacteria in biocontrol and fermentation

pathogen control and eradication



A commensal Lactobacillus *isolate from chicken GI tract adhering to tissue-culture cells.*

This proactive dimension to the Food Safety Theme is concerned with biological approaches to the control and eradication of food-borne pathogens. We investigate commensal bacteria (species that have a positive influence on health and the capacity to exclude pathogenic bacteria) that inhabit the gastrointestinal tracts of food animals and people, as well as those involved in food fermentation. We develop natural antimicrobial products for the biocontrol of pathogens and exploit lactic acid bacteria for the competitive exclusion of pathogens, and delivery of vaccines and bioactive compounds.

Lactic acid bacteria (LAB) in competitive exclusion

The gastrointestinal tracts of man and animals contain a complex bacterial ecosystem. Commensal strains of LAB have a history of use with the intension of enhancing health in the form of probiotics and controlling human pathogens in farm animals. We have initiated the isolation and characterisation of commensal bacteria from poultry in order to obtain strains that will persist in the chicken gastrointestinal tract and compete with key human pathogens. In collaboration with the Veterinary Laboratories Agency, we have undertaken challenge experiments with an interesting isolate of *Lactobacillus* and demonstrated its capacity to control a range of human pathogens including *E. coli, Campylobacter jejuni* and *Clostridium perfringens.*

Lactic acid bacteria as probiotics

A wide range of bacterial isolates are used as human and animal probiotics and there is a need to ensure that individual strains are safe. There is a particular concern about the safety of strains of *Enterococcus,* because this genus is associated with serious nosocomial (hospital-acquired) infections and multiple drug resistance. The basis of pathogenicity in *Enterococcus* is poorly understood, making the evaluation of individual strains difficult. We have developed molecular methods based on gene probes and PCR amplification to screen for the presence of virulence genes in enterococci and used these to investigate strains from different ecosystems, including hospitals and food. We have also investigated strains that are used as probiotics and as starter cultures in dairy fermentations. Results suggest that there are more virulence determinants in hospital isolates. The molecular tools that we have developed are of value for the safety assessment of individual strains of *Enterococcus.*

Lactic acid bacteria as delivery vehicles

Commensal LAB can be exploited to deliver vaccines and other biologically active material to the gastrointestinal tract. Their use for vaccine delivery is of especial value in stimulating mucosal immunity that is protective at the site of pathogen entry. The advantages of LAB delivery include: ease of administration; survival in stomach acid; inherent safety; particulate nature and size for uptake by M cells; economic technology in that the bacteria manufacture the vaccine or therapeutic agent. We have assembled a unique skill base at IFR that is developing the potential of this novel approach and addressing a variety of applied targets.

Further reading

Eaton, T. J. & Gasson, M. J. (2001) Molecular screening of virulence determinants and potential for genetic exchange in food and medical isolates of *Enterococcus.* Applied & Environmental Microbiology **67** 1628-1635

Contact

Mike Gasson (Programme leader)

gene expression and metabolism in plants and fungi

molecular profiling for safety evaluation of GM food

One of the most prominent and topical issues in GM food safety assessment concerns substantial equivalence, the comparative approach that is used universally to guide the safety evaluation of GM foods and food constituents. Future developments in GM technology involving plants with multiple traits and more complex output traits may challenge the robustness of substantial equivalence. One of the most promising approaches to improving comparative analysis is the use of molecular profiling, but it has not been developed sufficiently in this context. We are contributing to a detailed evaluation of molecular profiling for future use in the safety assessment of GM foods.

At IFR we are investigating three molecular profiling approaches: DNA microarrays to monitor gene expression at the level of transcription; proteomics to reveal protein content; and metabolic profiling to reveal the metabolite pool. This work takes advantage of our state-of-the-art facilities for post-genomic analysis and our advanced capabilites in chemometrics.

Proteomics involves the use of two-dimensional gel electrophoresis to separate proteins on the basis of their isoelectric point and molecular weight. The quantitation and comparative analysis of the data is challenging, but substantial progress has been made with this technique. One advantage of proteomic analysis is that individual spots can be extracted from the gel and characterised using MALDI-TOF and Q-TOF mass spectrometry. For a model transgenic tobacco plant, we found the unanticipated production of two protein spots that were identified as the same plant lectin. The two proteins differed with respect to post-translational modification and this is an important variable that needs to be accommodated in comparative analysis of composition.

We are investigating the relationship between the plant cell's pool of messenger RNAs (the transcriptome) and the cell's profile of proteins (the proteome). This relationship is not simple and we have shown that in the case of the key enzyme, S-adenosylmethionine decarboxylase, abolition of translational regulation (i.e. deregulating the relationship between the messenger RNA and the corresponding protein), results in a massive 400-fold increase in the reaction product of the enzyme, decarboxylated S-adenosylmethionine (dcAdoMet). This deregulation of translation causes severe growth perturbations, indicating that translational regulation is integral to the maintenance of normal plant and GM plant physiology.

The result of abrogation of translational control of S-adenosylmethionine decarboxylase through a single nucleotide alteration.

The smaller, darker flowers (on the right) are from transgenic plants carrying the single nucleotide alteration; the lighter-coloured flowers (on the left) are from control plants that have segregated without the transgene. Superimposed on the images are high performance liquid chromatography (HPLC) traces of extracts from transgenic (lower trace) and syngenic (upper trace) leaves, showing the substrate (AdoMet) and the product (dcAdoMet) of S-adenosylmethionine decarboxylase. (The prominent central peak present in both traces is unidentified)

Further reading

Franceschetti, M., Hanfrey, C., Scaramagli, S., Torrigiani, P., Bagni, N., Burtin, D. & Michael, A. J. (2001) Characterisation of plant monocot and dicot S-adenosylmethionine decarboxylase gene families including identification of a pair of conserved overlapping upstream open reading frames. Biochemical Journal **353** 403-409

Hanfrey, C., Sommer, S., Mayer M. J. & Michael, A. J. (2001) *Arabidopsis* polyamine biosynthesis: absence of ornithine decarboxylase and the mechanism of arginine decarboxylase activity. The Plant Journal **27** 551-560

Mayer, M. J., Narbad, A., Parr, A. J., Parker, M. L., Walton, N. J., Mellon, F. A. & Michael, A. J. (2001) Rerouting the plant phenylpropanoid pathway by expression of a novel bacterial enoyl-CoA hydratase/lyase enzyme function. The Plant Cell **13** 1669-1682

Thompson, A. & Gasson, M. J. (2001) Location effects of a reporter gene on expression levels and native protein synthesis in *Lactococcus lactis* and *Saccharomyces cerevisiae*. Applied & Environmental Microbiology **67** 3434-3439

Contact

Tony Michael (Programme leader)

food safety and social science

communication, trust and institutional change

Further reading

Frewer, L. J. & Salter, B. (2002) Public attitudes, scientific advice and the politics of regulatory policy: the case of BSE. Science and Public Policy (in press)

Frewer, L. J., Scholderer, J. & Bredahl, L. (2002) Communicating about the risks and benefits of genetically modified foods: Effects of different information strategies. Risk Analysis (in press)

Frewer, L. J., Hunt, S., Brennon, M., Kuznesof, S., Ness, M. & Ritson, R. (2002) The views of scientific experts on how the public conceptualise uncertainty. Journal of Risk Research (in press)

Eiser, R. J., Miles, S. & Frewer, L. J. (2002) Trust, perceived risk and attitudes toward food technologies. Journal of Applied Social Psychology (in press)

Frewer, L. J. (2001) Environmental risk, public trust and perceived exclusion from risk management. Research in Social Problems & Public Policy **9** 221-248

Contact

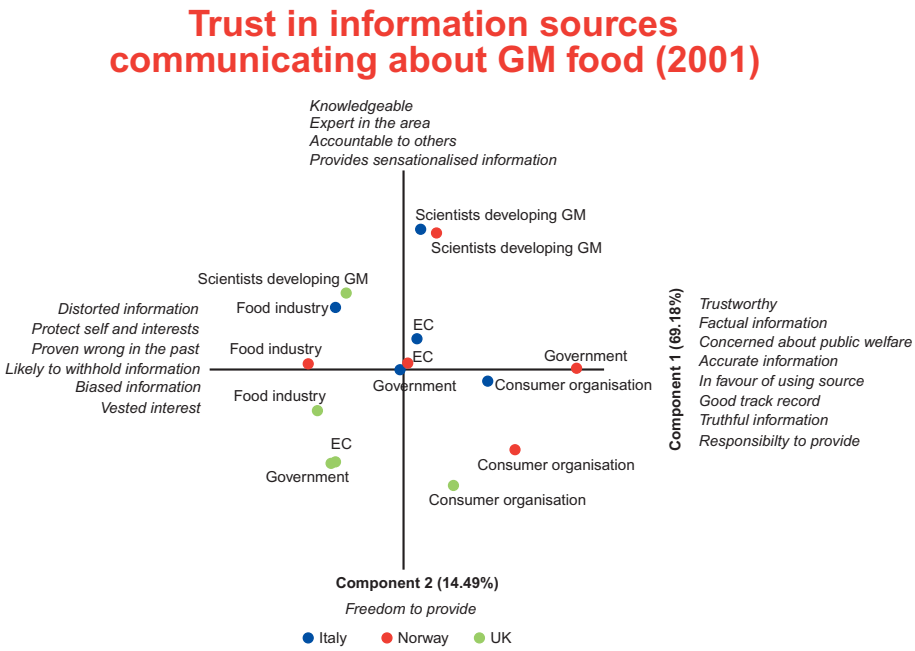
Lynn Frewer (Programme leader)

The way in which we perceive risks associated with a particular hazard will have a direct impact on our attitudes towards that hazard. It is important to understand the psychology of risk perception not only to optimise risk communication practices, but also to develop an effective public consultation strategy which explicitly involves the general public in policy development associated with science innovation and risk management.

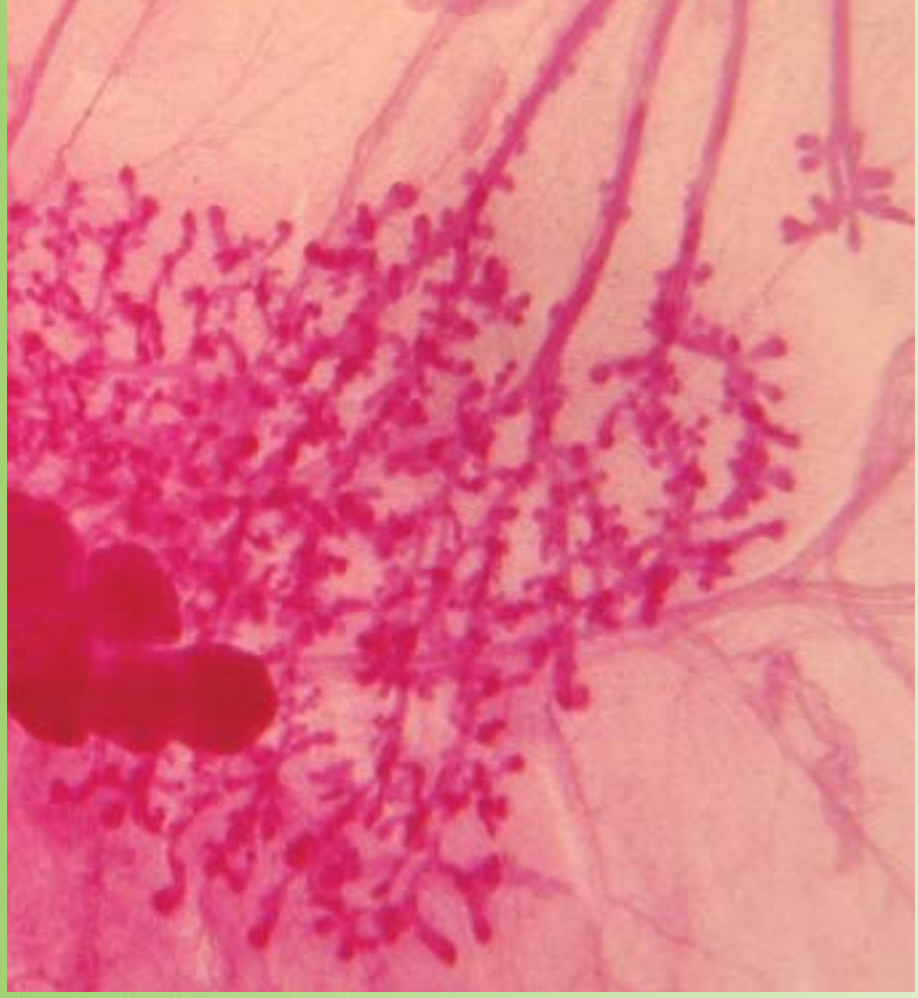
Communication of risk uncertainty is an important issue for the FSA. In collaboration with the University of Newcastle we have found that, although members of expert communities believe that communication of information about scientific uncertainty will increase public distrust in science and regulatory practices, the public's belief that experts are hiding uncertainty information actually increases their distrust in experts' motives. The public are very aware that uncertainty exists as part of risk management processes.

Advances in science, medicine and technology mean that people in the developed world are living longer and healthier lives, but they have increased expectations of greater regulatory protection from risk. Increased anxiety is likely to be linked to changes in hazards (increasing globalisation with potential for negative impact on food security; large-scale production of commodities; uncertain technological implications regarding safety and ecological impact).

There is a need to broaden the base of public consultation and dialogue on risk issues, particularly in the food domain as the food we eat, its taste, safety, price, and so on, are of fundamental, unavoidable and everyday interest to society. We are developing methods to determine whether consultation processes are effective, whether the outputs inform policy decision-making, and whether public consultation does have a substantial impact on public confidence in science and technology.



There are big cross-cultural differences in the extent to which people in different countries trust information sources to provide information about genetically modified ingredients. In particular, Norwegian consumers trust their government to be truthful, which is not the case in Italy and the UK. Consumers in all three countries agree that they do not trust industry to be honest about food risk.



What mechanisms are responsible for the suggested protective effect of hormonally active soy components against breast cancer? - the image is of a mammary gland from a young female rat; at this stage the gland is undifferentiated, consisting primarily of proliferative structures (Photomicrograph by Paul Cairns, IFR)

Diet and Health Theme Leader

Professor Sue Fairweather-Tait

diet and health

Theme overview

The post-genomic era provides us with an unprecedented opportunity to study the effects of diet on human health. Establishing optimal health for individuals requires an understanding of the links between food, genetic make-up and diet-related diseases. Research takes an integrated approach, combining molecular, cellular, whole-body and consumer sciences and is problem-driven, focused on public health issues, and contributes to the development of health-promoting policies. It crosses between the other themes e.g. gut immunology and food allergy work is part of both the Food Safety and the Materials and Ingredients Themes.

We also study psychological barriers to dietary change in order to facilitate strategies aimed at improving health through better nutrition; consumer science research is consolidated in a single programme in the Safety Theme in order to provide critical mass for our social scientists.

Our objective is:

- **Enhancing food quality in relation to nutrition, preventing diet-related disease, and improving the health of individuals throughout life through optimal nutrition**

May 2002

diet and health

establishing optimal health for individuals

Diet and gastrointestinal health
Dietary microcomponents interact with intestinal epithelial cells in ways that profoundly affect the growth, integrity and function of mucosal tissue. We aim to increase our understanding of the molecular basis for these effects and to assess their practical significance for human health. Current research is focused on the effects of flavonoids, glucosinolates and n-3 polyunsatuated fatty acids.

Phytochemicals and health protection
Two classes of phytochemicals, the polyphenols, and the glucosinolate breakdown products derived from Brassica vegetables, have potent effects on carcinogenesis *in vitro*, but possible toxic effects at very high doses. We are interested in polyphenol bioavailability, the form in which phytochemicals reach target tissues and how they affect gene expression. We are investigating whether the beneficial and toxic effects of phytochemicals in foods, functional foods, and supplements, are different, and how much of the difference is related to the individual's response rather than absorption and metabolism.

Vitamins and health
One of the strongest epidemiological associations is that between higher intakes of fruits and vegetables and lower risk of chronic diseases; it is widely suggested that the causative agents for this relationship are microcomponents in these foods. We aim to elucidate and exploit relationships between fruits and vegetables and health, in particular, the influence of food structure on the release of organic micronutrients during digestion, their bioavailability and effects on cells and tissues.

Minerals and health
Public health issues drive the research programme; iron and calcium are the minerals of current interest because of the high prevalence of iron deficiency and interest in iron fortification, and the increasing problem of osteoporosis. Generic information on bioavailability, required to assess dietary adequacy, is obtained using stable isotopes. Improved methods are being developed for measuring iron bioavailability and homeostatic mechanisms, and for monitoring bone calcium metabolism.

Immunological response to food
In nutritional immunology, we are investigating the ability of selected dietary components to redress changes in immune cell gene expression and phenotype that might predispose individuals to an increased risk of infection, chronic inflammation or age-related diseases. Gut immunology is focused on food allergy and specific aspects of food safety, for instance the interaction between lymphocytes and epithelial cells and bacteria in the gut.

Nutritional genomics
In this underpinning, technology-driven programme we are preparing microarrays of 14,000 human genes which will be used to study changes in gene expression in relation to diet, and will complement the Taqman (ABI 7700 sequence detection system) assays that are being undertaken to investigate the effects of phytochemicals on gene expression including DNA repair enzymes, transcriptional factors, and phase I metabolising and phase II detoxification enzymes in human cells. Standardised protocols will maximise our ability to perform meaningful analyses with data from different experiments and enhance the construction of a broad-spectrum diet-gene interaction database.

Research governance
Recognising the growing importance of research governance, we have a Human Research Committee which deals with research on human volunteers. This facilitates interactions with the Norfolk and Norwich University NHS Trust's District Ethics and Research & Development Committees, both of which include IFR representatives.

helping us live healthier lives

answers to frequently asked questions

People expect to live for longer, but how can we ensure our lives are healthy ones and that the risk of diet-related disease is minimised? We are all individuals, so is good advice for one person applicable to everybody? Or, are we just programmed by what's in our genes? Our science contributes to the development of Government policies for health promotion and disease reduction in all population groups.

Flavonoids are natural chemicals that often add colour to foods - for instance the red colour in wine and the brownish colours in tea.

? Are people eating a healthier diet nowadays?

We certainly have a much wider variety of foods available to us, but many scientists are concerned about the amount of fat, sugar and salt in our diets. Obesity is becoming increasingly widespread. Making the changes to a healthier diet seems to be difficult for many people, and social scientists at IFR are trying to find out why.

? Why are you interested in fruits and vegetables?

Because a high intake of fruits and vegetables can halve the risk of many cancers, and because there is good evidence from laboratory experiments to show that it is particular natural chemicals in plant foods that help us to stay healthy. As pure chemicals in a laboratory experiment, flavonoids can stop some of the processes that result in cancers, but what does this mean for people eating real food?

? What are free radicals and antioxidants?

Free radicals are unstable molecules that contain an unpaired electron. Our bodies produce them as a by-product of normal metabolism. The free radical goes on a "search and destroy" mission, stealing an electron from a healthy molecule. They can cause damage to cells and our DNA, and are the starting point for many disease processes. Our bodies make enzymes that curtail the activity of free radicals; the mineral selenium is part of an enzyme that has antioxidant properties, and the main dietary sources of antioxidants are carotenoids, vitamin C and vitamin E.

? Do adults need to take supplements to stay healthy?

No, we don't think so, provided they are eating a balanced diet. But, if someone is planning a pregnancy, they will want to ensure that they consume enough folic acid - and in this case a supplement may be useful. There is currently a debate as to whether or not the Government should introduce legislation to routinely supplement a staple food such as flour with folic acid. IFR scientists are taking a lead role in the debate.

food components and health protection

how does intake relate to function?

In order to examine the relationship between dietary intake and function of vitamins, minerals and phytochemicals, it is essential to measure the absorption and metabolism of the microcomponent or its metabolite(s). We often use stable isotopes to label microcomponents and follow the movement from food to the target tissue. Compartmental modelling can be employed to predict changes in body pools that cannot be sampled directly, and hence estimate bioavailability. These approaches have been adopted to study the bioavailability of carotenoids, folates, calcium, iron, flavonoids and glucosinolates.

Carotenoids are associated with health promotion and disease protection when eaten as carotenoid-rich foods, but which foods?

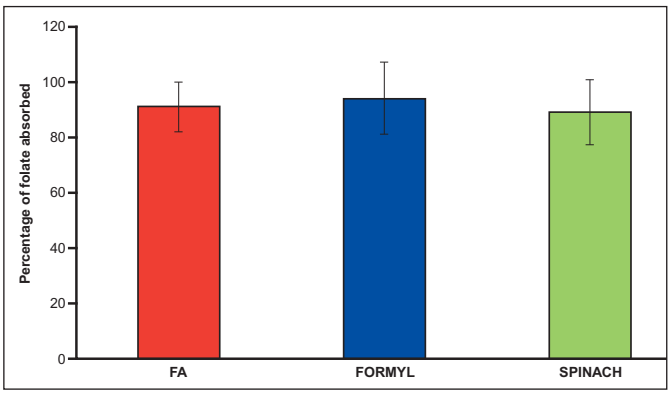
Carotenoids, being lipid-soluble, must be solubilised within the lipid phases within the gut during digestion. Mixed micelles act as the delivery vehicle for carotenoids to the point of absorption. We have developed an *in vitro* model gut (validated against *in vivo* studies with ileostomy volunteers) that mimics the enzymatic, pH and temperature environment of the gut, to measure the amount of carotenoid available for absorption. After a meal, carotenoids can be recovered from the ileal effluent with no colonic delay or degradation by colonic microflora.

Using the model we have shown that

- The physical form and location of carotenoids in plant tissues are the major determinants of bioavailability
- Cooking and processing of fruits and vegetables does not reduce the carotenoid content and in many cases will increase bioavailability
- The greater the extent of cell rupture during processing, the greater the carotenoid bioavailability
- Carotenoids can transfer directly to micelles during digestion, thus transfer to lipid emulsions in the gut is not the only route from plant cell to the absorptive surface of the gut, as assumed previously.

What are the best dietary sources of folate?

Low body stores of folate are associated with an increased risk of cardiovascular disease, and neural tube defects in children. Increased folate consumption could reduce the high burden of coronary heart disease and stroke but this requires information on the fraction of this vitamin that is usually absorbed from different natural/fortified food sources, or supplements. We have developed a novel LC-MS method that enables us to detect low concentrations of stable-isotope-labelled folate appearing in plasma after the consumption of an acute moderate dose, and measure folate absorption from supplements extrinsically-labelled with ^{13}C and spinach intrinsically-labelled with ^{15}N. Mathematical modelling of the plasma absorption data provides an estimate of the fraction of folate absorbed from the test doses. Using these techniques we have shown that



Percentage absorption, in adult volunteers, of folate from an acute dose (225 microgram) of ^{13}C-labelled folic acid (FA) or 5-formyl tetrahydrofolic acid (Formyl), and natural folate from a ^{15}N-intrinsically-labelled food model (Spinach). This shows that folate absorption from foods is apparently as good as that from supplements.

- Fortification vehicles, such as white bread or bran flakes, significantly reduce the absorption of added folic acid
- Bran flakes (a breakfast cereal matrix) are a significantly poorer fortification vehicle than white bread
- Absorption from different folate supplements is similar, and initial results suggest that absorption from spinach is just as good.

Calcium and bone health

Optimising dietary calcium supply is important for the prevention of osteoporosis. Various food components, such as caseinophosphopeptides (CPP's), have been proposed as absorption enhancers, but we have used stable isotope techniques to demonstrate that calcium absorption was not enhanced by CPP's. We are currently investigating the interactive effects of calcium and salt on bone metabolism in post-menopausal women, the ultimate aim being to provide dietary advice to reduce fracture risk in this 'at risk' population.

Who is at risk of iron deficiency?

Meat is a good source of absorbable iron and enhances iron absorption from foods taken with it; thus there is concern that the increasing avoidance of meat in the UK may lead to iron deficiency. Reassuringly, the results of a recently completed FSA-funded project demonstrated that iron status was similar in vegetarian and omnivorous women of childbearing age. However, iron stores were low in 26%, and absent in 44% of the women, iron depletion being associated most strongly with the amount of menstrual blood lost. Optimising iron bioavailability is an important strategy to prevent iron deficiency anaemia, which occurs in many pregnant women. The usefulness of prophylactic iron supplements in pregnancy is currently under evaluation in a study investigating whether commonly prescribed levels are necessary, or if the same health benefits could be achieved from a much lower iron dose.

How are phytochemicals delivered to target tissues?

Phytochemicals are plant-derived secondary metabolites that are associated with the maintenance of health and the prevention of degenerative diseases such as cancer, heart disease and cataract. Attempts to understand how they provide protection have been hindered by a lack of knowledge regarding their absorption and metabolism.

We have used a combination of *in vitro* studies, cellular models, and an *in situ* human perfusion model to elucidate the important steps in the absorption and metabolism that determine the bioavailability of flavonoids. Our work has shown that human ß-glucosidases and sugar transporters combine to deglycosylate dietary flavonoids and deliver them to epithelial cells where they are conjugated by UDP-glucuronosyl transferases. Further metabolism occurs in the liver to yield a mixture of methylated, sulphated, glucuronidated and mixed metabolites that are delivered to peripheral tissues. The *in vitro* data were confirmed when we identified the metabolites of quercetin in plasma of humans fed onions.

Sulphoraphane is a potent natural anticancer compound present in Brassica vegetables such as broccoli. We have developed robust LC-MS methods for analysis of sulphoraphane and sulphoraphane metabolites that have enabled us to investigate the absorption and metabolism of sulphoraphane from a broccoli extract in humans. Sulphoraphane enters the gut epithelial cells, is conjugated to glutathione, and then most of it is excreted back into the gut. Importantly, there are significant inter-individual differences in the amount entering the cells, and in responses of cells at the level of gene expression. The causes of the large differences in responses between individuals will be investigated using cellular models, human *in situ* perfusions and human intervention studies in combination with genomics and proteomics.

Further reading

Andreasen, M. F., Kroon, P. A., Williamson, G. & Garcia-Conesa, M.T. (2001) Intestinal release and uptake of phenolic antioxidant diferulic acids. Free Radical Biology & Medicine **31** 304-314

Dupont, M. S., Bennett, R. N., Mellon, F. A. & Williamson, G. (2002) Polyphenols from alcoholic apple cider are absorbed, metabolized and excreted by humans. Journal of Nutrition **132** 172-175

Fairweather-Tait, S. J. & Teucher, B. (2002) Calcium bioavailability in relation to bone health. International Journal of Vitamin & Nutrition Research **72** 13-18

Finglas P. M., Witthöft, C. M., Vahteristo, L., Wright, A. J. A., Southon, S., Mellon, F. A., Ridge, B. & Maunder, P. (2002) Use of an oral/i.v. dual-label stable-isotope protocol to determine folic bioavailability, from fortified cereal grain foods in women. Journal of Nutrition (in press)

Southon, S. & Faulks, R. M. (2001) Predicting the bioavailability of antioxidants in food: the case of carotenoids. In: Antioxidants in Food, Practical Applications (Pokorny, J., Yanishlieva, N. & Gordon, M. eds) pp 124-146 Woodhead Publishing Limited, Cambridge

Wolfe, C. A., Finglas, P. M., Hart, D., Wright, A. J. A. & Southon, S. (2001) Isotopic methods to detect food folates. Innovative Food Science and Emerging Technologies **1** 297-302

Wright, A. J. A., Finglas, P. M. & Southon, S. (2002) Proposed mandatory fortification of the UK diet with folic acid: have potential risks been underestimated? [Viewpoint Article] Trends in Food Science & Technology (in press)

Contacts

Sue Fairweather-Tait (minerals programme), Sue Southon (vitamins programme), and Paul Kroon or Yong-Ping Bao (phytochemicals programme)

diet, gastrointestinal health and phytochemicals

how can our diet protect against cancer?

Franziska Kramer from the Danish Food Administration is spending 9 months with Elizabeth Lund studying mechanisms responsible for the suggested protective effect of hormonally active soy components against breast cancer.

One widely held misconception is that cancer "just happens", and that nothing can be done to avoid it. However, many cancers that are common in the west are rare in the developing world. The ongoing revolution in our understanding of the birth, development and death of cells is also revealing the complex protective mechanisms of diet. Much of our work is focussed on the role of dietary microcomponents as protective factors against carcinomas of digestive organs, which cause about 25% of all cancer deaths in the UK.

Understanding cancers of the digestive system

The incidence of stomach cancer has fallen dramatically in the west over the last 50 years but the total number of deaths from cancers of the mouth, oesophagus and stomach is still about equal to those from colon cancer. The protective effects of plant foods against cancer of the upper digestive tract may be even stronger than against colon cancer.

Colorectal cancer

This develops from a stepwise progression of abnormal cells called the *adenoma-carcinoma* sequence. Polyps (non-cancerous growths, a small proportion of which develop into cancer) are very common in the UK; the discovery of reliable dietary strategies to suppress the appearance and growth of polyps would be a major contribution to public health.

We are studying the role of diet in *mutation,* in which a change in DNA sequence disrupts the function of a gene product, and *epigenetic* effects in which a gene is silenced by a chemical modification (methylation) of its promoter region. If a cell acquires genetic or epigenetic damage, it must survive and multiply in order to develop into a polyp. Immature cells often die by apoptosis (cell suicide) soon after stem cell division, and this mechanism is an important route for the removal of genetically damaged cells from the mucosa.

Our recent studies show that phytochemicals exert protective effects at all of these early stages of cancer development.

Suppressing cell growth:
In EU-funded work we have shown that phytochemicals have a profound effect on the growth of intestinal epithelial cells, both before and after they have undergone DNA damage. Flavonoids are an important group of phytochemicals, of which one, quercetin, found in fruits, vegetables and tea, mostly in the form of glucosides, is a powerful inhibitor of cell proliferation both *in vitro* and in the intact mucosa. We have recently used an *in vitro* technique to screen over 70 different flavonoids, and explore the relationships between chemical structures and function. Another important group, the glucosinolate breakdown products found in Brassica vegetables, can induce crypt cell apoptosis, but only in cells that have been damaged by chemical carcinogens.

Blocking mutations:
The mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is formed during cooking and occurs in “well-done” meat. After activation in the liver, PhIP can attack DNA to form PhIP-DNA adducts, which can generate G→T transversion

mutations in mammalian cells.

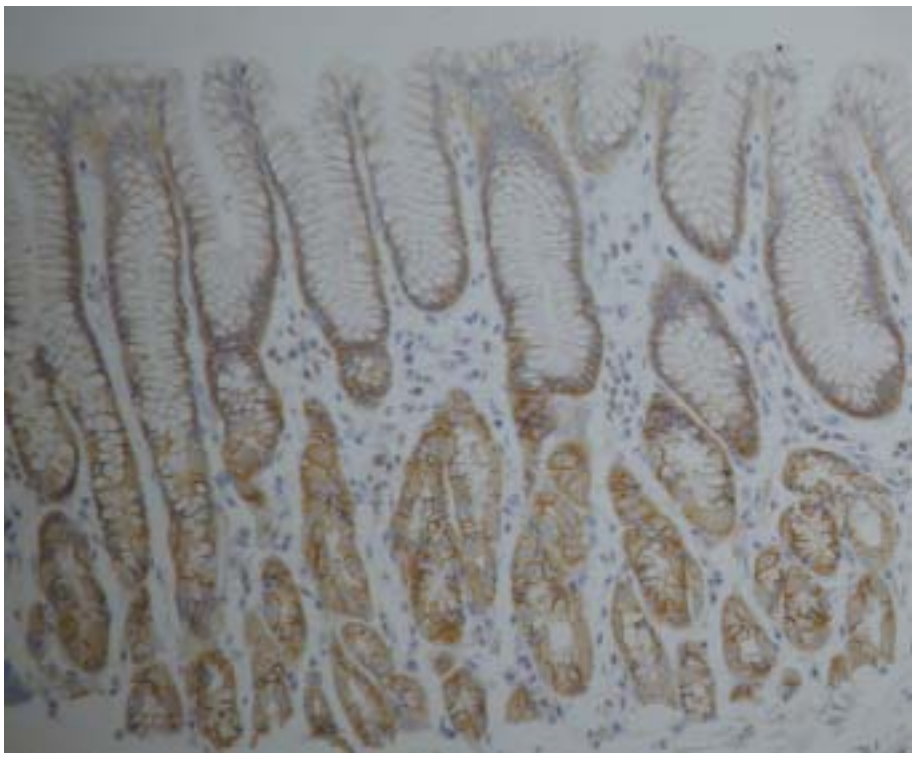
We have examined the effects of two phytochemicals, the isothiocyanate sulphoraphane, and quercetin, on PhIP-DNA adduct formation in human liver cells, using the most sensitive technique available, Accelerator Mass Spectrometry (AMS). Our work has shown that both quercetin and sulphoraphane can inhibit DNA adduct formation in a dose-dependent manner. The protective mechanism of quercetin is through the inhibition of the phase I enzyme CYP 1A2, while sulforaphane acts through the induction of phase II detoxification enzymes such as glutathione transferases and UDP-glucuronosyl transferases. Future work will focus on the gene-specific site of DNA damage and mechanisms of protection and repair, using genomic and proteomics approaches.

Oesophageal cancer

Adenocarcinoma of the oesophagus – the passage between the throat and the stomach - was extremely rare 20 years ago, but it is now increasing more rapidly than any other cancer in the western world. Like colon cancer, it develops from a common precancerous lesion, in this case called 'Barrett's oesophagus', the main risk factor for which is chronic back-flow of stomach contents. Increasing rates of obesity, which favours reflux, may be an indirect, diet-related risk factor for the disease, or there may be some more subtle diet-related mechanism, leading to increased inflammation in response to acid.

Inflamed tissues and cancer cells more actively express one of the cyclooxygenase enzymes, COX-2. Aspirin and similar drugs, which inhibit COX-2, suppress both bowel cancer and oesophageal adenocarcinoma in people. Many flavonoids are also powerful COX-2 inhibitors.

Working with clinicians at the Norfolk and Norwich University Hospital we have confirmed that high levels of COX-2 are expressed in Barrett's oesophagus. We have now begun a search for dietary microcomponents that inhibit the enzyme, and perhaps suppress progression of the disease. Preliminary results show that quercetin suppresses COX-2, and induces apoptosis in oesophageal tumour cells, even more effectively than some non-steroidal anti-inflammatory drugs.



Immunostaining showing COX-2 expression in Barrett's metaplastic epithelium of the human oesophagus (N+NU Hospital)

Further reading

Basten, G. P., Bao, Y. P. & Williamson, G. (2002) Sulforaphane and its glutathione conjugate but not sulforaphane nitrile induce UDP-glucuronosyl transferase (UGT1A1) in HepG2 and HT29 cells. Carcinogenesis (in press)

Bao, Y. P., Bacon, J. & Williamson, G. (2001) Effect of phytochemicals on PhIP-DNA adduct formation in human Hep G2 and hepatocytes. In:- Food: Analysis, Metabolism, Bioavailability & Function (Pfannhauser, W., Fenwick, G.R. & Kokkhar, S. eds.) Royal Society of Chemistry, London pp 589-591

Gee, J. M. & Johnson, I. T. (2001) Polyphenolic compounds: interactions with the gut and implications for human health. Current Medical Chemistry **8** 1245-1255

Johnson, I. T. (2001) New food components and gastrointestinal health. Proceedings of the Nutrition Society **60** 481-488

Lund, E. K., Smith, T. K., Clarke, R. G. & Johnson, I. T. (2001) Cell death in the colorectal cancer cell line HT29 in response to glucosinolate metabolites. Journal of the Science of Food & Agriculture **81** 959-961

Johnson, I. T. (2001) Mechanisms and possible anticarcinogenic effects of diet related apoptosis in colorectal mucosa. Nutrition Research Reviews **14** 229-256

Johnson, I. T. (2002) Glucosinolates: bioavailability and importance to health. International Journal for Vitamin & Nutrition Research **72** 26-31

Further information

A more detailed article is at:
www.ifr.bbsrc.ac.uk/Diet/GITract.html

Contacts

Ian Johnson (diet and gastrointestinal health programme) and Yong-Ping Bao or Paul Kroon (phytochemicals and health protection programme)

searching out biomarkers for diet-related disease

techniques to investigate how diet affects health

Ruan Elliott

Biomarkers are chemical and biological 'signatures' which provide indicators of processes in cells. New and very powerful techniques will allow us to validate biomarkers for changes in metabolic processes, cell function or dysfunction and ultimately health. Within our research, DNA microarrays, proteomics and real-time PCR are being exploited to underpin established programmes.

Prior to the development of microarray technology, researchers could examine, at most, a few genes in any one study; the transcriptional regulation of many thousands of genes can now be analysed in parallel, but the vast quantities of data generated require new approaches for data handling, interrogation and interpretation. Even state-of-the-art software is not sufficient to extract all the useful information, and the integration of microarray data with those obtained from other high-throughput techniques such as proteomics and NMR-based metabolomics is challenging. We are developing collaborative proposals to devise innovative new mathematical approaches and tools for data analysis.

Diet-gene interaction database

Over the next few months we expect to complete the validation and optimisation of standard protocols for analysis of our IFR human microarrays. Most current protocols require substantial amounts of sample RNA. While appropriately sized samples can be obtained routinely using a wide range of *in vitro models* there is a real problem in isolating sufficient RNA from blood and tissue samples that can be obtained ethically during human intervention and population studies. Because of the importance of such studies to IFR's nutrition research, we are assessing alternative strategies that would provide sufficient sensitivity to permit RNA profiling from very limited samples. Success here will substantially broaden our scope to perform gene expression analyses within the context of human studies.

All users of the arrays will adopt the final standardised protocol, enhancing the power of the data generated by maximising our ability to make meaningful comparisons, not only between data sets generated from a single study but also between samples obtained from discrete studies. These standardised data will form the basis of an IFR diet-gene interaction database, which will also be designed to incorporate linked data obtained using other techniques and approaches.

European network

It will be critical for nutrition research to exploit the opportunities provided by the post-human genome sequencing era and the new molecular technologies. With this in mind, we are actively promoting the development of this area across Europe. One of the key results arising from a European workshop on "Nutrigenomics" hosted by IFR in November 2001, was universal agreement from the participants to form a network of research centres. The aims of this network are to raise the profile of nutrigenomics research, seek funding to cement and expand the network and to develop collaborative research projects.

Further information

www.ifr.bbsrc.ac.uk/Safety/Microarrays/

www.ifr.bbsrc.ac.uk/metabolomics

Contact

Ruan Elliott (Programme leader)

food allergy

what makes food allergenic, and what makes people allergic to foods?

Along with other forms of allergic disease, allergies to foods appear to be on the increase. Early onset, but persistent, peanut allergy is a particular problem. As well as health effects, food allergies are a food safety issue and can have considerable social impact on the sufferers and their carers.

About seven foods are responsible for the majority of allergies, usually triggered by the protein components. In peanut not all the proteins are allergens - abundance seems to be one factor, but is not sufficient on its own. Through an EU project co-ordinated by IFR (Protall) it is clear that those plant food allergens that trigger a reaction *via* the gastrointestinal tract belong almost exclusively to one of two structurally related protein superfamilies, which share remarkable stability to processes such as heating (being stable to temperatures between 75-95°C, compared with 45-50°C for most proteins), and the extremes of pH and the proteolytic processing environment found in the digestive tract.

Superfamilies

Prolamin - includes the prolamin storage proteins of cereals, non-specific lipid-transfer proteins, $\propto$-amylase inhibitors, 2S albumins
Cupin - specifically the 11S and 7S globulin storage proteins

Whilst we do not understand the sum of the properties that make a protein become an allergen, it is clear that membership of these protein families increases the likelihood of a protein being a food allergen. Such information is highly relevant to the allergenic risk assessment process performed for novel foods. In order to assist in this process the Protall partnership have developed an on-line searchable database of biochemical and clinical information on allergens from many plant species.

Some important genetic factors are also involved in the development of food allergy in people, but we do not understand the mechanisms involved. And, although we do know that a specific type of T helper immune cells (Th2) have a central role in both inducing and maintaining the allergic inflammatory cascade that results in the well-recognised symptoms of allergy, we do not understand the factors involved here either. The current thinking is that production of the different types of T cells (Th1 and Th2) is influenced by both environmental and genetic factors, and that certain cells have a profound influence on the nature of the T helper responses.

Dendritic cells (DC) are a particular type of immune cell that have an unique potential for absorbing and processing antigens at extremely low exposure levels, to produce highly immunogenic forms that stimulate the naïve T cell system. Several studies have suggested a role for dendritic cells in the pathogenesis of allergic diseases, but most of these studies focused on asthma and very little is known about adverse reaction to food. We have started to address the role of DC in the sensitisation process in adverse reactions to food at the cellular and molecular level, and have data suggesting that DC are heavily involved in the host's sensitisation to food components. In addition, we want to test the hypothesis that human DC might be used to establish an *in vitro* cell-based method for determination of the allergenic potential of food allergens.

Further reading

Mills, E. N. C., Madsen, C., Shewry, P. R. & Wichers, H. J. (2001) Plant food protein allergens – the role of structure and function in allergenic potential. Food Allergy & Intolerance **2** 194-209

Further information

www.ifr.bbsrc.ac.uk/protall
www.akh-wien.ac.at/safe/

Contact

Claudio Nicoletti and Clare Mills

Communication is a key element for EU-funded projects on food allergy issues

working with people

what people think and what they eat

Further reading

Lambert, N., Dibsdall, L. A. & Frewer, L. J. (2002) Poor diet and smoking: the big killers. British Food Journal **104** 63-75

Dibsdall, L. A., Lambert, N. & Frewer, L. J. (2002) Understanding the experiences of a low-income group of UK women towards aspects of food and cancer prevention: a qualitative study. Journal of Nutrition Education (in press)

Lambert, N. (2001) Food choice, phytochemicals and cancer prevention. In: Food, People and Society (Frewer, L. J., Risvik, E. & Schifferstein, H. eds.) pp. 131-154, Springer-Verlag, Berlin

Contacts

Lynn Frewer

David Hughes
(Scientist in Charge, HNU)

If people don't believe they have a problem

Poor diet is a major risk factor for the development of many cancers in the UK, and increasing the consumption of fruit and vegetables is the key component of Government initiatives aimed at improving the nations' diet – the messages are particularly targeted towards low-income groups. But what really determines peoples' attitudes and behaviours towards healthy eating?

We interviewed white, female tenants from a large East Anglian Housing Association, where information characteristics, egocentric systems and control issues governed attitudes and behaviour towards nutrition and cancer protection. Phase 2 involved testing the themes of access, affordability and motivation to eat fruit and vegetables that arose in the qualitative phase. In one such study, questionnaires were sent to over 3000 Housing Association properties. We identified distinct demographic clusters regarding fruit and vegetable consumption; for example young, male jobseekers who smoked were the least likely to eat fruit and vegetables. Access and affordability were not major barriers to fruit and vegetable consumption for the tenants, and only 4% felt they were not eating healthily.

Selected questionnaire responses for Housing Association tenants

Descriptor	% of Tenants
Regularly shop at large supermarkets	90
Unhappy with their major food shop	4
Unhappy with choice of fruits and vegetables	1
Thought fruit and vegetables too expensive	4
Thought they had a problem with healthy eating	4

Although studies are ongoing, it is clear that a major barrier facing those promoting healthy eating is convincing target groups that they have a problem.

Human nutrition

Our 'volunteer database' contains the names of 850 people willing to help with nutrition research. This often takes place in our Human Nutrition Unit (HNU), a detached building on-site, providing a people- and research-friendly environment. The HNU consists of a medical room, clinical laboratory, diet kitchen, living room with dining area and 5 bedrooms used either for residential studies (each with *en suite* facilities) or as interview rooms, laundry and food storage rooms.

About 20 projects are on-going, funded from external sources (e.g. FSA, EU, industry) or the BBSRC core strategic grant. Work varies, from a single blood sample for *in vitro* experiments, to long-term dietary interventions with meals formulated and prepared in our diet kitchens. We welcome approaches from outside organisations that wish to use the HNU's facilities.

IFR's Human Research Committee reviews and approves all proposed studies involving volunteers, in accordance with the principles of the Department of Health Research Governance Framework for Health and Social Care, prior to their submission to the appropriate Ethical Committee. We have adopted the principles of Good Clinical Practice, with a view to accreditation when the GCP Monitoring Authority extends the scheme to include nutritional studies.

Food Material & Ingredients Theme Leader

Dr Reg Wilson

Within the European Community, 500,000 tonnes of environmentally unfriendly onion waste is produced every year. IFR science is helping to understand how this wasted material can provide useful products. One such product is quercetin crystals from onion (pictured left); quercetin and related compounds are flavonoids, and there is good evidence that these types of compounds protect against heart disease and cancer (Scanning Electron Micrograph by Mary Parker, IFR; see for example Waldron, K. W. et al. *(2001) Food Science & Technology Today* ***15*** *38-41)*

food materials and ingredients

Theme overview

The Food Materials and Ingredients Theme aims to develop a basic, fundamental understanding of the relationship between food structure and functional properties to enable the rational design of foods of improved quality. This understanding will be used to develop predictive models and tools that we can exploit for industrial and consumer benefit, via alliances with organisations having skills and experience of food processing and engineering.

Our science will underpin the development of improved foods offering greater eating pleasure combined with more efficient nutrient release and resistance to microbial growth, combined with optimum shelf life.

Our objectives are:

- **To improve and control composition and structure of manufactured and plant based foods in relation to quality and performance, and to develop new approaches for analysis and control**

May 2002

food materials & ingredients

improving functionality and enjoyment

The formation, stability and breakdown of structures within foods are extremely important in determining key quality attributes such as texture, flavour release, nutrient availability, moisture migration and microbial growth. Our research programmes are aimed at understanding how important structures such as protein and carbohydrate networks, interfacial structures, plant cell walls and tissues determine the functional properties of food.

We are interested in understanding how the structures can be formed or modified naturally or during processing and subsequent storage as well as during digestion. The understanding we will acquire will be used to develop predictive models to allow the rational design of raw materials and foods for consumer and industrial benefit.

Key strategic alliances are being developed with organisations possessing pilot plant or food engineering capability to assist the rapid exploitation of our fundamental science, in addition to new and existing interactions with the food industry. We are well equipped with advanced and unique biophysical characterisation tools that provide us with an extraordinary capability to probe food structure.

The research within the theme is divided into three programmes.

Understanding complex foods

This is the theme's largest research programme, primarily concerned with processed foods of which foams and emulsions are a major category. We have continued to develop new methods to visualize interfacial networks in these systems and our work is leading to new thinking on the basic mechanisms of interfacial stability that are now being exploited industrially.

Starch is a major food component. We are undertaking new studies aimed at developing design targets for new starches, and our work in crystallisation and processing of starch products is enabling us to predict and control the shelf-life of starch-rich foods.

A new initiative involves the application of advanced physics to predict the behaviour of particulate foods and to explain storage events. This work will explain stability and breakdown of a range of important processed food types.

In addition to this work, which is explained in greater detail on pages 27 and 28 and on the IFR website, we are also actively investigating protein functionality in relation to the bread-making process and allergenicity.

Plant tissue structure

Plant-based products are another important range of food products eaten raw or processed e.g. cooked, frozen or canned. The texture of such products is still of major concern and we are studying the relationship between composition and structure of plant tissues and quality parameters.

Now concentrating on the mechanical properties of the plant cell wall and cell adhesion, our work on plant tissue structure is advancing the understanding of the molecular basis of texture, and assisting us to develop models and means to control the quality of raw materials or to improve processing and post-harvest treatments, leading to better quality products.

In the past year we have made significant progress using *Arabidopsis* as the main model system, backed up with selected food-based systems. We are also proposing new approaches to characterising and locating important molecules responsible for cell adhesion; a primary determinant of texture in cooked produce.

In the future we intend to exploit our knowledge to increasing benefit in food plants.

Measurement and data analysis

This programme has backed up the main strategic thrust of all IFR's research themes with underpinning science in areas such as metabolite profiling, authentication and data analysis. Recent developments are highlighted on page 30.

For the future we will concentrate on developing methods to guarantee food authenticity, particularly in new areas such as organic food, and to provide the industry with new sensing techniques to improve processing and control assurance for the consumer.

our eating experience

answers to frequently asked questions

Food is very complicated. Raw ingredients such as cereals, fruit and vegetables are made up of thousands of chemicals.

The way these materials behave during processing – whether in your home, or in a food factory, determine whether the finished food has good texture, aroma and taste, keeps well and is pleasurable to eat.

If we can understand how the structure of food (like the architecture of a building) links to its properties, then we have a rational way to improve quality, nutrition and safety.

? What sort of foods do you work on?
We're interested in raw ingredients including cereals, fruits and vegetables; processed foods like bread and dairy products; and food additives – for instance emulsifiers (which stop food mixtures such as salad dressing separating).

? Why?
Because this research can result in better quality foods, less wastage and a more satisfying eating experience.

? What does 'fresh' really mean?
Fresh means different things to different people. We're interested in changes in eating quality during storage, for instance fruit crunchiness or mealiness.

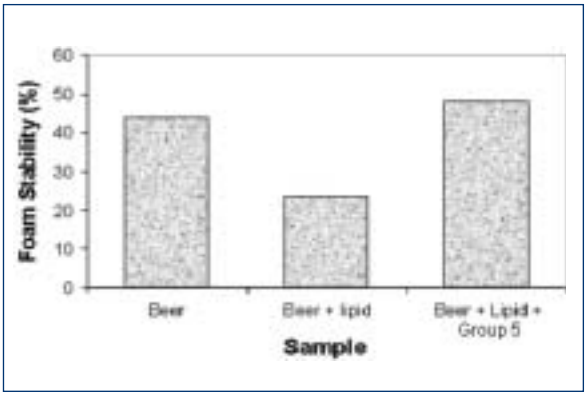
? The bread we buy seems OK, so why would you be interested in that?
Bread could be made to stay fresher for longer without any changes in quality or nutrition, if we understood how the ingredients aged.

? Can we trust what's on the label?
Food fraud costs the industry millions, and they use methods developed at IFR to assure the purity of products such as coffee, olive oil, fruit juice, jams, meat and fish products. They, and you, need to know you are paying for the contents as described.

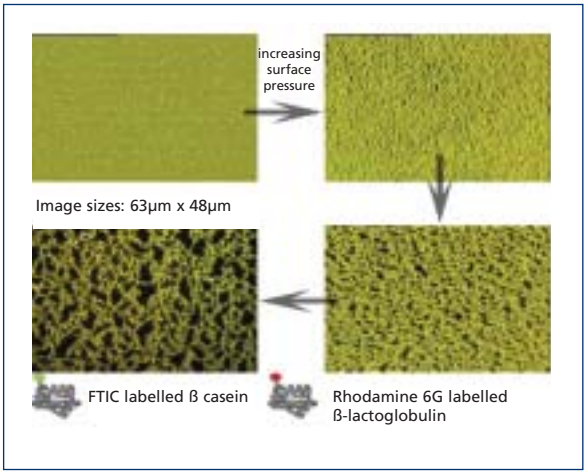
? Why is waste an important issue?
Nobody likes paying for food that isn't used, whether it's industrial landfill or the kitchen bin – if we use raw materials more effectively we can reduce the environmental burden.

understanding complex foods

satisfying demands for better quality



The effects of lipid and the restoring effect of a lipid-binding protein fraction (group 5) on the foam stability of beer.



Fluorescent microscopy images of mixtures of proteins from an air-water interface displaced by lipid (increasing surface pressure). Current resolution is about 100 nm. (Images by Alan Mackie and colleagues)

In order for the food industry to satisfy demands for better quality foods it needs to be able to rationally modify processes and enhance the value of raw materials. Our work on complex foods underpins understanding of texture, taste, aroma and long-term stability and supports the industry in manipulation and modelling of flavour release, and in prediction and control of long-term stability of low-moisture foods. At IFR we are undertaking research to define – and predict – the changes in structure that occur during food manufacture.

Foams and emulsions

Many processed foods are foams or emulsions, whose form and shelf life depend on the structure and stability of their air-water or oil-water interfaces. Despite a wealth of information on the interfacial action of individual proteins or lipids, it is still difficult to describe foods containing mixtures of proteins and lipids. At IFR we have developed new microscopic methods allowing imaging of the molecular structure of mixed interfaces. Visualisation of protein networks showed that resistance to fracture determines interfacial stability. This led to the discovery of a new molecular mechanism by which lipids displace proteins, causing instability of foams and emulsions. It is now possible to fluorescently label proteins and visualise changes within mixed protein networks as lipids disrupt them. Images of fluorescently labelled protein mixtures show a uniform colour with no evidence of phase separation (see left). Displacement with lipids progressively changes the colour of the network, suggesting that the 'stronger' protein more ably resists displacement and determines the ultimate stability of the interface. Such studies provide a generic description of interfaces appropriate to foods. This is being exploited through industrial and LINK funding to investigate interfacial behaviour in dough liquors and beer foams, and during development of texture in whipped products and ice cream.

For example, the major factor determining the perceived quality of beer is the appearance of the foam. Beer foams are stabilised by proteins that come from malted barley and withstand the harsh processing during brewing. Lipid destabilisation of beer foams means that dirty glasses and traces of fatty foods can drastically reduce foam stability. We have identified proteins that protect the foam by binding lipids. This offers the potential to enhance quality through selective breeding of barley or rational modification of the malting or brewing process.

Interfacial networks are formed when proteins adsorb, partially denature and associate. Defining changes at the interface is key to understanding the ability of proteins to stabilise emulsions. Spectroscopic methods have been used successfully to probe unfolding and partitioning of sunflower protein at oil-water interfaces, to explain its ability to stabilise emulsions. This forms the basis for developing screening methods to identify new plant proteins as potential alternatives to animal proteins currently used by the food industry.

Highlighting starch

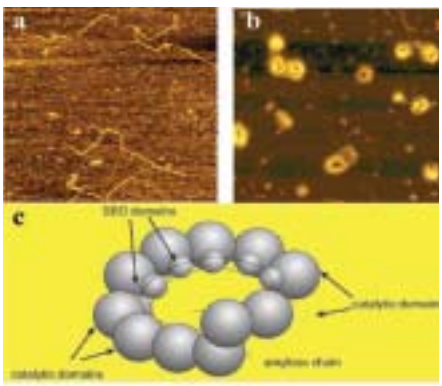
Major bulk components of foods are carbohydrates and proteins. Starch is by far the most important carbohydrate. Degradation of crystalline starch is of importance in its industrial use as a substrate for enzymatic modification, and for understanding fermentation of 'resistant starch' by gut micro-flora. Glucoamylase, which can degrade crystalline starch, is a multi-domain enzyme, and there is interest in the mechanisms by which the starch-binding domain facilitates hydrolysis. Our direct visualisation of complexes between genetically-inactivated glucoamylase and amylose has demonstrated that the binding domain alters the amylose conformation prior to hydrolysis.

We have developed predictive models of the melting of starch crystals and demonstrated how NMR can reveal the dynamics of water redistribution in starch during gelatinisation. Images of the ultra-structure of 'native starches' will be used for development of molecular models of starch gelatinisation. The ability to image granule structure will also allow investigation of the structural origins of functional changes induced in mutant starches, with a view to developing targets for design of new starches.

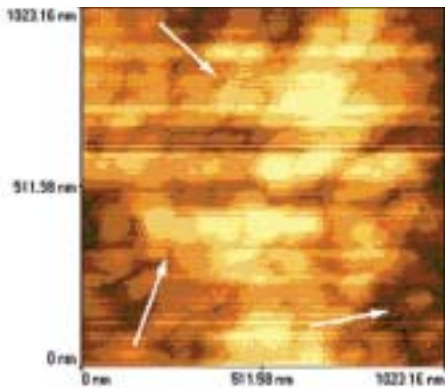
Changes in stiffness of stored starchy foods impact on their shelf life. A common occurence is slow phase separation and crystallisation of starch chains, leading to network formation and 'firming' (eg in bread staling). We have demonstrated another type of time-dependent change in processed, low-water starch products (pasta, snack foods), which are brittle, glassy solids. Using models based on synthetic polymers we can predict time-dependent embrittlement of starch-rich foods. The potential for this approach in optimising food storage is being examined via LINK collaboration.

A recurring theme

Despite the differences in the detailed structure of food products (gels, colloids, particulates) their behaviour can be described by using generic predictive models, developed as part of modern materials science. We have explored similarities between motion of grains in a powder, oil droplets in dense emulsions and molecules in super-cooled liquids such as sugar glasses. In all cases particular combinations of temperature, density and applied stress cause the particles to become 'jammed' and the system meta-stable. This is closely linked with formation of glass-like states and may explain ageing. In granular materials, the features (bridges or arches) are often responsible for stopped or intermittent flow in silos or hoppers. Understanding the physics of arrested structures provides opportunities for improved processing of foods.



Atomic force microscopy studies of the interaction of amylose with glucoamylose. The linear amylose molecules (a, scan size 1 x 1 µm) form circular complexes (b, scan size 0.8 x 0.8 µm) with the inactivated glucoamylose molecules. The schematic model of the interaction (c) provides evidence for the role of the starch binding domain (SBD) in the mode of action of the enzyme. (Images by Patrick Gunning and colleagues)



Atomic force microscopy image of the ultra-structure within a wheat starch granule, showing 'blocklet' structures (arrowed) in the vicinity of a growth ring. (Image by Mike Ridout)

Further reading

Burnett, G. R., Rigby, N. M., Mills, E. N. C., Belton, P. S. , Fido, R. J. , Tatham, A. S. & Shewry, P. R. (2002) Characterisation of the emulsification properties of 2S albumins from sunflower seed. Journal of Colloid & Interface Science **247** 177-185

Giardina, T., Gunning, A. P., Faulds, C. B., Juge, N., Furniss, C. S. M., Svensson, B., Morris, V. J. & Williamson, G. (2001) Influence of the two binding sites of the starch-binding domain of *Aspergillus niger* on amylose conformation. Journal of Molecular Biology **313** 1151-1161

Mackie, A. R., Gunning, A. P., Ridout, M. J., Wilde, P. J. & Morris, V. J. (2001) Orogenic displacement in mixed ß-lactoglobulin /ß-casein films at the air/water interface. Langmuir **17** 6593-6598

Parker, R. & Ring, S. G. (2001) Aspects of the physical chemistry of starch. Journal of Cereal Science **34** 1-17

Pugnaloni, L. A., Barker, G. C. & Mehta, A. (2001) Multi-particle structures in non-sequentially reorganized hard sphere deposits. Advances in Complex Systems **4** 289-297

Ridout, M. J., Gunning, A. P., Wilson, R. H., Parker, M. L. & Morris, V. J. (2002) Using AFM to image the internal structure of starch granules. Carbohydrate Polymers (in press)

Tang, H. R., Brun, A. & Hills, B. (2001) A proton NMR relaxation study of the gelatinisation and acid hydrolysis of native potato starch. Carbohydrate Polymers **46** 7-18

Wilde, P. J., Husband, F. A., Cooper, D., Mackie, A. R., Gunning, A. P., Morris, V. J., Woodward, N. & Mills, E. C. N. (2001) Interfacial mechanisms underlying lipid damage of beer foam. Proceedings of the 28th European Brewing Convention Congress, Budapest May 12-17th 2001. Fachverlag Hans Carl, Nurnberg

Further information

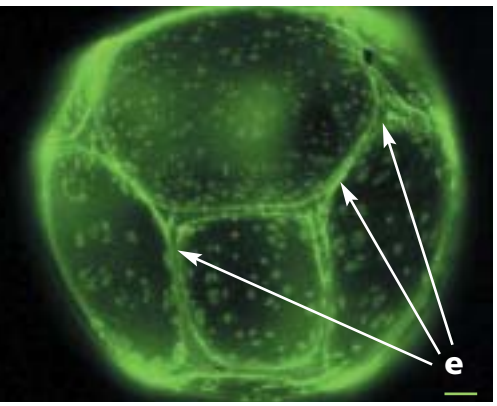
www.ifr.bbsrc.ac.uk/complexfoods2002

Contact

Vic Morris (Programme leader)

plant tissue structure

relating cell wall architecture to food quality



Localisation of JIM5 monoclonal antibodies on potato cell surface (Image by Mary Parker, Neil Rigby and Dr Keith Waldron)

Further reading

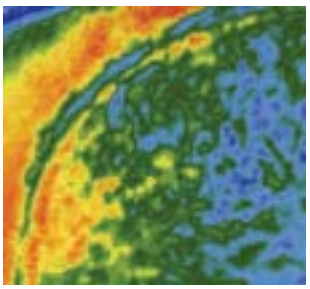
Parker, C. C., Parker, M. L., Smith, A. C. & Waldron, K. W. (2001) Pectin distribution at the surface of potato parenchyma cells in relation to cell-cell adhesion. Journal of Agricultural & Food Chemistry **49** 4364-4371

Toole, G. A., Smith, A. C. & Waldron, K. W. (2002) The effect of physical and chemical treatment on the mechanical properties of the cell wall of the alga *Chara corallina.* Planta **214** 468-475

Rodriguez-Arcos, R. C., Smith, A. C. & Waldron, K. W. (2002) Mechanical properties of green asparagus. Journal of the Science of Food & Agriculture **82** 293-300

Contact

Dr Keith Waldron (Programme leader)



The focal plane array detector on the JREI-funded infrared microscope allows us to map the distribution of components in tissues at a spatial resolution of 5 µm (image of wheat grain)

Why are some fruits and vegetables soft and others crisp when we eat them? Understanding structure will not only provide a basis for predicting and controlling the texture of fruits and vegetables, but will also help us understand how cell contents such as flavours and nutrients are released during and after the meal.

Plants are very complex structures with numerous tissues each of which has specific, or multiple, biological functions including water transport, photosynthesis, rigidity and support. We need to understand the relationships between cell-wall architecture, structure, and mechanical properties, and how these relationships change along the food chain. The textural quality of any fruit or vegetable is strongly dependent on the relationship between cell-wall rupture and cell separation. This relates to the relative strengths of the cell wall, and intercellular adhesion. During fruit ripening and thermal processing, the softening of plant tissues is dominated by biochemical or chemically-induced cell separation.

Cell-wall polymers and wall strength

Currently, no definitive model of the cell wall exists, particularly one that relates the cell wall composition to its mechanical properties. We have been investigating the role of cellulose, pectin and hemicellulose in determining cell wall mechanical properties using advanced spectroscopic techniques based around Fourier transform infrared spectroscopy combined with *in situ* mechanical deformation. Studies on systems such as onion epidermis, *Chara corallina, Arabidopsis,* and *Acetobacter* cellulose composites are shedding new light on the interactions between the biopolymer networks of the plant cell wall.

We have shown that cellulose and pectin networks are largely independent or only interact weakly through hydrogen bonding. Pectin facilitates the realignment of cellulose microfibrils in systems under strain, but hemicellulose interacts much more strongly with cellulose and makes the network more rigid. Studies on mutant and transgenic food plant and model systems will allow us to study the effect of compositional variation on wall strength. Using a new focal plane array infrared microscope we will be able to map samples in order to locate tissue-specific effects in mutants exhibiting a mechanical phenotype.

Cell adhesion in many plant tissues is dependent on polymers at the edges of cell faces. This was highlighted by previous work at IFR on Chinese waterchestnut (CWC) in which we found that cinnamic acid cross-links are probably responsible for the maintenance of crispness. More recently the use of antibodies such as JIM5, which bind preferentially to pectic polysaccharides with low levels of esterification, have provided similar patterning, reflecting the localisation of de-esterified pectic polymers in these locations. We know relatively little about the chemistry and cross-linking of cell-wall polymers at the edges of cell faces; purifying polymers from functionally-interesting wall locations will enable us to characterise these polymers with a wide range of spectroscopic and (bio)chemical techniques. This work is being complemented by the development of probes such as inactivated cell-wall-degrading enzymes for localising components within cell walls and for detailed structural characterisation of cell-wall polymers by Atomic Force Microscopy.

measurement and data analysis

quality assurance and chemical profiling

Used in conjunction with pattern recognition methods, chemical profiling can tackle a range of important issues, from adulteration detection in food products to metabolite profiling of raw materials (where a particular constituent may have an important influence on the value of the crop).

Our approaches are based on instrumental techniques such as infrared and nuclear magnetic resonance (NMR) spectroscopies, and gas and high performance liquid chromatographies (GC, HPLC), often linked to mass spectrometry (MS).

Authentication

A long-term programme of research at IFR has demonstrated the potential of infrared spectroscopy and high-resolution proton NMR spectroscopy for the authentication of a wide range of different foodstuffs. Most recently we have developed a rapid method for detecting adulteration of hand-pressed, fresh strawberry and raspberry purees. This methodology has been transferred to an independent analytical services company, which offers a commercial screening service. Work is ongoing on extending the 'unadulterated' database, with which unknowns are compared during the screening, to include a wider range of industrially-prepared specimens.

Metabolite profiling

NMR, GC and HPLC are also suitable for profiling a broad range of plant metabolites from soft fruits, green teas, tomatoes and potatoes.

Worldwide, the potato crop is hugely important, due to its high yield and ability to thrive in a wide range of environments and, for many potato processors, an important characteristic of a crop is its sugar concentration. High sugar levels result in an excessively dark-coloured product, due to Maillard reactions between sugars and certain free amino acids.

We are currently studying the effect of low (2°C) and high (20°C) temperature storage on the chemical composition of potato tubers. NMR is the main analytical technique employed in this study. As well as sugar concentrations, we are investigating the effect of storage temperature on minor metabolites, including amino acids, phenolic compounds and organic acids. To date, we have observed significant changes in the content of phenolic acids, and in certain free amino acids (glutamic acid, aspartic acid and alanine).

Further reading

Al-Jowder, O., Kemsley, E. K. & Wilson, R. H. (2002) Detection of adulteration in cooked meat products by mid-infrared spectroscopy. Journal of Agricultural & Food Chemistry **50** 1325-1329

Wilson, R. H., Colquhoun, I. J. & Kemsley, E. K. (2001) Screening food products for authenticity and adulteration: the use of infrared spectroscopy. American Laboratory (News Edition) **3** 54-55

Al-Jowder, O., Casuscelli, F., Defernez, M., Kemsley, E. K., Wilson, R. H. & Colquhoun, I. J. (2001) High resolution NMR studies of meat composition and authenticity. In: Magnetic Resonance in Food Science: a View to the Future, (G. A. Webb *et al.* eds.) Royal Society of Chemistry: Cambridge, pp232-238

Contact

Kate Kemsley (Programme leader)

Authenticity issues in a range of raw materials and products have been studied using chemical profiling

enterprise

building bridges across the science / business interface

IFR's recently established Enterprise Unit is led by Sue Southon. Sue will be looking for opportunities to add value from interactions with external fundholders, transfer of technology and science know-how, and international collaboration.

Intellectual property

We are establishing a more focused and robust framework to manage our intellectual property, and IFR is a participant in the successful ICENI bid of up to £3M to the University Challenge Seed Fund initiative with UEA, the University of Essex, The Sainsbury Laboratory, John Innes Centre and Plant Bioscience Ltd (PBL). This forms an important part of the overall "downstream" financing strategy, where network links have also been established with a number of funding sources.

The prime aim of ICENI will be to exploit the strong base of intellectual property flowing from the Norwich Research Park and from the University of Essex. The Fund will be used to support the protection of intellectual property where commercial potential exists, to take existing basic science forward to the proof-of-concept stage and to support the development of prototypes. The Fund will be used to support and nurture the flow of intellectual property, with a particular aim of starting new technology-based ventures. Where appropriate, licensing arrangements will be considered. Where the spin-out route is the chosen option, support for detailed business plan preparation and company launch will be an important part of the Fund's usage.

In order to identify and encourage exploitable IP, we will also benefit from a Biotechnology Platform Award, microBEP, led by PBL, focusing on the identification of microbiological IP in *Lactococcus* research, and complementing *Streptomyces* expertise in the John Innes Centre. Also working with PBL, IFR has won a Capacity Building Grant under the Department of Trade and Industry initiative to help organisations develop the exploitation of their intellectual property. The award has funded the appointment of a Technology Acquisition and Licensing Executive, Nicola Stockman. These two important activities represent a significant bridge between IFR commercial strategy and the external world that can benefit from, and exploit commercially, the results of IFR research.

Left to right: Anette Fillery-Travis (Enterprise Unit Support Officer), Sue Southon (Enterprise Manager) and Nicola Stockman (Technology Acquisition and Licensing Executive)

Food and Health Network

As the leading food research centre in the UK, we seek to develop our contribution to wealth creation in the UK and European food and drink and related Industries. IFR believes that the active creation of bridges with industrial companies will aid this process. We have set up the Food & Health Network (FHN) as a forum to improve knowledge transfer in areas where we can make a real contribution to industrial profitability.

In July 2001, FHN was launched at a very successful meeting in Norwich. Four expertise clusters:- Predictive Microbiology and Risk Analysis, Waste Upgrade, Shelf Life Extension and Barriers, are under way. A very strong industrial representation has already joined, with the plan to achieve an optimal industrial membership of around fifteen by the end of 2002. A number of other clusters, particularly in Diet and Health, are under active discussion and at least one is planned to be operational during 2002. Discussions have been very open and there is already a real expectation of progress being made on some of the more important industrial problems.

FHN website front page

Visits

Multinational and major national organisations continue to visit IFR to participate in a series of tailor-made discussions focused on their research needs. IFR's Industrial Team has played an important part in recognising the need to understand individual requirements, and to present IFR science in relevant ways.

Further information

www.foodandhealthnetwork.com

Contact

Professor Sue Southon (Enterprise Manager)

Launch of the FHN, July 2001

National Collection of Yeast Cultures

Good progress has been maintained in the marketing of the yeast strains in the National Collection of Yeast Cultures (NCYC), particularly those for the brewing industry via our collaboration with Cara Technology. We are building on this progress by developing other commercial opportunities in, for example, food-spoilage forensics, identification of clinical isolates and exploitation of non-conventional yeasts. A number of external organisations have already initiated collaborative efforts with us in these areas.

The scientific importance of sustaining NCYC in the post-genome era is widely recognised. We expect our ability to define yeast variation at the molecular level to be called upon with increasing frequency by stakeholders throughout the agri-food industry and biomedical sciences.

Further information

www.ncyc.co.uk

Contact

Dr Ian Roberts (Curator, NCYC)

international

networking to benefit IFR science and stakeholders

UK/Israeli Workshop, March 2002

The year has seen a continuation of the activity of Institute staff in projects funded by the European Commission. In addition to successful applications for RTD and Concerted Action funding, we have been recognised as a European Centre of Excellence in Training (see next page). We are especially pleased with our developing links to research organisations in Central and Eastern Europe and beyond; particularly welcome at a recent meeting of an INTAS project at IFR were researchers from Tajikistan and Azerbaijan.

Workshops

Two bilateral workshops have been held during the year. In July 2001 we hosted researchers from the USDA-ARS during a UK-North American Food Safety Workshop. Subsequently, Alastair Robertson and Mike Peck were invited to attend the annual ARS Food Safety Review in Williamsburg, Virginia; further meetings to discuss future US-UK co-operation are planned for later this year and a Memorandum of Understanding has been signed between IFR and the ARS.

In March 2002, a UK-Israel workshop on *Food in the Post-genomic Era* was held at Norwich and served to highlight future opportunities for co-operation in the areas of food safety and nutrition. These workshops were supported by BBSRC, and the British Council/Israel Ministry of Science, respectively.

The European Commission also gave financial support to the by-invitation *European Nutrigenomics Workshop* coordinated by Ruan Elliott (see page 21).

SAFE Consortium

IFR is a founder member of the SAFE Consortium – a network of European Centres of Excellence in food safety, with VTT (Finland), TNO Food and Nutrition Research and Wageningen University Research Centre (The Netherlands), INRA (France) and ISPA (Italy). SAFE members are working together to provide added value advice at an international level to the Commission and European Governments.

Sixth Framework

Currently much attention is being focused by staff on the forthcoming Sixth Framework programme of the European Commission. In order to harness relevant European experience, we initiated and hosted a workshop in July 2001 for Directors of leading food and nutrition centres. Attended by members of the European Commission, this served to identify key challenges and opportunities in the agri-food sector, discuss and compare best practice and determine relevant research targets for Framework VI. A number of subsequent meetings have been held with the aim of delivering appropriate Expressions of Interest to the Commission.

Further information

www.ifr.bbsrc.ac.uk/business/international.html

www.safeconsortium.org

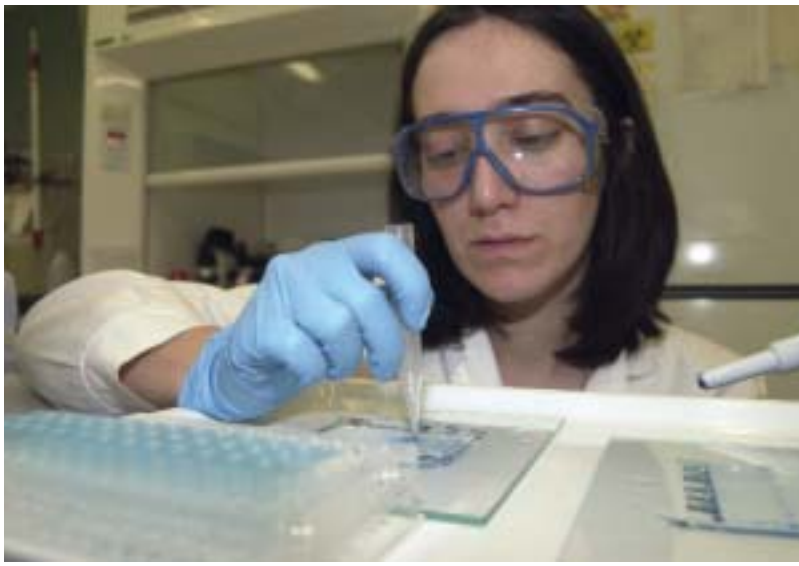
Contact

Roger Fenwick
(International Coordinator)

training

building for the future

IFR is a centre for training of young scientists in the underpinning sciences associated with food issues. At any one time about 20 students are undergoing PhD training, virtually all registered at the University of East Anglia. They have access to training in a wide range of transferable, employment-related skills provided both by UEA and Norwich Research Park staff, and additionally by BBSRC's central training section. Students complete a modular course of training 'Professional skills for postgraduate scientists' formulated by the University's Science Group in consultation with IFR and the John Innes Centre. Equally, supervisor training is an important issue, and imaginative briefing sessions for new and experienced supervisors have been developed by UEA, based on the discussion of case studies.



Typical of the Training Site post-graduate students is Eleni Pappa from The Natural Agricultural Research Foundation, Dairy Research Institute, Greece who worked with Jim Robertson studying protein profiles during ripening of Teleme, a type of Greek cheese.

Amongst recent successful PhD candidates were Susan Miles for a thesis on 'Public perception of five food hazards: investigating optimistic bias and perception of uncertainty information', and Tracy Smith for 'Modulation of colonic epithelial cytokinetics and apoptosis by glucosinolates and their breakdown products'. Ana Sancho from Consejo Superior de Investigaciones Cientificas, Madrid recently completed her EU-funded European PhD on 'Polysaccharide degradation in cereals: cinnamoyl esterase activity and protein inhibitors against xylanases and ∝-amylases'; her thesis was written in both Spanish and English versions.

IFR has also received EU recognition as a major site of excellence in training for young scientists by the award of four Marie Curie Training Sites, 2000-2004, funded through Framework V's Quality of Life Programme. The areas are: 'Bioavailability of micronutrients', 'Phytochemicals and health', 'Engineering carbohydrate functionality' and 'Food emulsions and foams'. A fifth award, 'Bacterial functional genomics', is a joint Site with JIC and the Syngenta Genome Centre.

IFR's scientific and support staff are continually encouraged to progress their academic and vocational qualifications, and technical skills.

Two staff achieved PhD's 'by publication' in 2001. Jenny Gee submitted an illustrated 11,000-word critical review of a selection of her publications - 40 papers, brought together under the title 'Interactions between dietary phytochemicals and epithelial cells of the mammalian gastrointestinal tract' which covered about 70% of her peer-reviewed publications with Ian Johnson over the past 20 years. Peter Wilde was awarded a PhD by publication, with the School of Chemical Sciences at UEA, for 'Interfacial mechanisms underlying the stability of protein stabilised foams and emulsions'.

Ben Piggott gained a 1st class honours degree in Computing and Computer Science from the Open University. And, following BTEC and HTEC, Abigael Polley has achieved an OU pass degree in Biology, but is taking a year out to nurse a new FSA-funded project before tackling the final 30 points she needs for her Honours.

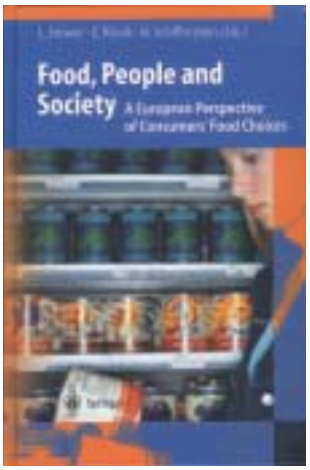
Examples from support include Dawn Barrett (Communications), who gained a Diploma in Administrative Management, and Marcus Yarham (Finance), who has passed examinations for parts 1, 2 and 3 leading to the Chartered Institute of Management Accounts qualification.

Contact

Human Resources

communicating science

communicating with stakeholders



IFR News redesign, 2002

Clare Mills uses the ISDN line from IFR to do a media interview

Scientific communications

A highlight of scientific communication in 2001/2 were the awards to Roger Fenwick and Vic Morris of 'Highly Cited Researcher' status by the Institute for Scientific Information (ISI) in Agricultural Sciences as two (out of four in the UK) of the 250 most cited authors worldwide in the field of Agriculture since 1981. This news validates our policy of publishing widely, using mechanisms most relevant to the particular stakeholder audiences. IFR staff and students interact widely with many stakeholder audiences, and we can give only a snapshot of our activity here.

Reaching more academic audiences, IFR has an extremely high publication rate in journals as varied as Applied and Environmental Microbiology (where a paper on the use of *Enterococcus* spp. in foods was selected as one of the American Society of Microbiology's 'best of the best' in 2001), Biochemica et Biophysica Acta, Nature – Reviews, American Journal of Clinical Nutrition, Clinical Science and The Plant Cell. IFR scientists also contribute to books that discuss different research approaches and methodologies – for example Lynn Frewer was a co-editor of 'Food, People and Society – a European Perspective on Consumer's Food Choices', published in 2001.

For industry audiences, briefing papers have appeared in publications such as Food Safety Express and Liquid Food and Drink Technology. Web-based communication is also increasingly popular – typical are www.Foodnavigator.com, and www.nature.com where Paul Finglas gave a briefing on the scientific issues underpinning the politically-sensitive option of fortification of flour with folic acid.

Science in print and on the web

In addition to our annual corporate publication 'IFR in….', we print IFR News quarterly, and mail out to nearly 5000 contacts worldwide. For all the EU contracts that IFR coordinates, we publish newsletters and brochures, and develop websites to describe aims and outputs, targeted at specific audiences. All this material is re-published in 'soft copy' and is also downloadable from our website.

Consultations

In February 2002, The Royal Society published an update to the 1998 report 'Genetically modified plants for food use', focusing on the effects that GM foods might have on human health and the use of the principle of substantial equivalence in GM food safety testing. IFR's Clare Mills was appointed to the working party that produced the update because of her expertise in plant food allergens; she was a leading contributor to media interaction for both UK and international media outlets.

In recent years the European Commission, UK Government and a variety of enquiries have used a consultation phase to elicit views and provide information to aid policy development. IFR is actively commenting on all consultations within its sphere of expertise and interest. All responses are also published on the IFR website.

Media interaction

Science news journals carry information about us, too – typical in New Scientist was an article 'Spotting the difference' on IFR substantial equivalence research, and

positive coverage in an article 'Laboratories braced for push on accreditation' in Research Fortnight was a timely reflection of our commitment to quality assurance.

Advice for newspapers, magazines, TV, radio and internet publications continues to be given on a wide variety of topics including *Salmonella* and *Clostridium botulinum*, genetics and many nutrition issues. IFR staff work both 'behind the camera' advising on scientific accuracy and in front of the microphone – typical is Ian Johnson, who contributed to BBC Radio 4's 'The Food Programme' on diet and cancer; our Press Officer, Jo Belsten is heard widely on the radio and has written a number of articles for lay audiences.

Search Publications; Consultations

www.ifr.bbsrc.ac.uk/science/

News releases

www.ifr.bbsrc.ac.uk/media/

A-maiz-ing

www.ifr.bbsrc.ac.uk/public/Maize.htm

Contact

Catherine Reynolds
(Head of Communications)

Conferences, advice and policy formation

IFR staff serve on a wide variety of national and international committees and editorial boards.

A sample of new committee memberships and editorial activities - 2001-2

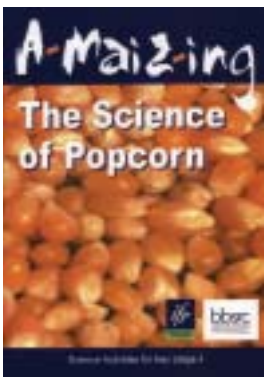
Alastair Robertson	• Food Standards Agency Advisory Committee on Research
	• Consumers' Association Council
Mike Gasson	• Advisory Committee on Microbiological Safety of Foods
Sue Fairweather-Tait	• Nutrition Society Council
	• Scientific Advisory Committee on Nutrition (Expert Group on Iron)
Roger Fenwick	• Federation of European Chemical Societies' Food Chemistry Division (Chairman 2002-5)
Jay Hinton	• Society for General Microbiology - Physiology, Biochemistry and Molecular Genetics Committee
Tim Brockehurst	• International Journal of Food Microbiology - Editorial Board
Paul Kroon	• Journal of the Science of Food and Agriculture - Executive Editorial Board, Subject Editor for Biochemistry

We contribute to information-sharing events for many audiences. Workshops and conferences in the UK included 'Where there's muck there's brass' - organised jointly with the Biochemical Society to stimulate interactions between industry and researchers in biochemistry and biotechnology in the field of bioremediation, and Eurofoodchem XI held on the Norwich Research Park, with 260 participants from about 40 countries addressing the issue of biologically-active phytochemicals in foods.

IFR staff have also travelled widely to share their knowledge and skills, including Vic Morris, who attended the Society for Chemical Industry's symposium on Nanomechanics and presented an invited talk 'Applications of probe microscopy in food science'. Steve Ring was a member of the International Organising Committee for the International Starch Conference organised by the Institute of Biochemical Physics, Russian Academy of Sciences and gave a plenary lecture. Mike Peck was an invited speaker at the US Institute of Food Technologists International Food Safety and Quality Conference in Atlanta, USA and gave an invited lecture 'The challenge of perfection'. József Baranyi spoke at the annual conference of the French Statistical Society. IFR science was well profiled at Bioavailability 2001, the latest in an international series of conferences launched by IFR jointly with the Royal Society of Chemistry back in 1988.

In 2002, IFR staff plan workshops and conferences including the joint IFR/RSC meeting on 'Nutrient release from foods – macro- and micronutrients', and the 'European Research on Functional Effects of Dietary Antioxidants' (EUROFEDA) conference, a major outcome for an EU-funded project.

IFR's Paul Gunning at the BA Discovery Day



Financial support from BBSRC meant we could produce a booklet to accompany web-based support materials, thus supporting our policy of activity where we can make a difference to knowledge and understanding at national level.

Science in education

IFR's contribution to UK Science Week 2001 was to trial in local schools an interactive Key Stage 4 (14-16) presentation and activity on maize. 'A-maiz-ing – The science of popcorn' explains the science underpinning this simple food-processing method that, in the case of sorghum, overcomes the problem of low protein-digestibility during conventional cooking. The project was also presented at the Royal Albert Hall in London during the national British Association Discovery Day, 2002

governing body

advising and supporting IFR

During 2001 we have been involved in preparations for the 'Institute Assessment Exercise' and we were delighted to see the very positive report on IFR science from the Visiting Group, chaired by Professor Julia Goodfellow, who is now the BBSRC's Chief Executive. Our plans for Knowledge Transfer have been supported by the BBSRC's KT Panel. We have a clear vision and can support senior staff in planning an evolving science strategy to suit stakeholder needs.

We are satisfied that IFR, as an organisation with charitable status and a company limited by guarantee, has presented a satisfactory Report and Accounts for the year 2000/2001. This is available on the IFR website. The recently revised SORP and Regulations have made a number of changes to the Trustees' Annual Report. There is a requirement for reports for 2001/2002 onward to contain a statement confirming that the major risks to which the charity is exposed, as identified by the trustees, have been reviewed, and that systems have been established to mitigate those risks. We have discussed business risk management at the Governing Body; at the time of writing individual statements on key business risks have been finalised for our formal approval. Major risks will continue to be reviewed at both IFR Executive and Governing Body meetings on a regular basis.

We record our thanks to Professors John Blundell and Derek Burke, and Baroness Judith Wilcox, who have retired from membership. We welcome Professor Malcolm Jackson from the University of Liverpool (appointed January 2002), and Ms Pauline Murphy from Nestlé's Product Technology Centre, York (August 2001). Both have also joined the Science sub-committee, where Professor John Mathers succeeds Derek Burke as Chairman; all members of the Governing Body are welcome to attend Science meetings.

Dr Alistair Penman
April 2002

Governing Body

Dr Alistair Penman (Chairman) - Director of Laboratory, Unilever Research, Colworth

*__Professor Athene Donald FRS__ - Head of Polymers & Colloids Group, Department of Physics, University of Cambridge

*__Professor Peter Fryer__ - Professor of Chemical Engineering, University of Birmingham

*__Dr Ian Gibson MP__ - Chairman House of Commons Science & Technology Committee and of the Parliamentary & Scientific Committee, and Vice-Chairman Parliamentary Food & Health Forum

Mr Chris Hart - Research & Development Director, Weetabix Ltd

†__Dr Deirdre Hutton CBE__ - Chair, National Consumer Council

Professor Malcolm Jackson - Dean, Faculty of Medicine, University of Liverpool

Professor John Mathers - Professor of Human Nutrition, University of Newcastle

Ms Pauline Murphy - Head of Chocolate Confectionary, Nestlé Product Technology Centre, York

*__Professor Robert Poole__ - West Riding Professor of Microbiology, Department of Molecular Biology & Biotechnology, University of Sheffield

Mr Mike Samuel - Finance Director UK, Unilever plc

Dr Geoff Spriegel - Technical Director, J Sainsbury plc

Dr John Stevens - Group Technical Director, Uniq

* missing from the April 2002 photograph

† resigned April 2002

Science sub-committee 2002

Athene Donald

Peter Fryer

Malcolm Jackson

John Mathers (Chair)

Pauline Murphy

Robert Poole

Geoff Spriegel

John Stevens

Finance and General Purposes sub-committee 2002

Chris Hart

Alistair Penman

Mike Samuel (Chair)