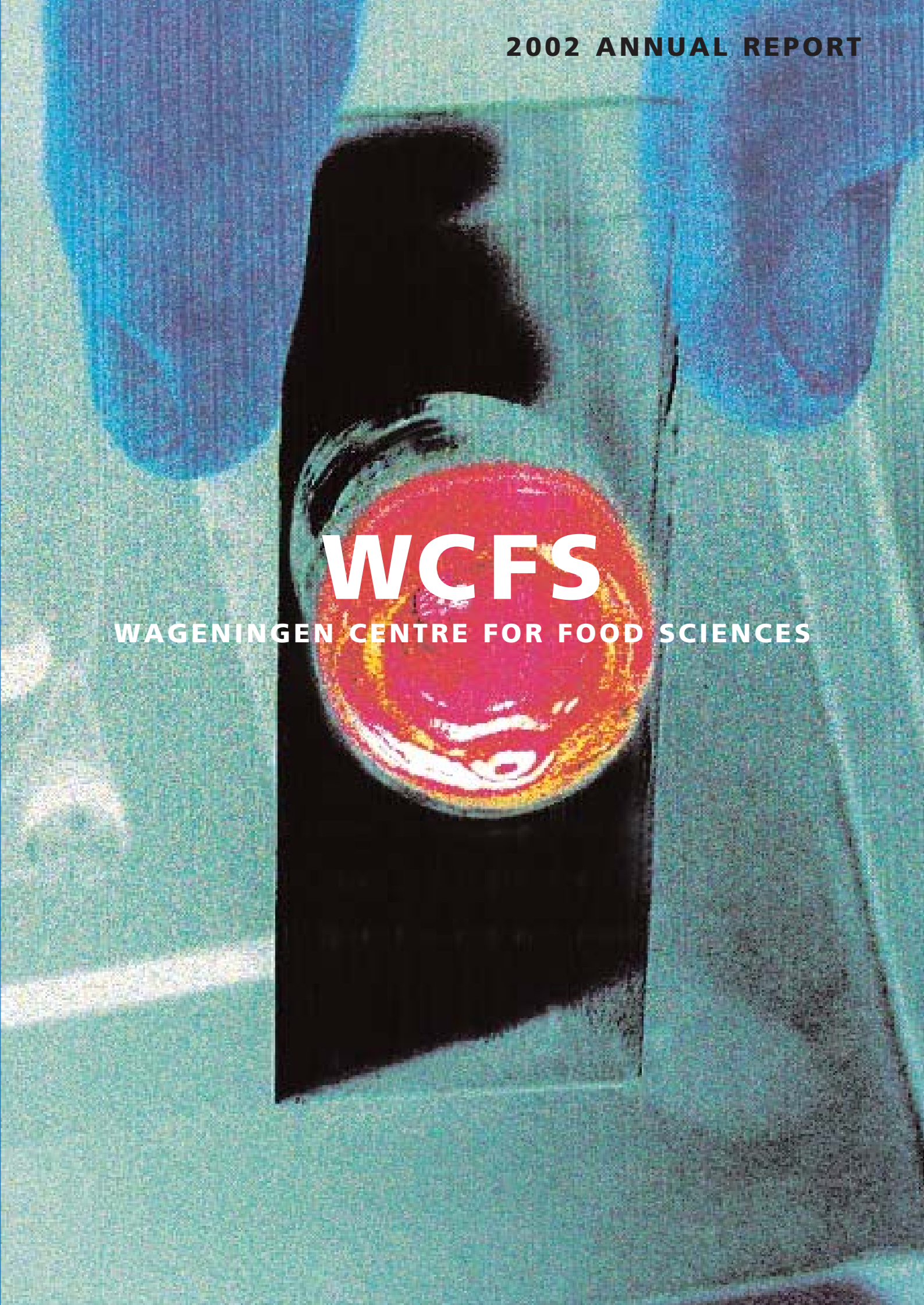
2002 ANNUAL REPORT



WCFS

WAGENINGEN CENTRE FOR FOOD SCIENCES

PUSHING THE BOUNDARIES

2002

THE PROOF OF THE PUDDING

2001

GETTING RESULTS

2000

'Five years on, WCFS has become a strong, focused entity in food research with public and private sector partners sharing responsibilities for innovative research. This concept also makes WCFS unique in Europe and the USA and will allow us to grow further',

Prof. Jo Hautvast, WCFS Director.

GAINING MOMENTUM

1999

BUILDING THE FOUNDATIONS

1998

WAGENINGEN CENTRE FOR FOOD SCIENCES

Pushing the Boundaries

Wageningen Centre for Food Sciences carries out strategic, fundamental research to contribute to the long-term commercial advantage of the Dutch food industry.

Contents

A complete list of WCFS publications and research activities in 2002 is published separately.

WCFS Partners

WCFS RESEARCH PARTNERS

DLO

NIZO FOOD RESEARCH

TNO NUTRITION AND FOOD RESEARCH INSTITUTE

WAGENINGEN UNIVERSITY

WCFS INDUSTRIAL PARTNERS

AVEBE

CEBECO GROUP (until 1-7-2002)

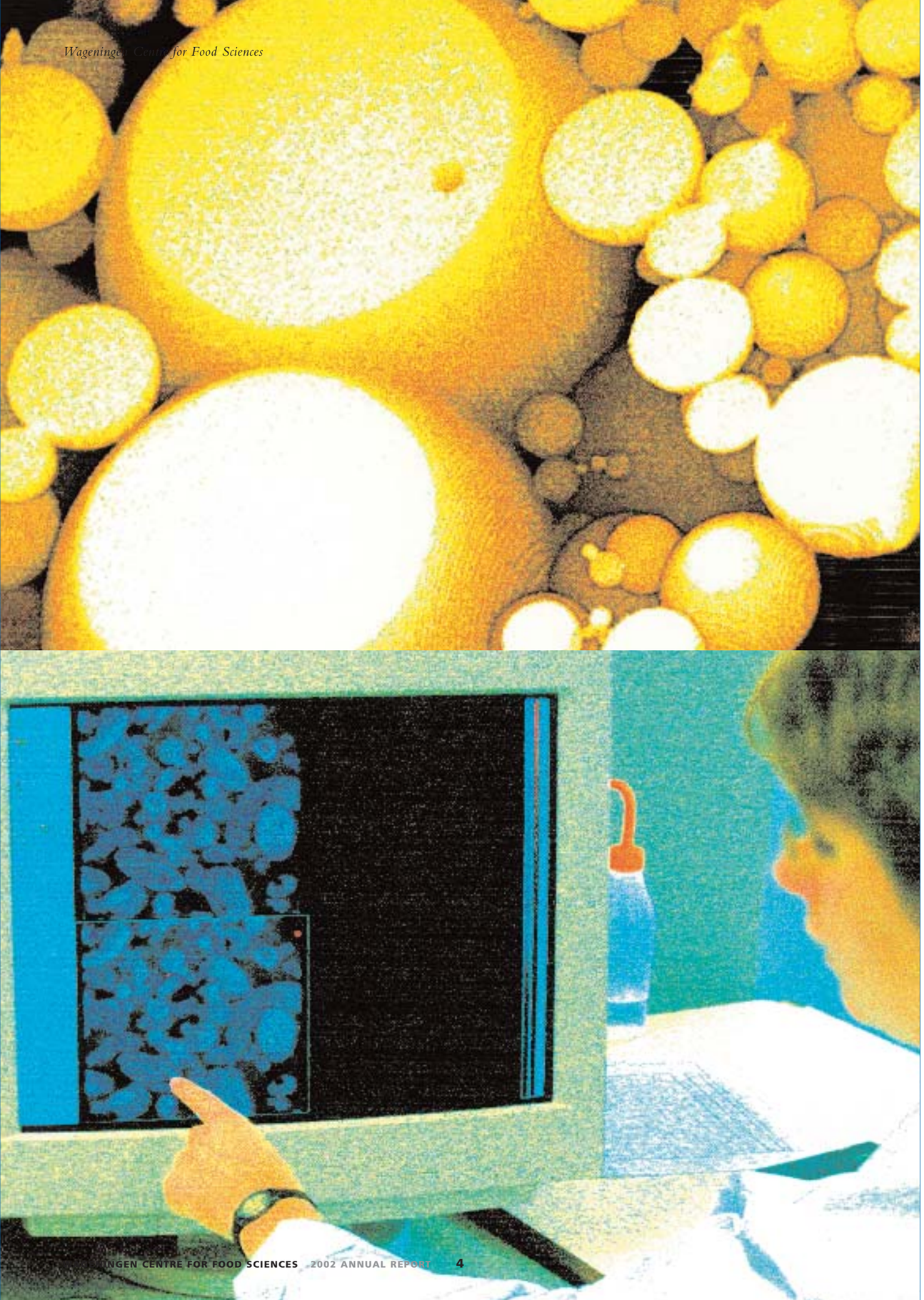
COSUN

CSM NV

DSM GIST BV

UNILEVER N.V.

NETHERLANDS DAIRY INDUSTRY ASSOCIATION (NZO) representing:
Campina bv / Coöperatieve Zuivelonderneming CONO BA / Coöperatieve Zuivelfabriek 'Rouveen' UA / 'DOC Kaas' BA / Friesland Coberco Dairy Foods BV / Koninklijke Numico NV / Leerdammer Company BV



Wageningen Centre for Food Sciences

Industrial Relevance; Scientific Excellence

WCFS was established five years ago by research and food industry partners to carry out groundbreaking fundamental research. The WCFS hub is located in the Dutch 'food valley', Europe's largest concentration of food-related research organisations.

This milestone year of 2002 is marked by a number of changes and new developments that firmly establish WCFS as a key force in food and nutrition research in the Netherlands. We are pleased to report these developments in this year's annual report entitled *Pushing the Boundaries*.

2002 is also marked by a change in the leadership of the WCFS Board. Drs Gerard van der Lely from NZO who has been Chairman of the WCFS Board from the start of our unique alliance stepped down in October 2002. WCFS wishes to acknowledge his valuable contribution in shaping the WCFS alliance in the formative years. Drs van der Lely is succeeded by Dr Theo Ockhuizen, who is Director of the Dutch Dairy Foundation for Nutrition and Health.

WCFS was set up in 1997 as one of the four Leading Technological Institutes at the initiative of and with support from the Netherlands Ministry of Economic Affairs. These public-private partnerships of government, industry and research aim to carry out fundamental research directed at strengthening the innovative power and competitive strength of Dutch industry.

By participating in WCFS, our partners have pooled resources to create the critical mass needed to carry out pioneering research faster and more efficiently than would be possible for them to achieve individually. The WCFS alliance also provides our partners with a window on the wider scientific scene, and direct access to the diverse range of research expertise in food sciences in the Netherlands and internationally.

Our research partners have the potential to use the knowledge generated in the research programmes for the benefit of medium and small food industry players, thus enabling them to play a significant role in strengthening the innovative capacity of the Dutch food industry.

Prof. Joseph Hautvast ~ *WCFS Director*

How Successful is the WCFS Concept

Pushing the Boundaries

'WCFS is now long past the phase of being a "promising concept". We have the mechanisms in place for exemplary interaction between research and industry; and we are learning how to use these mechanisms to the full benefit of all partners', explains Prof. Jo Hautvast.

RESEARCH PROGRAMME

In the last five years, WCFS has done very promising work in the three research areas selected for their future relevance to the food industry partners. Research on links between health and nutrition is of increasing significance as industry partners move towards developing functional foods. Fundamental research that opens new horizons on consumer perceptions of food texture is highly relevant to WCFS industry partners as demand grows for healthy foods that look and taste good. More knowledge about the role that microbes play in food flavour and texture, as well as food shelf life and their contribution to gastrointestinal health is self-evident.

Genomics has always been an element in WCFS research programmes, offering great potential for further understanding and development of mechanisms. Genomics is not a strategic goal in itself; it is an enabling technology and a tool to strengthen research in the three WCFS research programmes. 'We started applying genomics approaches in bacteria five years ago, and we are now moving to humans in gaining further understanding of the relationship between nutrition and health', explains Jo Hautvast.

INDUSTRIAL RELEVANCE

'WCFS must focus first and foremost on research that is relevant to the industry partners', stresses Programme Council Chairman, Ir Joop Roels, who is Director Life Sciences Products, DSM Research. 'Of course, these companies do their own research directed to product development and improving production processes. In this type of R&D, fundamental and applied research often tend to merge because it can take anything from five to ten years before the market launch of a new product. In reality, there is very little room for "free" research that is not specifically directed to product development.'

Most large companies are willing to set aside a small proportion of their total R&D budget for "visionary" research as they regard this as an investment for the future. They hope that such research will lead to significant breakthroughs. But the stakes are high and so too are the risks. These are compelling reasons for the industry partners to invest in the WCFS alliance. In 1996, a group of food companies was prepared to pool resources to create the critical mass to do groundbreaking research. Of course, pooling resources also means sharing the fruits.

WHAT IS GROUNDBREAKING RESEARCH?

'The most direct answer would be research leading to breakthroughs that have commercial value', says Joop Roels. 'In such cases, we need to protect this knowledge with patents for the benefit of the partners. But we should also bear in mind that not all breakthroughs are patentable or even worth patenting.

'Apart from patenting research discoveries of commercial value, the ultimate test of whether research is groundbreaking is that the results are used by the industrial partners', continues Joop Roels. 'But we will only see the effect of the new knowledge generated by WCFS in five to ten years when new products start to appear.'

Research breakthroughs can be presented in scientific publications that demonstrate advances in technology, and that show WCFS is in the forefront. Of course, this is rather subjective, but the scientific directors need to be proactive in stimulating publications that demonstrate the industrial relevance of their research. A very positive step has already been taken in this direction with the annual WCFS patent and publication prizes.

WHY CAN WCFS CARRY OUT GROUNDBREAKING RESEARCH?

According to Prof. Rob Hamer, Scientific Director Structure and Functionality, WCFS is well positioned to carry out innovative research. 'We have developed a philosophy of introducing rigorous scientific principles to research that has the potential to lead to industrially relevant breakthroughs. Such an approach is bearing fruit in the groundbreaking work we are now doing on food texture and perception', explains Rob Hamer.

'Another innovative factor is WCFS' commitment to multidisciplinary research, which can sometimes be difficult to achieve in a university environment. Once again, the texture perception work is a good example as it draws on disciplines as far apart as food physics and physiology in an integrated approach to gaining understanding of complex processes', Rob Hamer continues. 'And finally, WCFS research projects are extremely focused – they have to be. We have established procedures that allow enquiring scientific minds to pursue exciting new leads, while enabling the core research team to stay focused on clearly established goals. Patents and the potential for patents also help to keep research focused on industrial relevance.'

HOW DOES WCFS DECIDE WHAT IS INDUSTRIALLY RELEVANT AND GROUNDBREAKING?

'The industrial partners want WCFS to succeed; they have invested heavily in the alliance and want to see a return on investment', says Joop Roels. 'Success comes through involvement, commitment and communication by both research and industry', stresses Rob Hamer. This is a view shared by CSM Focal Point, Dr Chris Huscroft, Divisional Head of Research, CSM Bakery Supplies Europe.

Since CSM joined WCFS in October 2000, Chris Huscroft has been actively involved in Focal Point discussions with Rob Hamer in defining new research on food texture perception. Initially, this work was carried out using soft solids as the model food and now in close interaction with the Focal Points, it has been decided to extend the food texture perception research to a crispy/crunchy food model.

This illustrates how the company Focal Points contribute to WCFS research, and the pivotal role they and the more informal discussions with the experts within the companies can play in the success of the WCFS alliance. 'There are many people involved as Focal Points and Experts and so it's not realistic to think that we can meet the specific interests of all parties in every research project', explains Rob Hamer. 'But we do need to reach a consensus on each research direction, and of course, all parties can share the results.'

Each partner organisation has a Focal Point for each of the three WCFS research programmes. Although, in fact, the Focal Points have only an advisory role, their input is pivotal and their task is multifunctional. As key researchers in their own organisations with close contacts with WCFS scientific directors, the Focal Points are very well placed to communicate the research objectives and results within their companies. Part of this task is advising their Programme Council member of the opportunities for patenting and protecting knowledge generated by WCFS research.

WHAT IF RESEARCH TURNS OUT NOT TO BE GROUNDBREAKING?

'What is needed is the vision and courage to see beyond the short-term commercial gains, of say, the next five years', explains Dr Harry Raaijmakers, Cosun Focal Point. 'Leading edge research is long haul research. But by its very nature, groundbreaking research is risky. There is always the chance you don't make a breakthrough. Even so, the work may not necessarily be entirely useless', says Harry Raaijmakers. 'All research is valuable. Sometimes, it's important to know why something doesn't work because maybe this knowledge will lead to finding out how to make it work in future.'

But leading edge research also requires scientists to take risks. These risks can be high for scientists who need to ensure that they can publish and establish their scientific reputations. While research results may be disappointing to industry, they may, nevertheless, be exciting scientifically.

HOW IS KNOWLEDGE TRANSFERRED?

A new concept in research and industry partnership such as WCFS automatically implies a learning process. From the start, the mechanisms have been in place for two-way transfer of information and knowledge between research and industry partners. But it is a two-way learning process in which all partners need to be proactive. Success comes through involvement, commitment and communication. All partners have acknowledged that an important spin off from the WCFS alliance is the increased opportunities for interaction between companies and research institutes within the alliance as well as in the broader scientific context.

'Fundamental research of industrial relevance is by definition scientifically innovative; it's a top sport in which only the very best is good enough', Hans Nieuwenhuis, former WCFS Director.

New Initiatives in 2002

In 2002, a number of new initiatives were implemented based on intensive interaction between the research and industrial partners and have contributed to strengthening the WCFS mission of industrial relevance and scientific excellence. The key body in the deliberations on the various actions and developments was the Programme Council.

All WCFS partners are represented on the Programme Council, which has responsibility for the overall direction of WCFS research policy and programme. Thus, one of its key tasks is review of the project portfolio. During the year, the Programme Council met four times under the chairmanship of Ir Joop Roels (Director Life Sciences Products, DSM Research).

Strategic review

As part of the deliberations on proposed new developments, in May 2002, the Programme Council conducted a strategic review and SWOT analysis of WCFS. This re-affirmed the primary focus on research of industrial relevance with potential for scientific breakthrough. The critical mass has been built up to carry out a scientific programme of excellent quality. The strategic review also supported further attention to extending WCFS' international reputation through recruitment from outside the Netherlands and greater involvement in European networks.

NEW RESEARCH PROJECTS

During the year, the Programme Council approved seven new Outline Project Proposals. Extensions were approved to three ongoing projects in Nutrition and Health: on the work on homocysteine lowering ingredients on the risk of cardiovascular disease; on folate availability; and on the nutrigenomics of the mucosal barrier against bacterial pathogens (see Nutrition and Health, page 23).

In extending research in Microbial Functionality and Safety, the Programme Council approved a new project to investigate the effects of mild physical and biological preservation methods on these food-borne microorganisms. The project is entitled *Food preservation and safety* (see Microbial Functionality and Safety, page 39).

Three new interrelated research projects were approved in the Structure and Functionality programme. The project *Integration of sensory perception* will focus on how texture-related attributes are integrated with other stimuli leading to the desired perception. The other two projects will focus on the physical and chemical origins of these attributes, and will build on the research work on emulsions and foams, and dispersions and gels. Research on *Dynamics of biopolymer networks and textures* will investigate the mechanical properties of biopolymer networks in the continuous phase of composite food products. The behaviour of the dispersed phase is central to the project entitled *Engineered textures of emulsions and foams* (see Structure and Functionality, page 30).

NEW PARTNER: UNIVERSITEIT MAASTRICHT

Following extensive preparations and negotiations, the Programme Council recommended to the WCFS Board to accept Universiteit Maastricht as a new research partner. On 6 December 2002, Chairman of the WCFS Board, Dr Theo Ockhuizen and WCFS Director Prof. Jo Hautvast signed the formal agreement on behalf of WCFS, which made Universiteit Maastricht a partner in the WCFS alliance as from 1 January 2003.

The university's nutrition group and access to an academic hospital contributes to making WCFS a very strong public-private alliance for food and nutrition research in the Netherlands. The university will make a significant and complementary contribution to the WCFS research programme. The university's research area encompasses the relationship between nutrition and health in healthy subjects, in subjects with specific health risks, and in patients with specific health problems. Several research areas have been selected as potentially complementary to WCFS' research objectives such as chronic metabolic stress and nutrient interactions in the gastrointestinal tract, and will contribute to the research programmes: Nutrition and Health, and Microbial Functionality and Safety.

As well as access to an academic hospital, the university offers access to additional expertise and facilities including the Stable Isotope Research Centre (SIRC) and proteomics centre and *in vivo* intestinal function methods and laboratory.

WCFS EXPANDS EXPERTISE IN GENOMICS

Genomics has always been an important element in the WCFS research programme, particularly the work on lactic acid bacteria. Two new initiatives in 2002 will expand the use of this powerful technology. The one is WCFS' participation in the Kluyver Centre for Genomics of Industrial Fermentation. The other is the proposal lead by WCFS industrial partners on Nutrigenomics, which is directed to using genomics in gaining more understanding of the relationship between nutrition and health.

Kluyver Centre

WCFS is a partner in the Kluyver Centre for Genomics of Industrial Fermentation, one of four designated centres of excellence set up by the Netherlands Genomics Initiative to implement a national genomics strategy. The Kluyver Centre, which is a consortium of universities, institutes and industrial companies, will focus on industrial fermentation and production processes.

Prof. Willem de Vos, WCFS Scientific Director Microbial Functionality and Safety, is a board member of the Kluyver Centre, and WCFS project leader, Dr Jeroen Hugenholtz, is a member of the management team and programme leader for the work on the genomics of lactic acid bacteria. The research programme will focus on improving production of B vitamins by lactic acid bacteria and will incorporate the work in the new WCFS research project on *Engineering of microbial functionality*.

Nutrigenomics
The WCFS industrial partners were invited to submit a detailed business plan for the setting up an Innovative Cluster in Nutrigenomics to the Netherlands Genomics Initiative. The business plan is based on the Letter of Intent prepared in September 2002. The proposed research programme aims to discover and validate molecular biomarkers for the early detection of metabolic stress and novel food components for dietary management and prevention of this syndrome.

In the last decade, nutrition research has undergone a major shift from dominance by epidemiological and physiological approaches to become increasingly oriented on molecular biology and genetics. This switch is due to a number of factors that have led to an increasing awareness that the effects of nutrition on health and disease need to be understood in terms of how nutrients act at the molecular level. Several major genome projects have drawn attention to the importance of genes in human nutrition, and have provided a wealth of new genetic information. Micro- and macronutrients can serve as potent dietary signals that influence metabolic programming of cells and have a major impact on homeostasis. It is becoming clear that, in addition to environmental factors such as diet and life-style, genotype is a major contributing factor in cardiovascular disease, diabetes type 2, and cancer.

The nutrigenomics programme aims to provide tools for molecular phenotyping by measuring gene and protein expression and by determining metabolite patterns in response to diet (dietary intervention). New biomarkers will enable very early detection of instability in homeostatic control due to sustained metabolic and pro-inflammatory stress and will enable dietary interventions aimed at preventing metabolic syndrome to be developed.

PATENTS: THE ULTIMATE IN KNOWLEDGE TRANSFER

Under the WCFS mandate, it is implicit that high priority be given to patenting as well as to publishing. Thus wherever feasible, the results of breakthrough research must be patented if the partners are to reap the commercial advantage of their investment in WCFS.

During the year, various patent applications were reviewed by the Programme Council. WCFS has filed ten patent applications including two new applications in 2002.

Patent procedures
At the initiative of the Programme Council, Prof. Rob Hamer, Scientific Director Structure and Functionality, organised two workshops - one in April and the other in July - attended by members of the Programme Council and patent experts from the partner organisations. The April meeting focused on the WCFS strategy with regard to patents, and particularly the transfer of patents to the partners. The discussion continued in July and provided the basis for the draft patent procedure, which was reviewed by the Programme Council in September.

The draft patent procedure sets out the rights and obligations of the inventors and partners; conditions of transfer of patents (costs and waiver of the rights of other partners); ownership of the patent; and the potential to sub-license to other partners and third parties.

Attention was given to clarifying the roles of the various parties involved in the patent process including the knowledge institutes, the role of the Focal Points in identifying patent opportunities; increasing the industrial relevance of WCFS patent applications, possible conflicts of interest; and financial aspects.

Patent or perish
The potential for patents needs to be identified as early as possible in a project to prevent a breach in confidentiality through leakage of results. However, a patent application of commercial significance is not necessarily the same as an outstanding piece of scientific work. Thus patenting should not prevent publication of scientific papers. WCFS screens all scientific papers for potential patentable material before submission to journals for publication.

It is also important to guard against too many patent applications. Apart from the cost of the patent application itself, few patents will actually make money for the industry partners. Thus, WCFS has safeguards against accumulating a long list of patents with a system by which all interested partners share the costs of patenting.

Patent & Publication Prizes

In a special ceremony held at Wageningen University on 16 May 2002, the first WCFS Patent & Publication Prizes were presented to WCFS scientists. The authors of four publications and two patent applications were presented with their prizes by Jury Chairman, Ir Joop Roels. This is to be an annual event and strongly underlines the importance WCFS places on scientific excellence within its mandate of industrially relevant research.

The prizewinners were selected by an independent jury of eminent scientists from the ten publications and five patent applications nominated in 2001. The jury members were:

Ir J.A. Roels, Chairman of the Jury and Chairman of WCFS Programme Council, Director Life Sciences Products, DSM Research;
Prof. Dr M.A. Cohen Stuart, Laboratory of Physical Chemistry and Colloid Science, Wageningen University;
Dr O. Korver, former Chief of Nutrition Research, Unilever Research, Vlaardingen;
Prof. Dr J.T.M. Wouters, Emeritus Professor Dairy Science, Wageningen University.

The prizewinning publications and patent applications were:

PATENT APPLICATIONS

R. van der Meer, P. Verhoef, G. Steenge, T. van Vliet, E. Boelsma, M.R. Olthof, M.B. Katan. Patent no: 01205069.6: Modified methionine rich food products and process for their manufacture.

W.F.H. Sybesma, M. Kleerebezem, I. Mierau, M.J.C. Starrenburg, W.M. de Vos, J. Hugenholtz. Patent no 01202013.7: Production of bioavailable folic acid.

PUBLICATIONS

A. Voortman, A. Melse-Boonstra, J.M. Schultz, J. Burema, M.B. Katan, P. Verhoef . Optimal time interval between repeated blood sampling for measurements of total homocysteine in healthy individuals. *Clinical Chemistry* 47(10): 1839-1841 (2001).

W. Kloek, T. van Vliet, M.B.J. Meinders. Effect of bulk and interfacial rheological properties on bubble dissolution. *Journal of Colloid and Interface Science* 237(2): 158-166 (2001).

F. van de Velde, H.A. Peppelman, H.S. Rollema, R.H. Tromp. On the structure of κ/ι-hybrid carrageenans. *Carbohydrate Research* 331(3): 271-283 (2001).

E.G. Zoetendal, A.D. Akkermans, W.M. Akkermans-van Vliet, J.A.G.M. de Visser, W.M. de Vos. The host genotype affects the bacterial community in the human gastrointestinal tract. *Microbial Ecology in Health and Disease* 13: 129-134 (2001).

Management Report

WCFS Director Prof. Joseph Hautvast

2002 was a very productive and eventful year for WCFS, with the preparations for the admission of a new research partner, and important new initiatives to extend the research programme into challenging new areas. With Universiteit Maastricht, WCFS has become a very strong alliance for food and nutrition research in the Netherlands. By the end of the year, preparations were well advanced for a new research focus – nutrigenomics – to investigate the diet-genetic basis of metabolic stress, the early and reversible stage of metabolic syndrome.

2002 was a landmark year for the project portfolio, with two major research projects on food structure and functionality, and the two projects on lactic acid bacteria completed during the year. By the end of the year, some 100 scientific papers had been accepted for publication in peer-reviewed journals and four PhD theses have been completed. A full list of WCFS publications and research activities in 2002 is published separately.

During the year, the Programme Council approved new projects in all three research programmes and these will start in 2003 (see reports of the research programmes). Yet, another important development in 2002 was the drafting of the patent procedure setting out the rights and obligations of WCFS and partners. During the year, two patents were filed

CEBECO GROUP

Reorganisation within the Cebeco Group has had consequences for the company's corporate research and for its participation in WCFS. Regrettably, the Cebeco Group withdrew as a partner in WCFS as from 30 June 2002, however, the company will continue to meet its financial obligations to WCFS until 31 December 2003. WCFS appreciates the involvement of the Cebeco Group during the first five years of the development of this unique research and industry alliance.

FINANCE

WCFS income from subsidies and contributions from the partners amounted to ¤ 15.46 million in 2002, which is an increase of 13% in the income for 2001 (¤ 13.68 million).

As indicated in the 2001 Annual Report, all research budgets in 2002 were under considerable pressure as a result of the substantial increase in costs of approximately 19%, resulting from WCFS' change in status with regard to value added tax (BTW). WCFS gratefully acknowledges the decision of the Ministry of Economic Affairs to provide additional financial support to partially cover the increase in costs arising from the change in VAT status. In 2002, WCFS partners appealed the decision of the Department of Taxation with regard to WCFS' status on value-added tax. A final decision on this matter is expected early in 2003.

KNOWLEDGE TRANSFER

Two-way communication between WCFS and partners is essential to the success of this research-industry partnership. In 2002, researchers made an increasing number of visits to the industry partners for discussion with expert groups, and Scientific Directors and Focal Points met frequently during the year. This two-way communication occurred in all three research programmes as major research projects were completed during the year. Discussions intensified in the start up of new projects and the preparations for new projects approved by the Programme Council during the year, as presented in the reports on the research programmes.

During the year, the WCFS intranet was upgraded to give faster, easier access to the results of research work for the partner companies. There is intensive use of the WCFS intranet, which now has some 260 users. The intranet is only accessible to WCFS researchers and designated persons in the industry partner companies.

Food Summit

WCFS' fourth Food Summit entitled *Texture Dynamics* was held in Wageningen from 11 to 13 April 2002. During the three-day meeting, some 45 scientists from 10 countries discussed the little known phenomenon of food texture, processes in the mouth and brain, and physiochemical properties of foods that trigger these processes. WCFS Food Summit is an annual event that brings to Wageningen experts to discuss a specific aspect of the WCFS programme in the broader international context.

Food Colloids 2002

The biennial conference on the structure and physical chemistry of foods held in Wageningen from 14 to 17 April 2002 was organised by VLAG Graduate School in cooperation with WCFS. This was the ninth conference in a biennial series of conferences on food colloids (10 nm – 100 micron scale). The 2002 conference was attended by more that 230 scientists from 22 countries working in academic and industrial research.

PATENT & PUBLICATION PRIZES

The first WCFS Patent & Publication Prizes were presented in May 2002 by Programme Council Chairman, Ir Joop Roels, Director, Life Sciences Products, DSM research. The prize winning publications and patents were selected by an independent jury from a large number of nominations for these prizes (see page 15).

EXTERNAL RELATIONS

Following the positive interim evaluation and discussions about the position of the Leading Technological Institutes, a delegation from the Ministry of Economic Affairs made a working visit to WCFS on 23 October 2002. The delegation comprised Drs R. Bemer, Director General of Innovation of the Ministry of Economic Affairs; Drs H.J.T. Nieuwenhuis, Deputy Director Infrastructure and Innovation; and Drs M. Ottolander, Senior Policy Advisor.

During the year, a consolidated effort was made to develop and strengthen international relationships. WCFS received several visitors from government and research organisations that had expressed interest in WCFS public-private research partnership. This unique structure positions WCFS favourably for potential international cooperation such as Networks of Excellence under the Sixth Framework Programme.

WCFS welcomed a number of distinguished scientists and researchers from countries worldwide. There was, in fact, a two-way process of both formal and informal visits, as many WCFS scientists made visits to research organisations in other countries.

PERSONNEL

The number of staff under contract or seconded to WFCS staff increased from 125 in 2001 to 150 in 2002. This includes an increase in 18 senior scientists and postdoctoral fellows as well as the appointment of a further seven PhD fellows. WCFS has 37 PhD fellows under contract. While staff are being recruited for new projects starting in 2002 and 2003, it is WCFS policy to maintain a small core of senior scientists with established reputations and to recruit high potential graduates at postdoctoral and PhD levels.

The first PhD fellows appointed in 1998 and 1999 are expected to graduate in 2003. Four PhD fellows whose research was incorporated into WCFS completed their work and graduated in 2002.

It is also pleasing to note that several of the PhD fellows and postdoctoral fellows have been recruited to the R&D departments of the industry partners. In 2002, twelve researchers completed their contracts and took up appointments with one of the industrial partners, joined the core staff of research partners or are now working in research organisations abroad.

TRAINING

All PhD students participate in the courses organised by the VLAG Graduate School. Accredited by the Royal Netherlands Academy of Arts and Sciences, VLAG conducts postgraduate training in the food chain in the broadest sense, from food processing to nutrition and health. Again in 2002, members of the WCFS core staff contributed to the training, giving courses on subjects ranging from protein engineering and nutrigenomics to healthy foods.

WCFS attaches great importance to teamwork, personal development and social awareness. In the annual staff performance reviews, the individual training needs were assessed and where appropriate, arrangements made to meet these needs. During the year, training sessions were organised on a variety of topics such as media training, writing scientific papers and report writing in English. In addition, each of the research programmes organised ‘days away’ with the express aim of teambuilding.

OUTLOOK

The initiatives in 2002 have set the scene for exhilarating research challenges. Increased interaction between industry partners and researchers has led to better understanding of one another’s needs, and thus to new research directions and fine-tuning of the research proposals. During 2003, WCFS will work at intensifying two-way communication and knowledge transfer between the research and industry partners.

‘Being located in the Wageningen “food valley”, WCFS is strongly positioned to be catalyst as well as a platform to make innovative developments happen’, Jo Hautvast.

Financial Highlights

		2002*	2001	2000	1999
Income *(in millions of euros)*					
	Government				
	Ministry of Economic Affairs	6.31	6.04	5.22	3.73
	Research organisations				
	Wageningen University	1.05	1.01	0.96	0.62
	DLO	1.05	1.01	0.96	0.62
	TNO Nutrition and Food Research Institute	1.05	1.01	0.96	0.62
	Industry partners				
	NZO	2.93	2.22	2.45	1.62
	Unilever N.V.	1.15	0.89	0.98	0.65
	DSM Gist B.V.	0.59	0.45	0.49	0.32
	CSM nv	0.51	0.39	-	-
	AVEBE	0.27	0.22	0.25	0.17
	Cebeco Group	0.27	0.22	0.25	0.17
	Cosun	0.27	0.22	0.25	0.17
	Total	**15.46**	**13.68**	**12.77**	**8.69**
Research expenditure *(in millions of euros)*					
	Research contracted to:				
	Wageningen University	5.01	4.42	3.72	1.91
	NIZO food research	3.85	2.86	2.50	2.31
	TNO Nutrition and Food Research Institute	1.97	1.77	1.50	1.09
	DLO	1.52	1.27	1.18	1.77
	Other research costs	1.91	1.36	2.72	0.77
	Total	**14.27**	**11.68**	**11.62**	**7.85**
Expenditure per research programme *(in millions of euros)*					
	Nutrition and Health	4.31	3.87	3.13	1.41
	Structure and Functionality	5.64	3.91	3.72	2.77
	Microbial Functionality and Safety	4.00	3.72	4.08	3.45
	Other programme expenditure				
	(Leads, patents, Food Summit)	0.33	0.18	0.69	0.23
	Total	**14.27**	**11.68**	**11.62**	**7.85**
Overhead and provisions *(in millions of euros)*					
	Overhead costs	1.19	0.86	1.15	0.84
	Provisions for VAT, etc.	0.00	1.13	-	-
	Total	**1.19**	**2.00**	**1.15**	**0.84**

**Preliminary financial results for 2002 without an Accountant's Statement. Financial results with the Accountant's Statement will be presented to the WCFS Board on 24 April 2003.*

Food Summit

Texture Dynamics: Perception and Measurement

The creaminess of a dessert, the crispiness of a biscuit, the melting of ice cream on the tongue are all mouth sensations determined by the texture of the food. Texture related attributes are becoming increasingly important in consumer choice and purchase behaviour.

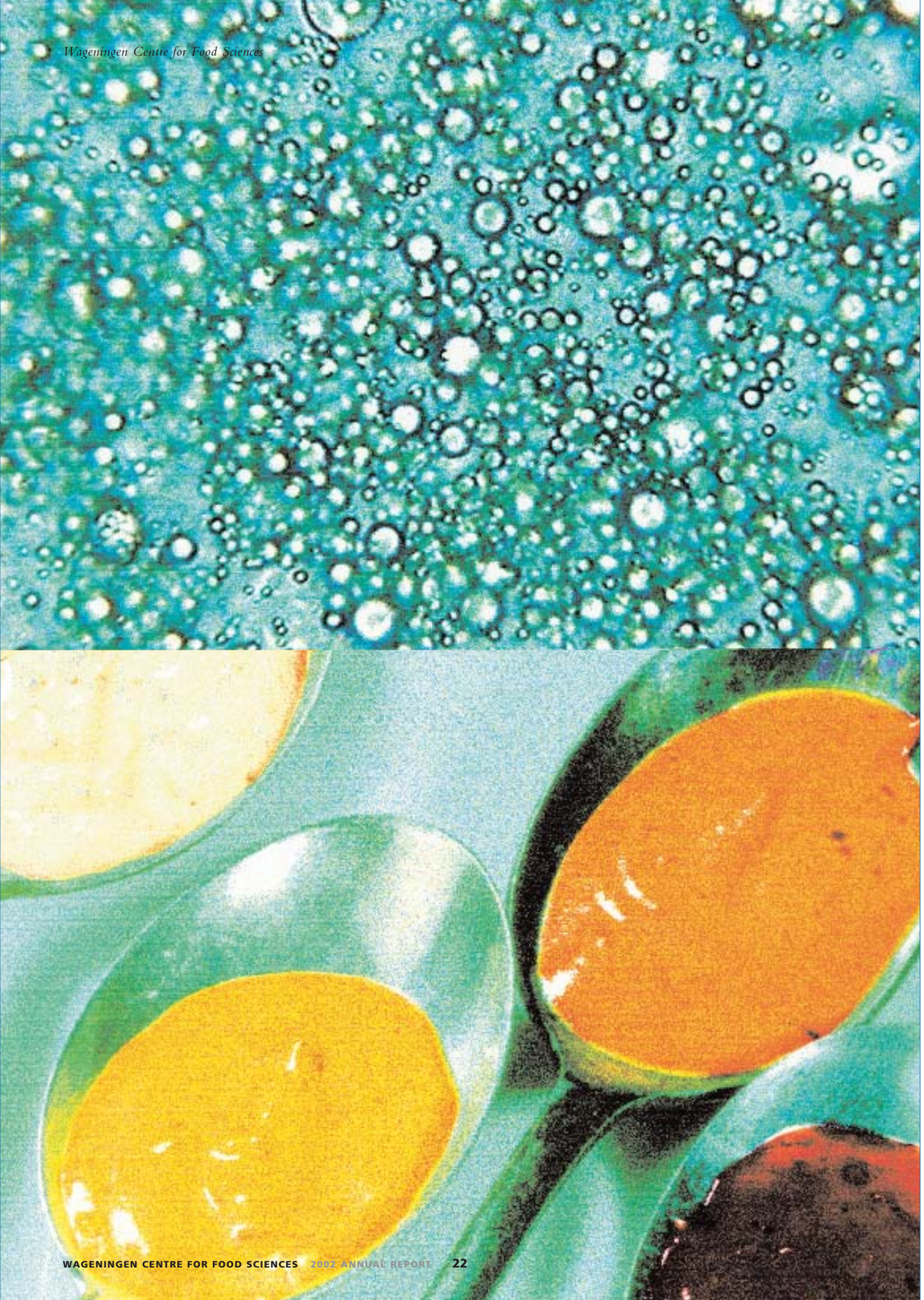
The Structure and Functionality research team under the Scientific Director Prof. Rob Hamer are studying the relationship between sensory characteristics of foods and the molecular properties and structure of food ingredients. This was the topic of WCFS' fourth Food Summit held in Wageningen from 11 to 13 April 2002.

This year's Food Summit, entitled 'Texture Dynamics', considered the perception of food texture, the processes in the mouth and brain, and physiochemical properties of foods that trigger such processes. During the three-day meeting, some 45 scientists from 10 countries discussed state-of-the-art research and the research needs for the coming five to ten years including potential breakthroughs in these areas. The keynote addresses by prominent scientists and discussion sessions focused specially on the texture properties needed to create the desired mouth sensations, whether these texture properties can be generated with current technology and what further knowledge is required. How are the food properties that create the desired mouth sensations affected by production, packaging, transport and storage? How do we cope with such constraints and safety issues while still maintaining the sensory properties?

The Food Summit identified long-term needs in fundamental research on the functionality of food structures. Little is known about the oral sensoric perception of food structures such as gels, emulsions, foams and solids, and how these structures can be engineered to trigger desired oral sensoric perceptions. Thus, developing products with more attractive structures is still largely a process of trial and error.

Also, there are individual differences in oral perceptions based on physiology, age, sex and culture, as well as experience and expectation. The challenge is to map the oral sensory receptors related both to food texture and to consumer preferences.

The Food Summit concluded with a number of recommendations. First and foremost, the meeting stressed that research on texture perception should take more account of information on the breakdown of food. Research should aim to connect these aspects of texture directly with perception. This will require a multidisciplinary approach focusing more on real-life situation. In this respect, it was generally agreed that 'real' food structures should be used in oral receptor studies because many sensoric properties cannot be obtained in simplified food structures.



Nutrition and Health

Scientific Director Prof. Martijn Katan

Research on nutrition and health integrates laboratory and clinical studies to identify food components that affect the risk of cardiovascular disease, cancer and infectious diseases. We need to understand the mode of action of such food components in relation to the risk of these common diseases, which offer good prospects for nutrition intervention. Research projects may start from either the laboratory or the clinical side but should involve both approaches. A crucial aspect of the research is establishing biomarkers and this may be the end point of laboratory studies or the starting point of human studies.

All research in nutrition and health is directed to providing industry with leads for the development of healthy foods. Leads come from insights provided by current research or through patented knowledge. There are, however, limited opportunities for protecting knowledge on health effects with patents because most food components and their potential health effects have already been discussed, often in the public domain. Another important objective of the research programme is to develop new concepts and techniques that can be applied in studying new issues in health and nutrition. This includes the development and validation of biomarkers and techniques to study dietary effects on biomarkers.

In 2002, the Programme Council approved extensions to three research projects in Nutrition and Health. Flow Mediated Dilation recognized as an early marker of cardiovascular disease will be used to investigate the effects of alternative homocysteine lowering ingredients on the risk of cardiovascular disease. The Programme Council also approved work on the validation and application of stable isotopes to study folate availability in humans *in vivo*. Research on dietary modulation of intestinal infections will be extended with a study on the effects of bacterial pathogens on the expression of mucosal barrier genes.

A brief report is presented of the key research activities in 2002.

n-3 FATTY ACIDS AND CARDIAC ARRHYTHMIAS

WCFS researchers led by Dr Peter Zock are investigating whether and how dietary n-3 fatty acids can prevent cardiac arrhythmias and related risks of heart disease. The work involves studying the effects of n-3 fatty acids on the electrical activity of the heart at all stages of the pathway from diet to disease prevention. The overall objective is to develop reliable biomarkers and new models and methods for studying arrhythmia and heart disease risk. These tools can then be used in investigating the effects of various dietary components on heart health.

Dr Peter Zock working in cooperation with Dr Evert Schouten and Dr Ingeborg Brouwer is organising a multi centre, Europe-wide study to investigate the effects of n-3 fatty acids on the incidence of life-threatening arrhythmias in patients at risk. Patients with implanted cardioverter defibrillators are being recruited for this study from 30 cardiological centres throughout Europe. After initial delays in obtaining regulatory approval and organising infrastructure, patient recruitment has greatly increased in the past year.

Brouwer, I.A., et al., European Journal of Clinical Nutrition, in press.

Risk indicators of arrhythmia

WCFS researcher, Dr Evert Schouten and PhD fellow Anouk Geelen are investigating the effects of different n-3 fatty acids on electrocardiographic (ECG) outcomes as predictors of arrhythmia risk in humans. These studies will validate and develop biomarker tools for studies in humans on diet, arrhythmia risk, and heart health. The first study in 84 healthy subjects found no significant effects of a daily dose of 1500 mg n-3 fatty acids on heart rate variability and baroreflex sensitivity. This suggests that n-3 fatty acids do not affect arrhythmia in healthy people through modulation of cardiac control by the autonomic nervous system. This outcome prompted the research team to initiate a second study on the effects of n-3

fatty acids in 100 patients with premature ventricular complexes, a population with more susceptible electrophysiology. The effects of 12-week supplementation with a mix of very-long-chain n-3 fatty acids (eocosopenta ennoic acid and docosohexa enoic acid) will be measured in 100 subjects who frequently have premature ventricular complexes.

Geelen, A., et al., American Heart Journal, in press.

Mechanisms

A pig heart model has been developed by Dr Ruben Coronel at the Academic Medical Centre Amsterdam to study anti-arrhythmic effects of diet during the early 'injury current' phase of ischemia (first ten minutes after artery occlusion) and the delayed 'myocardial stretch' phase (after 30 minutes). A pig model was chosen because life-threatening arrhythmias are caused by re-entry currents (a mechanism most relevant to the human situation) and because they cannot be studied in smaller rodent hearts. Pilot experiments suggest that crucial *in vivo* electrophysiological properties, such as conduction velocity and length of the refractory period, can be measured with high reproducibility.

Experiments on acute administration of n-3 fatty acids in the pig heart model are now being carried out to fine tune the protocol for electrophysiological testing in feeding studies. The aim is to develop and validate a model for screening the anti-arrhythmic potential of different n-3 fatty acids and other food substances.

Bioconversion of alpha-linolenic acid in humans

A study was carried out to quantify the conversion of alpha-linolenic acid to very-long-chain n-3 fatty acids in humans, and to determine how this is affected by other polyunsaturated fatty acids in the diet. This study in 30 healthy subjects using stable-isotope-labelled alpha-linolenic acid was done in collaboration with Universiteit Maastricht. Isotope enrichment of fatty acids in 360 plasma phospholipid samples were determined by GC-IRMS and data are now being analysed and interpreted. A compartmental model for quantifying conversion was developed in cooperation with Prof. D.M. Foster from University of Washington, Seattle (USA). The study is unique because of its duration. Earlier results suggest that shorter studies on the bioconversion of alpha-linolenic acid may not represent what actually happens in the longer term.

Brouwer, I. A., et al., American Journal of Cardiology 89: 629-631 (2002).
Geelen, A., et al., Journal of Nutrition 132: 3051-3054 (2002).

HOMOCYSTEINE: A RISK FACTOR FOR CARDIOVASCULAR DISEASE

WCFS researchers led by Dr Petra Verhoef at Wageningen University, in collaboration with TNO Nutrition in Zeist, are investigating food components that can lower homocysteine concentrations in plasma, and the relationship between homocysteine and cardiovascular disease. High homocysteine levels are associated with cardiovascular disease but it is still unknown whether there is a causal link.

Diet and homocysteine

Several dietary factors influence plasma homocysteine concentrations. Homocysteine is a sulphur-containing amino acid and dietary methionine is the sole dietary precursor. After oral administration of methionine at a dose of 0.1 g/kg bodyweight (a classical methionine-loading test developed to diagnose subjects with enzymatic defects in homocysteine catabolism) the plasma concentration of homocysteine reaches a peak value after about six hours. However, the acute homocysteine-raising effect of pure methionine does not translate into a similar effect of protein-rich foods. High intakes of animal protein are not associated with high homocysteine levels in epidemiological studies. This implies that protein-rich foods contain other factors, in addition to methionine, that might affect homocysteine metabolism, such as B vitamins and other amino acids. In December 2001, a patent was filed regarding a specific combination of dietary amino acids to reduce the homocysteine-raising effect of methionine-rich foods.

Folic acid lowers plasma homocysteine. WCFS research revealed that at least 400 µg folic acid per day is needed to lower plasma homocysteine concentrations adequately. However, the amount in food is thought to be inadequate and the bioavailability of dietary folate is less than that of synthetic folic acid. The reason for the lower bioavailability of dietary folate might be the polyglutamate chain attached to dietary folate. Synthetic folic acid lacks the polyglutamate chain. PhD fellow Alida Melse carried out two human intervention trials to investigate whether the lower bioavailability of dietary folate compared to synthetic folic acid could be explained by the polyglutamate chain of folate in the diet.

Betaine is a dietary compound that also lowers plasma homocysteine and occurs in foods, such as bread, pasta and spinach. The effects of betaine on plasma homocysteine concentrations have only been tested at high doses (6 grams/day). Daily intake of betaine is estimated at 0.5-2.0 grams/day. WCFS researchers at TNO Nutrition and Wageningen University are investigating whether betaine doses in the range of normal dietary intake lower plasma homocysteine concentration in healthy volunteers.

Van Oort, F.V.A., et al., American Journal of Clinical Nutrition, in press.

Homocysteine and cardiovascular disease

Methylene tetrahydrofolate reductase (MTHFR) is an enzyme involved in homocysteine remethylation. A common polymorphism exists in the gene that encodes MTHFR. Individuals who are homozygous for the MTHFR 677C→T mutation generally have higher homocysteine levels. The advantage of studying a polymorphism is that the metabolic effects of a particular genotype start early in life, and therefore the level of the risk factor – in this case, homocysteine – cannot be influenced by the presence of disease. Therefore, investigating the association between the MTHFR 677C→T polymorphism and the risk of coronary heart disease can potentially reveal whether the association between homocysteine level and cardiovascular heart disease risk is causal.

In a meta-analysis of 40 observational studies, Dr Mariska Klerk has shown that the MTHFR 677TT genotype was associated with a 16% increase in risk of coronary heart disease. This result supports the hypothesis that impaired homocysteine metabolism is causally related to coronary heart disease risk.

The mechanism by which homocysteine could increase cardiovascular disease risk is still unclear. Studies have suggested that homocysteine might damage the vascular endothelium, and could consequently lead to increased blood clotting. In a trial with 118 healthy volunteers, Dr Mariska Klerk investigated the effect of supplementation with high doses of folic acid, vitamin B6 and vitamin B12 for eight weeks on clotting activation markers. Despite a considerable reduction in homocysteine concentrations (~25%), no significant effect on clotting activation markers could be observed.

The effects of lowering homocysteine levels on the wall thickness of the neck artery are being investigated in the FACIT study of 835 subjects over a period of three years. Wall thickness of the neck artery is a validated marker of cardiovascular disease. Baseline measurements are completed, and data on wall thickness of the neck artery have been provided by the Julius Centre, Utrecht University Medical Centre. PhD fellow Jane Durga is carrying out a cross-sectional analysis of the relationship with homocysteine and wall thickness of the neck artery. The effects of one-year folic acid supplementation on markers of blood clotting were investigated using data from the

first 276 subjects in the FACIT study.
Another biomarker for cardiovascular disease investigated by WCFS is vascular function. Vascular function is measured as Flow Mediated Dilation. This is the increase in arterial diameter in the arm induced by increase in arterial blood flow. Flow Mediated Dilation is measured non-invasively with high-resolution ultrasound in collaboration with the Julius Centre, Utrecht University Medical Centre. Dr Margreet Olthof is carrying out two studies each with 40 healthy volunteers to investigate the potential short and long-term effects of lowering plasma homocysteine levels on flow-mediated vasodilation.

Klerk, M., et al., Journal of the American Medical Association 288: 2023-2031 (2002).
Klerk, M., et al., Thrombosis Haemostasis 88: 230-235 (2002).
Klerk, M., Homocysteine and coronary heart disease; the role of polymorphic genes and hemostatis, PhD thesis, Wageningen University.

DIETARY MODULATION OF COLON CANCER RISK

Epidemiological studies indicate that diet can modulate the incidence of colon cancer, which is the second leading cause of cancer deaths in Western societies. WCFS researchers led by Dr Roelof van der Meer are investigating how non-absorbed nutrients, such as haem (in red meat) and calcium modulate colonic cytotoxicity and thus epithelial cell turnover, which affects endogenous mutations and thus the risk of colon cancer.

Red and white meat
Studies have shown that feeding either raw or cooked meat induced cytotoxicity and hyperproliferation in rat colon. However, these parameters were drastically increased by isolated haem. Thus, the bioavailability of haem in these freeze-dried meat diets was much lower than of isolated haem. While dietary cysteine, cystine or nitrate have been shown to increase the bioavailability of haem in freeze-dried haemoglobin added to rat diets, none of these dietary treatments induced the haem-specific effects in colon. Binding of haem to hemoproteins in the diet seemed to prevent intestinal formation of the cytotoxic haem factor, indicating that isolated haem does not have the same effect as red meat in this animal model.

Diet and colonic genomics
Researchers led by Dr Jaap Keijer at RIKILT are working on identifying genes that are molecular markers of mucosal damage and thus of colon cancer risk. Expression of these genes can then be used in screening the effects of nutrients on the health of colonic mucosa.

Sequencing and annotation of the cDNA microarray of known cell-turnover genes and unknown genes differentially expressed in rat colon mucosa have been completed. The rat colon microarray has been used to identify those genes expressed in response to dietary calcium. Preliminary analysis indicates that rats fed with or without calcium have comparable expression profiles except for a twofold upregulation of our new pentraxin gene.

Dietary haem had stronger effects on colonic gene expression than did calcium. Most striking was the tenfold downregulation of a novel rat sequence that encodes a pentraxin protein, mucosal pentraxin (Mptx). This gene had an unprecedented effect on response to dietary haem while only modest changes were observed in expression levels of all other genes on the array. A set of Affymetrix chips was hybridised to the RNA samples from rats fed with or without calcium or haem. These chips contain short synthetic stretches of nucleotides encoding about 8000 known rat genes. The results showed differential gene expression in response to dietary calcium as well as to haem and validated the rat model for nutrigenomic studies.

***Haem and calcium-regulated rat gene* Mptx**
The gene mucosal pentraxin (Mptx) is a potential biomarker for colonic cell turnover, the genomic organisation of the gene was determined by comparing the sequence with the rat genomic database. Based on this information, specific primers for the gene have been designed and used in RT-PCR experiments. These primers were also used to clone the full-length rat Mptx in a multicopy vector and the sequence analysis showed that the construct was correct.

Dr Cindy van der Meer has shown in homology modelling that colonic pentraxin has a very similar structure to the human pentraxin Serum Amyloid P Component (SAP) which was used as the template and that the β-sheets are likely to be organised in a similar way. In addition, the binding pockets for calcium molecules as well as the regions in between the five subunits are highly conserved. However, the region on top of the Mptx pentamer has a gap of four amino acids compared to human SAP. Homology modelling showed that this region contains many fewer negatively charged amino acids, which could be related to a different function of both proteins. Using the three dimensional model of Mptx to select regions in the protein likely to have antigenic activity, at least four regions were selected for the presence of polar residues and for their position at the exterior of the protein. The effects observed in array experiments were verified using quantitative real-time RT-PCR, which is an independent molecular technique to quantify mRNA levels. A patent concerning our mucosal pentraxin (*Mptx)* gene was filed in December 2002 and is entitled: Nucleic acid sequences for use as biomarker of damage to the intestinal epithelium.

DIETARY MODULATION OF INTESTINAL INFECTIONS

With the growing resistance of food-borne bacterial pathogens to antibiotics, the focus is changing from treatment to prevention of gastrointestinal infections. Dr Ingeborg Bovee is carrying out studies to identify nutrients that can prevent or inhibit intestinal bacterial infections, and to developing mechanistic concepts that can be used in designing food ingredients to improve host resistance to gastrointestinal infections.

Calcium and intestinal infections
Researchers led by Dr Ingeborg Bovee and Dr Roelof van der Meer have shown that dietary calcium inhibits intestinal colonisation of Gram-negative bacterial pathogens, for instance enterotoxigenic *Escherichia coli*, and reduces infection symptoms such as diarrhoea. There are two possible mechanisms involved. One is the direct binding of food-borne bacterial pathogens to amorphous calcium phosphate formed in the proximal small intestine. The other is an effect of calcium on the composition of the autochthonous microflora, in particular stimulation of endogenous lactobacilli and inhibition of enterobacteria.

Studies with salmonella-infected rats have shown that acute calcium administration just before infection is as effective as a continuous high calcium intake in inhibiting salmonella colonisation and translocation as well as lowering the specific serum immune response to this pathogen. However, acute calcium supplementation changed the gut flora composition by stimulating the endogenous lactobacilli and inhibiting the enterobacteria *in vivo*. When the protective effect of the endogenous microflora was excluded, calcium was shown to have absolutely no protective effect on germ-free rats in a salmonella infection experiment. Thus, it is most likely that the protective effect of calcium is mediated by interaction with the endogenous microflora.

Carbohydrate fermentation and intestinal infections
Non-digestible carbohydrates (prebiotics) may stimulate the endogenous lactobacilli and bifidobacteria, which are thought to improve resistance to intestinal infections. But despite stimulation of the endogenous lactobacilli and bifidobacteria, some prebiotics increased intestinal translocation of salmonella and thus aggravated the systemic infection in rats.

As reported earlier, this adverse effect is dose-dependent and counteracted by supplemental calcium. Because increased intake of slowly fermented resistant starch has no effect on the resistance of rats to salmonella,

rapid fermentation is possibly the cause of the impaired barrier function.

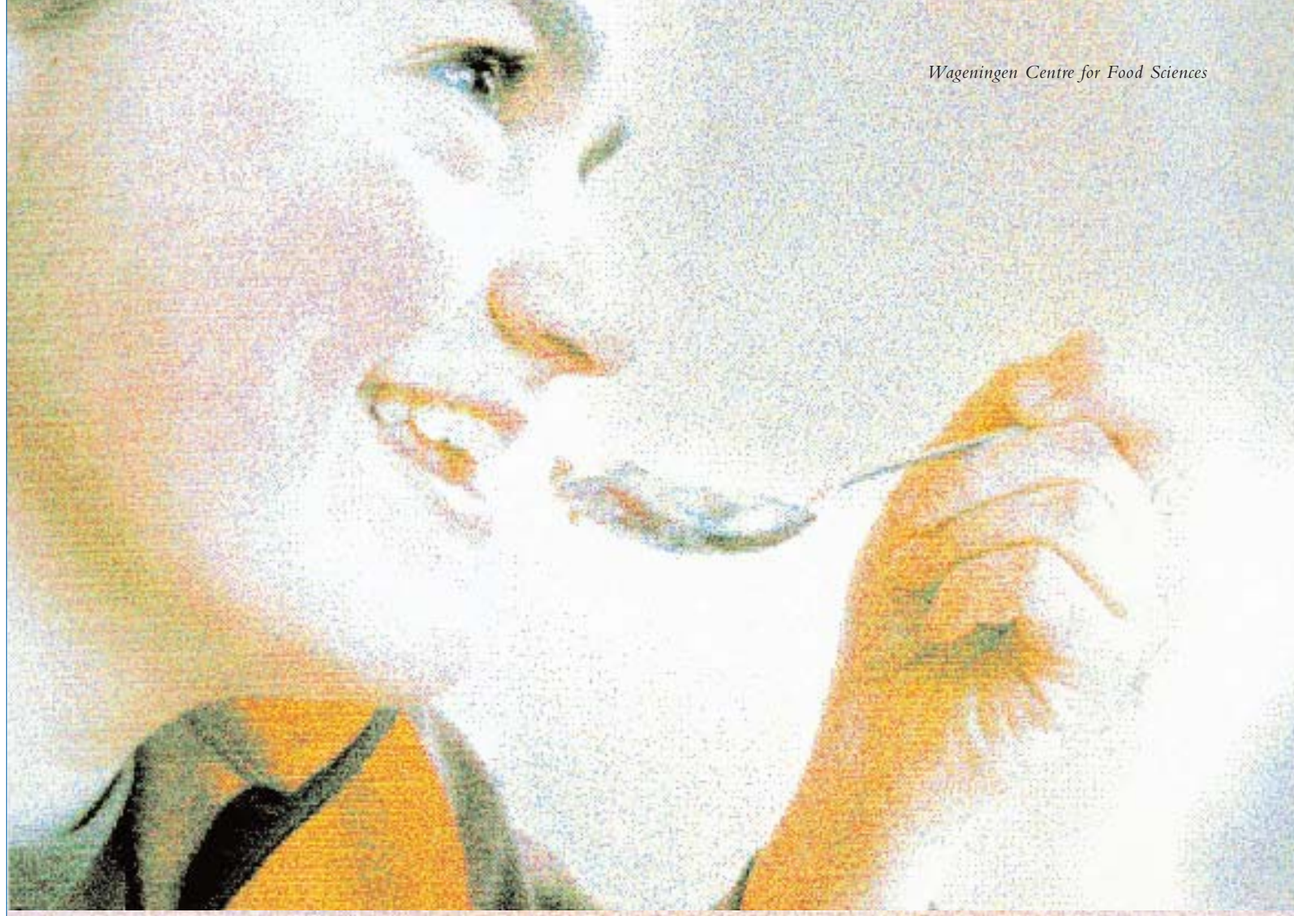
Dr Ingeborg Bovee and PhD fellow Sandra ten Bruggencate are carrying out experiments to determine the role of excessive bacterial fermentation in rats fed oligofructose, lactulose or cellulose and infected with salmonella. Both the oligofructose and lactulose groups had a three-fold higher myeloperoxidase activity in their faecal and colonic mucosa than those on the cellulose control diet. No supplement-induced differences were observed in the ileum where fermentation is limited. These results indicate that fermentation likely impairs the mucosal barrier in the distal gut, increasing translocation of invasive salmonella and leading to a concomitant inflammatory response. Moreover, a pilot study showed that normal intestinal histology was severely disturbed in infected rats fed oligofructose. In accordance with the data on myeloperoxidase activity, the intestinal coupes of infected rats showed massive infiltration of leucocytes, edema, and derangement of crypt structure.

At the end of the year, the Programme Council gave approval to extend the work on the effects of non-absorbed nutrients on the intestinal mucosal barrier to allow studies of differential gene expression to be carried out.

Sprong, R.C., et al., International Dairy Journal 12: 209-215 (2002).
Sprong, R.C., et al., Journal of Nutrition 132: 1269-1274 (2002).

WCFS success factors: involvement, commitment and communication.

Wageningen Centre for Food Sciences



Structure and Functionality

Scientific Director Prof. Rob Hamer

2002 was a landmark year for research on structure and functionality, with two important projects completed and three new projects starting. The year was particularly productive with over 35 new publications in peer-reviewed journals. In addition, a new system for patenting was implemented leading to the identification of six new patent ideas by the end of 2002.

During the year, considerable attention was given to implementing a new research strategy by scientific director Rob Hamer in close consultation with the Focal Points. This strategy aims to expand product control during processing to include generation of the desired sensory attributes. The three new projects started in 2002 are investigating how foods can be produced within constraints of production logistics and safety while generating attributes such as 'creamy' and 'full' when consumed.

The integrated research approach to food texture and perception was reinforced during this year's WCFS Food Summit, which was on Texture Dynamics: perception and measurement (see page 21). During the three-day expert meeting, it became clear that WCFS is in the forefront of research in this area. The meeting gave wide support to an approach in which generation of food textures is studied in relation to processing, desired storage and handling properties, and to desired sensory attributes.

Another important development following consultation with the Focal Points and the recommendation of the Programme Council in 2002 was the extension of the research scope from soft solids only to soft solids *and* solids with the start of the new crispy/crunchy project (see page 32).

Two-pronged approach
With the start of three new projects, research on Structure and Functionality enters a new phase in which the study of food texture is closely linked to research on perception. The study of the formation of food texture in relation to changes in process conditions (such as temperature, pressure and shear) is now closely linked to a more psychological/physiological approach. Selected sensory attributes will be linked to relevant in-mouth changes that trigger sensations leading to these attributes. In this way, optimum texture will be redefined. Creaminess, for example, can be related to specific functions of fat in the mouth (flavour delivery, coating, lubrication). One of the new projects will study these functions in detail and how optimal fat functions can be engineered.

NEW RESEARCH ON TEXTURE AND PERCEPTION

In 2002, the Programme Council approved three new interrelated research projects, all based on soft-solids as the model food. The three projects will focus on the creation and perception of textures responsible for sensory attributes, such as body/fullness, creaminess, fattiness and stickiness. The research entitled *Integration of sensory perception* will focus on the integration of primary sensations (texture, taste and aroma) in complex sensory attributes such as creamy, fatty and full.

The two other projects will focus on the physical and chemical origins of these attributes building on the research work on emulsions and foams and dispersions and gels. Researchers in both projects will work in close cooperation to model changes in the structure and rheological properties on ingestion of the food.

In one of these projects entitled *Dynamics of biopolymer networks and textures*, researchers will investigate the mechanical properties of biopolymer networks in the continuous phase of composite food products. The research is directed to designing and producing the microstructures responsible for specific mouth-feel performance and stability of soft-solid composite food products based on biopolymer networks. The research team will define the contribution of biopolymer networks to the physical and chemical origin of the perception of sensory attributes such as body/fullness, stickiness, and creaminess.

The behaviour of the dispersed phase is subject of the other research project, which is entitled *Engineered textures of emulsions and foams*. The research team will describe the physico-chemical mechanisms in emulsion droplets and foam bubbles under oral conditions in relation to hypothetical physico-chemical origins of sensoric attributes, such as velvety after-feel, fatty taste and lubricating ability. This is a relatively new area of research, in which few systematic investigations have been reported.

STABILITY OF EMULSIONS AND FOAMS

Research on the stability of emulsions and foams led by Dr Ton van Vliet was completed in 2002 except for three PhD fellows who will submit their theses in 2003. The overall research aim was to establish the mechanisms responsible for the stabilisation of emulsions and foams at molecular and mesoscopic scale under selected conditions, especially systems with a high volume fraction in the dispersed phase. The work done in 2002 is described below.

Stabilising high phase volume foams
WCFS researchers led by Dr Marcel Meinders investigated the contribution of the mechanical properties of the continuous phase and the adsorbed protein layer at the interface to stability against disproportionation (Ostwald ripening) of high volume fraction foams. Time-dependent relaxation effects in the rheological properties were incorporated in a previously developed model, thus improving correspondence between experiment and theoretical calculations significantly. Based on this model for single droplets/bubble dissolution, an advanced mean field theoretical model was developed to describe disproportionation in an emulsion and foam. The model describes experimental data on disproportionation in foam well, except for the latter stages.

Meinders, M.B.J., et al., Royal Society of Chemistry, Cambridge, in press.

Phase behaviour of protein-stabilised emulsions
During the year, PhD fellow Theo Blijdenstein worked on the role of ingredients such as protein and salt on the creaming stability of emulsions in the presence of dextran. At high concentrations, dextran-induced depletion flocculation led to the formation of a weak network of emulsion droplets that contracts rapidly. Creaming stability at low dextran concentration and high network contraction was affected by calcium concentration. This could be explained in terms of the relative rates of droplet flocculation and creaming, and the firmness of the bonds between the droplets. In addition, in systems stabilised by β-lactoglobulin and whey protein isolate, higher concentrations of dissolved protein were found to increase the polysaccharide concentration needed to induce depletion flocculation. This effect seems to be related to an increase in the low-shear viscosity of protein-dextran mixtures.

Blijdenstein, T.B.J., et al., Food Hydrocolloids, in press.

Coalescence stability
Dr George van Aken and team completed the work on elucidating the mechanism responsible for flow-induced coalescence in high volume fraction oil-in-water emulsions stabilised by proteins and in emulsions containing aggregated droplets. This mechanism has not been described previously. Surfactants were shown to reduce flow-induced coalescence by displacing adsorbed protein from the droplet interface. Results indicate that flow-induced coalescence can be prevented or induced during processing and handling by adapting the emulsion stabilising mixture.

Aken, G.A. van, Langmuir 18: 2549-2556 (2002).
Aken, G.A. van, et al., Langmuir 18: 7364-7370 (2002).

Adsorption of emulsion droplets at air-water interfaces
PhD fellow Natalie Hotrum identified the role of the mechanical properties and specifically the fracture properties of the adsorbed protein film at the air-water

surface on the spread of an emulsion droplet. Spreading behaviour on surfaces with an adsorbed flexible protein such as β-casein differed to that on surfaces with adsorbed globular proteins such as β-lactoglobulin and soy glycinin. The latter formed networks at the interface that fracture at large deformations. Fracture behaviour is important with regard to the insertion and spread of emulsion droplets at the air-water surface under aeration conditions where extension rates are low or moderate such as stirring, pouring and chewing. This aspect has not been reported previously.

Hotrum, N.E., et al., Journal of Colloid and Interface Science, 247: 125-131 (2002).

Properties of adsorbed protein layers

Dr Martin Bos and PhD fellow Anneke Martin have made significant progress in establishing the relationship between molecular properties of proteins and the mechanical properties of adsorbed protein layers, and the relationships between these mechanical properties and the stability of emulsions and foams. Conformational changes on adsorption and formation of interfacial layers were studied using Infra-Red Reflection Adsorption Spectroscopy (IRRAS), a technique developed at WCFS. A conformational change was observed for β-lactoglobulin, ovalbumin and soy glycinin at pH 3, for glycinin at pH 6.7 and for β-casein, no change was observed. Minor changes of 10% occurred in protein structures within minutes before the first measurements could be made. There is little correlation between these observed conformational changes and the mechanical properties of the adsorbed proteins. Other factors are likely to play a role, such as the density of the adsorbed layer and the hardness of the adsorbed molecules.

Martin, A.H., et al., Journal of Colloid and Interface Science, 254: 175-183 (2002).
Martin, A.H., et al., Langmuir, in press.

CRISPY/CRUNCHY BEHAVIOUR OF CELLULAR SOLID FOODS

Research on crispy/crunchy behaviour of cellular solid foods started in 2002 under the leadership of Dr Ton van Vliet. The research aims to substantially extend the period in which cellular solid food products with a crispy or crunchy crust retain these sensory properties. This requires unravelling the mechanisms responsible for the crispy/crunchy crust of cellular solid food products at molecular, meso- and macroscopic scale and specifically the mechanisms responsible for the loss of these sensory properties.

Model systems

Based on extensive discussions with WCFS partners, it was decided to work with two model food systems – a baked flat bread and a fried model product. Variations in molecular, meso- and macroscopic properties can be achieved by varying the composition and the processing conditions. The baked model product represents products with a crust that had originally the same basic chemical composition as the food product itself, but the water content and distribution have altered during crust formation. The flat bread model is prepared from standard quality flour and yeast, sugar and salt. The fried product model represents food products with a coated crusty layer after frying. The basic coating is a starch or wheat flour batter that becomes crispy/crunchy when fried in oil. The use of a traditional 'honey cake' filling allows the production of products with an even flat crust, and adjustable moisture content on the inside.

Sensory study

A trained expert panel will be used to establish terms to describe sensory characteristics of cellular solid food products with a crispy or crunchy crust. The perceived differences in crispy/crunchy characteristics of different foods will be studied as well as the changes in these characteristics with time after baking and frying. Another experimental panel will be trained in the perception of crispy/crunchy characteristics and changes in these characteristics.

Substantial progress has been made in establishing a technique for determining the mechanical characteristics in combination with analysis of the acoustic signal produced during the fracture process.

Luyten, H., et al., Proceedings of the Third International Symposium on Food Rheology and Structure, Zurich, Switzerland (2003).

FOOD BIOPOLYMER DISPERSIONS AND GELS

The texture of many soft-solid foods arises from interactions between proteins and polysaccharides. The WCFS research team led by Dr Ronald Visschers is investigating how food textures are affected by processes, such as aggregation, formation of space-filling networks and mutual repulsion resulting in phase separation. These processes determine the way in which food texture develops during manufacturing and storage.

In 2002, the various lines of research were integrated further. In close collaboration with microrheology research, a continuous shear-cell was developed to study the effect of shear on food subjected to shear while gelling or in phase separation. The role of calcium and electrostatic repulsion in protein denaturation and aggregation was investigated in collaboration with the researchers working on biomolecular studies.

Simons, J.W.F.A., et al., Archives of Biochemistry and Biophysics 406: 143-152 (2002).

Protein aggregation and cold gelation

PhD Fellows Mireille Weijers and Arno Alting worked on aggregation of ovalbumin, whey protein isolate and β-lactoglobulin at neutral pH. Several differences were observed between the heat-induced denaturation and in the aggregation kinetics. The denaturation kinetics of ovalbumin does not show concentration dependence. So, denaturation seems to be a first order reaction, while for β-lactoglobulin an overall reaction order of 1.5 was found. But as the structures of the aggregates and gels formed were dependent on the protein concentration, sodium chloride concentration and pH, different techniques were used to characterise ovalbumin aggregates including size exclusion chromatography, light scattering, calorimetry and *in situ* measurements. These results were incorporated in a kinetic model in which a small fraction of ovalbumin does not contribute to the network formation.

Weijers, M., et al., Biomacromolecules 35: 4753-5762 (2002).

The effect of aggregate size on the rheological properties of cold-set whey protein isolate gels was established using thiol-blocked aggregates of different sizes produced by varying the protein concentration at heating. This indicated that the number of thiol groups rather than the size of the aggregates determined the hardness of cold-set whey protein isolate gels.

Alting, A.C., et al., Food Hydrocolloids, in press.
Alting, A.C., et al., Journal of Agricultural and Food Chemistry 50: 4674-4681 (2002).

Comparison of the cold gelation of whey protein isolate and ovalbumin clarified the generic and protein-specific phenomena in cold gelation of globular proteins including the role of aggregate shapes (globular versus linear filaments) and the reactivity of exposed thiol groups. These results present a detailed picture of the processes that occur when proteins are used as thickening agents in food systems. The results also indicate how the resulting properties can be closely controlled, particularly by monitoring and adjusting the reactive thiol content of the protein aggregates.

Protein and polysaccharide interactions

WCFS researchers led by Dr Hans Tromp are studying how fractionation of molar mass affects the rheological properties of a product in which phase separation

occurs. For gelatine-dextran mixtures, fractionation of the ingredients across the co-existing phases was found to be an exponential function of molar mass. Together with PhD fellow Marijke Edelman, this was interpreted as the direct result of the free energy of mixing of gelatine and dextran solutions, and a free energy of mixing was found to be related to the extend of fractionation. This was confirmed independently by experiments measuring the surface tension between co-existing phases. Comparison of a gelatine-dextran-water system and a dextran-polyethylene oxide-water system showed that both systems display similar behaviour, thus indicating that this type of phenomena can occur in many different systems. Since the molar mass fractionation has a direct effect on the rheological properties of the two phases, it is important to understand these phenomena in foods where the texture depends on phase-separation processes.

The key parameters involved in phase separation and texture formation under shear are now being further understood. Different micro-rheological set-ups were further explored in 2002. In the formation of directional structures such as ellipsoids and needles, referred to as non-isotropic structures, shape fixation rapidly follows shape formation. Shape is determined by elongation while rotation causes breakdown of the particle. These design rules indicate new possibilities for engineering specific food textures.

Further integration of the research has resulted from work on the role of polysaccharides in determining the structure and strength of cold-set protein gels by post-doctoral fellow Dr Maarten van der Wielen and Ing. Anne van de Pijpekamp. Polysaccharides have been found to accelerate the effect of protein denaturation and aggregation in a way that only partly depends on their molecular charge and other properties. The gelation behaviour of pre-formed milk protein (β-lactoglobulin or whey protein isolate) aggregates in the presence of dextran, carrageenan (non-gelling conditions) and carboxymethyl cellulose have been investigated. In the presence of charged polymers, more open gel structures are formed with consequently, more closely packed protein clusters and less connectivity when polysaccharide concentration is increased. Fracture also appears to be more closely related to the mesoscopic gel structure than to the microscopic interactions.

Gelation can obstruct phase separation as well as enhance it. The gelling behaviour of one of the components induces network formation, which viscosifies the whole system. As a result, the kinetics slow down, and eventually the system is kinetically trapped into a non-equilibrium state. Gelation increases the effective molar mass of the gelling component. This influences the thermodynamics of the phase separation increasing the drive to phase separation. The final structure is determined by the relative rates of phase separation and gelation.

Postdoctoral fellow Dr Els de Hoog has determined the effect of various parameters on the kinetics and structure of phase separation in the presence of gelation in an aqueous mixture of gelatine and dextran. The effect of the parameters quench depth in temperature, molar mass, and cooling rate were quantified using small angle light scattering and the results used to control texture of mixed gels. The structure evolution was visualised with confocal scanning light microscopy. The cold gelation of globular proteins of two types of proteins was compared in the analysis of the structural properties. The results showed that, although a gel had already formed, further structural re-arrangements took place. Since these re-arrangements can significantly affect key textural properties, such as syneresis, it is important to establish how these can be controlled in order to design better food structures.

PHYSICAL AND CHEMICAL ASPECTS OF SENSORY ATTRIBUTES

The research group led by Dr Hugo Weenen follows a three-pronged approach to understanding the relationship between the textures of semi-solid foods (desserts, sauces, mayonnaise) and the perception of specific attributes such as creamy and fatty. In the last two years, extensive studies have been carried out with a wide series of model products to generate the necessary data to enable properties and attributes to be linked. Oral physiological studies have unravelled interactions between food, saliva and the human sensing system. In addition, physical studies have focused on developing more sensory measuring protocols and understanding the relative importance of mechanical versus enzymatic breakdown of texture upon mastication. In 2002, this has led to a number of important breakthroughs.

Firstly, a specific role of fat was discovered. The amount of fat retained in the mouth after swallowing could be related to the perception of creaminess. Secondly, the role of salivary enzymes in changing the properties of the food during oral manipulation has been established. The relevance of starch breakdown in the mouth has often been discussed, since the period of oral manipulation of semi-solid foods is only a few seconds. WCFS researchers have succeeded in proving its relevance since experiments using an amylase inhibitor demonstrate that amylase breakdown of starch significantly affects texture perception. In addition, methods have been developed to characterise food product and saliva mixing. Good correlations have been found between these mixing parameters and texture attributes. This work highlights the relevance of oral processing in texture perception. Using custom methods, we can now follow relevant events during mastication and investigate their relationship to specific sensory attributes. With four instrumental measurements, we can predict the creaminess mouth-feel of custard desserts, mayonnaises and sauces.

Food texture and perception

In order to link food texture to perception, the sensory model for sensory creaminess had to be refined. Creaminess is a complex attribute. Univariate and multivariate correlation analysis of creaminess with other sensory attributes led to a quantitative model for creaminess mouth-feel based on other sensory texture attributes. These models have been used successfully to predict the creaminess of commercial products. More importantly, the parameters in these models can be linked with intra-oral and custom rheological measurements. Although not yet completed, significant progress was made in 2002 with custom rheological measurements. These physical studies have two aims: firstly, to develop *in vitro* measurements that better predict sensory attributes, and secondly, to distinguish between the importance of mechanical and enzymatic breakdown of food texture in relation to perception. These studies will be completed in 2003 and integrated with the results of the physiology and sensory studies.

Texture perception in the young and elderly

In the framework of the EU Health Sense project, WCFS is studying how changes in sensory physiology, sensory psychology and socio-cognitive factors influence food choice. PhD fellow Stefanie Kremer is investigating the interactions between texture, flavour and trigeminal stimuli, and how these interactions change with ageing, and on compensation opportunities. A number of experiments have been conducted with liquid, semi-solid and solid foods. Different sensory attributes have a high positive or negative contribution to a liking of food products for elderly and young subjects.

Wilkinson, C. et al., Trends in Food Science and Technology 442-450 (2001).

Engelen, L., et al., Journal of Oral Science 110: 412-416 (2002).

MICROSTRUCTURE AND FUNCTIONAL PROPERTIES OF FOOD SYSTEMS

At the end of 2002, WCFS researchers Drs Marcel Paques and Dr Yves Nicolas had completed the design and construction of deformation cells configured with Confocal Scanning Laser Microscopy (CSLM) and Diffusion Wave Spectrometry (DWS). These cells were developed to enable WCFS researchers to characterise microstructures and functional properties of food systems at the micrometer level (microrheology). Because of the wide range of experimental conditions needed, three new types of deformation cells were developed.

The first is an Oscillatory Shear cell, designed by the WCFS team, and built by colleagues at Strasbourg University. As there is no interference between CSLM mapping and DWS measurements, this deformation cell can be used simultaneously to monitor dynamic formation and breakdown of macromolecular networks.

The second is a Continuous Shear cell designed in collaboration with the Technical University Twente, Forschungs Zentrum Juelich, and Unilever Research and built at the technical workshop of Unilever Research in Vlaardingen. This cell can be used to visualise structural changes in a material and film at a micrometer level during and following deformation.

The third configuration is a Compression Cell with an Instron compression system that requires interfacing with CSLM. The optimum alignment has been established for the observation of materials under deformation. The interface between the Instron and CSLM enables observation of the stagnation point during compression or extension, and simultaneous recording of stress measurements.

BIOPOLYMER STABILITY AND FUNCTIONALITY

The research team led by Dr Harmen de Jongh is investigating the key mechanisms that control formation and stabilisation of microstructures in foams, emulsions and gels by assessing the intrinsic functionality of the most dominant ingredient in the process. These investigations will provide insight into the potential of ingredients to influence these mechanisms and thus to better specification of ingredients than now possible.

The work in 2002 focused on preparing ingredients with specific properties based on our current understanding of what is important in controlling the properties of an emulsion, foam or gel. This work was very successful and enabled the researchers to test the ingredients under relevant conditions. Some highlights are given here.

Interface systems

Dr Marcel Meinders and PhD fellow Peter Wierenga increased the solvent-exposed hydrophobicity of ovalbumin by introducing small acyl chains into the protein. A direct relationship was established between exposed hydrophobicity and the kinetics of net adsorption, with the aid of a model developed by WCFS describing adsorption behaviour in terms of a limited number of molecular parameters, such as net adsorption rate, area per molecule at the interface, compressibility of the protein at the interface, energy of adsorption and relaxation phenomena. Moreover, acylation of the poor foam-forming protein ovalbumin changed the protein into a reasonably well-foaming agent. This work is now being extended to establish other intrinsic properties of a protein that can be controlled in order to improve foam formation.

Gels

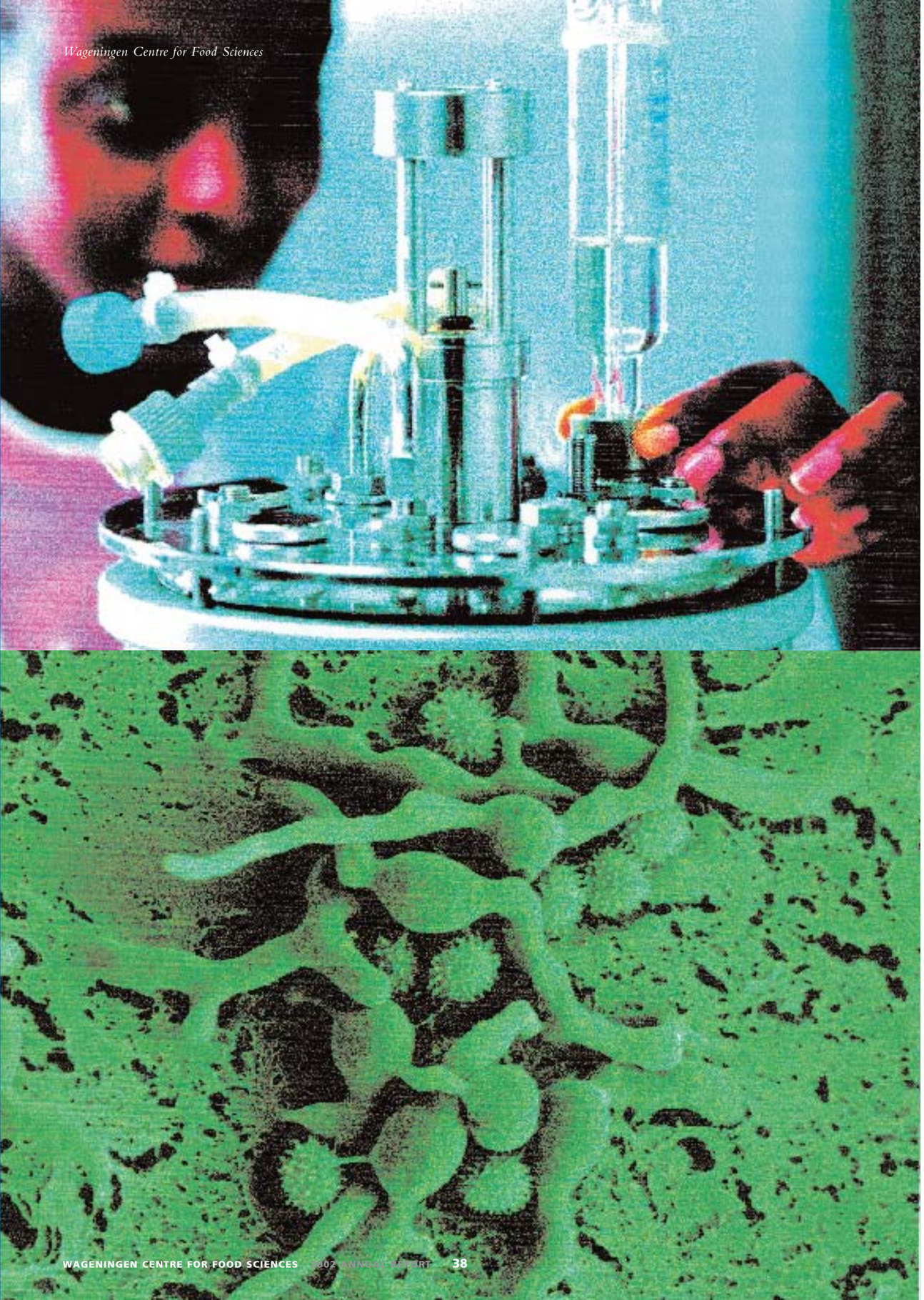
Proteins gel only after part of their conformation is changed as a result of heating, for example, and reactive particles are formed. These particles aggregate further to form the final gel. The first step, however, holds the key to improving the efficiency of proteins with respect to gel formation with the possibility of controlling the process under certain external conditions. PhD fellow Kerensa Broersen has made significant progress in understanding how to control this first step of heat-induced aggregation, and her work on glycosylated proteins highlights the importance of unfolding kinetics.

IRRAS

WCFS has developed a unique system Infra-Red Reflection Absorption Spectroscopy (IRRAS) for collecting quantitative data on the behaviour of proteins at an air-water interface. In 2002, use of this technique in a number of projects demonstrated its potential to provide information on protein-protein interactions and the apparent pH in a surface layer. Used in combination with time-resolved fluorescence techniques, IRRAS has been successfully applied to collect information on the lateral mobility of proteins at an interface. Use of IRRAS has enabled WCFS researchers to gain better understanding of how proteins control the properties of an interface and thus insight into designing ingredients with the precise functionality required.

Daas, P.J., et al., Journal of Agricultural and Food Chemistry 50: 4282-4289 (2002).
Meinders, M.B.J., et al., Biopolymers 67: 319-322 (2002).

'To get results that are not expected, you need to push science to the limit. That requires scientists with vision and a clear idea of how industry can use their results.'



Microbial Functionality and Safety

Scientific Director Prof. Willem de Vos

During the year, there were substantial changes and adjustments to the project portfolio. Two major projects were completed, one on regulation mechanisms and the other on metabolic engineering of lactic acid bacteria. In the final discussions with the Focal Points, it became clear that the scientific impact was rated higher than the industrial impact; the work provides a sound basis for contract research.

Research on this important group of food-grade fermentation microorganisms continued with the start of two new projects in 2002. One of these projects is on the functionality of lactic acid bacteria in the human gastrointestinal tract. The transcriptome-based research is directed to identifying and studying molecular responses of host and microbe, using as model microbe *Lactobacillus plantarum* WCFS1 that can survive passage in the human gastrointestinal tract in an active form.

The other project will build on the successful work on metabolic engineering of lactic acid bacteria and addresses the overproduction of vitamins and other cofactors in a functional genomics approach. This project will be incorporated in the Kluyver Centre for Genomics of Industrial Fermentation, one of the designated genomics centres by the Netherlands Genomics Initiative, in which WCFS is a partner.

In 2002, the Programme Council approved a new research project to investigate the effects of mild physical and biological preservation methods on the food-borne microorganisms *Bacillus cereus* and *Listeria monocytogenes*. Entitled Food preservation and safety, the project will use genomics-based approaches to identify key parameters in stress response and its regulation in the spore-forming *B. cereus* and *L. monocytogenes,* and also molecular mechanisms of stress adaptation and selection of variants.

2002 was a particularly productive year with over 30 papers published and a patent application resulting from the work on stress response was filed.

An overview of the year's research activities is presented.

REGULATION MECHANISMS IN LACTIC ACID BACTERIA

In December 2002, the research project on regulatory mechanisms in lactic acid bacteria was completed. These bacteria are important in industrial food fermentation. They provide flavour, texture and longer shelf-life of food products, and contribute to gastrointestinal health. WCFS researchers led by Dr Michiel Kleerebezem carried out the four-year research project at NIZO food research, Wageningen University and TNO Nutrition and Food Research Institute.

One of the key achievements of this project was the complete genome sequencing and full annotation of *Lactobacillus plantarum* WCFS1. A first generation, clone-based DNA microarray has been constructed and used for transcriptome profiling of *L. plantarum* grown under variable conditions and to compare wild-type cells with specific mutants. Second generation, ORF-based DNA microarrays are being constructed and will generate data sets that will improve the efficiency of the transcriptome profiling in further research to be undertaken by WCFS in this area.

Kleerebezem, M., et al., Proceedings of the National Academy of Science. USA 100: 1990-1995 (2003).

Regulation of sugar metabolism

Work carried out by PhD fellow Patrick van den Bogaard on catabolite repression in *Streptococcus thermophilus* has generated insights into how to engineer the sugar metabolism of this microorganism. This work has concentrated on the role of CcpA and HPr in the regulation of lactose and galactose metabolism. The genetic organisation of the *gal-lac* locus was shown to be highly conserved in *S. thermophilus* strains, and the infrequently encountered capacity to

ferment galactose appeared to reside in single nucleotide changes within the *gal*-operon promoter. A *ccpA* disruption mutant was constructed in both wild-type and galactose fermenting mutants of *S. thermophilus*. Studies on the role of CcpA in the regulation of lactose metabolism provided new insights and tools to study the regulation of the important genes involved in xylose and lactose/galactose transport, degradation and acid formation.

Finally, the role of CcpA in *L. plantarum* was investigated using a DNA microarray to compare gene expression of a wild-type strain and a C*cpA* mutant. The results confirm the global regulatory role of CcpA in regulation of gene expression.

Van den Bogaard, P.T.C., Catabolite control of sugar metabolism in Streptococcus thermophilus*, PhD thesis, Wageningen University (2002).*

Growth phase dependent gene regulation and environmentally regulated processes

Prolonged subculturing of *Lactococcus lactis* mutants deficient for lactate dehydrogenase (*ldh*) under anaerobic conditions resulted in recovery of the lactate producing capacity. PhD fellow Roger Bongers showed that this recovery was due to the transcriptional activation of a normally silent paralogous *ldhB* gene. The mechanism of *ldhB* activation was shown to involve the site specific and directed insertion of an *IS* element, *IS*981, in the upstream region of the *ldhB* gene. These results underline the determinant role of NADH regeneration in the metabolism and growth rate on these starter bacteria for industrial dairy fermentations.

The research focus has shifted to oxidative, bile and high-cell density stress in *L. plantarum*. Research on the high cell density stress aims to differentiate among the stress responses induced by high lactate concentrations, low pH and high osmolarity, which are all relevant for cells grown to high cell densities in fermenters. DNA microarray analyses were performed to gain an initial insight into the response to such stresses in this organism.

Clone-based DNA microarrays were shown to be useful in stress-response profiling. Multiple changes were found in global transcription profile between non-stressed and stressed cells, and cells grown aerobically and anaerobically. Currently, the data sets obtained from these experiments are being analysed and this work will be continued in the IOP Genomics project entitled Comparative and predictive transcriptome analysis of Gram-positive microorganisms.

Lactic acid bacteria in the gastrointestinal tract

Research on *L. plantarum*, which displays good survival traits, persistence, and activity during passage through the gastrointestinal tract, has concentrated on elucidating specific activities expressed by this bacterium during its passage. PhD fellow Peter Bron has developed and exploited systems for *in vivo* and *in vitro* selection of conditionally active promoters of *L. plantarum*. These systems were used to identify promoters activated in the gastrointestinal tract *in vivo*, which was done at the Pasteur Institute, Lille (France) as part of the EU collaboration project. This work continues to validate these results and to develop methods for *in situ* detection of specific *L. plantarum* mRNAs in the gastrointestinal tract.

Vaughan, E.E., et al., Antonie Van Leeuwenhoek 82: 341-352 (2002).

In collaboration with Lund University (Sweden), WCFS is working on elucidating specific *L. plantarum* gene expression *in situ*. DNA microarrays are being used to evaluate expression profiles in human biopsy samples containing significantly high numbers of *L. plantarum* cells. These studies will elucidate the specific activities expressed by this model microbe in this highly complex environment, and also provide the tools for specific *in situ* delivery of desirable health-promoting compounds in the gastrointestinal tract. This work will be continued in the new research project entitled Host-microbe interactions.

Bron, P.A., et al., Applied Environmental Microbiology 68:5663-5670 (2002).

In situ ***production and delivery in food products***

After proteolytic degradation of substrate proteins by proteinase and a range of peptidases, the resulting amino acids serve primarily as substrates for protein biosynthesis. They are also the precursors of important flavour compounds. The work of visiting scientist Dr Maria Fernandez focused on the regulation of gene coding for cystathionine β-lyase (*metC*) from *Lactococcus lactis* MG1363. This enzyme is involved in methionine biosynthesis and involved in the generation of sulphur-containing flavour compounds by converting methionine to methanethiol, an important precursor for the flavour compounds – dimethyl disulphate and trimethyl disulphate.

Expression of the *metCcysK* operon appeared to be regulated by the concentration of cysteine or methionine in the medium. Both *cis*- and *trans*-acting regulatory factors involved in *metCcysK* regulation were identified. The regulator CmbR (encoded by the gene *cmbR*) was shown to activate the *metC* promoter by binding to a direct repeat in the promoter and the binding appears to be strongly influenced by the presence of the co-factor O–acetyl-serine *in vitro*. This study clearly exemplifies the importance of understanding the regulation of transcription of flavour forming enzymes in microorganisms used in food fermentation.

Fernandez, M., et al., Journal of Bacteriology 184: 82-92 (2002).

HOST-MICROBE INTERACTIONS

A new research project started mid-2002 on the gastrointestinal tract with the aim of identifying the molecular response of host and microbe to one another. This will provide insight in host-microbe communications mechanisms using global and genomics-based approaches for developing fermented and other functional foods that affect the host physiology. The model microbe to be used is *Lactobacillus plantarum* WCFS1, an isolate of human origin that survives passage in the gastrointestinal tract in an active form, and contains a sequenced genome for which DNA microarrays are available.

While the ultimate objective is to elucidate host-microbe interactions in humans, initially animal model systems will be used to analyse the host response to consumption of live lactobacilli. Response in humans will be studied using *in vitro* (human cell lines), *ex vivo* (human biopsies) and eventually using *in vivo* systems.

Zoetendal, E.G., et al., Applied Environmental Microbiology 68: 3401-3407 (2002).

Research will also be carried out on commensal microbiota in the gastrointestinal tract using high-throughput, genomics related technologies to obtain insight in specific, dominant activities of this complex microbial ecosystem. The research led by Dr Michiel Kleerebezem will be carried out partly in cooperation with the new initiative on Nutrigenomics.

METABOLIC ENGINEERING OF FOOD-GRADE MICROORGANISMS

Many important components in food products are metabolites that are produced by microorganisms during fermentation. These can be components that attribute to flavour, to structural characteristics or to nutritional value of the food product. In a four-year project, headed by Dr Jeroen Hugenholtz and which

was concluded at the end of 2002, metabolic engineering was applied to lactic acid bacteria with the aim to increase production of these desired components. The work was carried out at NIZO food research, Wageningen University and ATO. This strategy involved physiological analysis of the relevant production pathways and, using this knowledge condensed in descriptive models, to control and increase the metabolic flux in the direction of the metabolite of choice. This was generally achieved by a combination of selected fermentation conditions and genetic engineering.

Metabolic models
The kinetic model developed for pyruvate metabolism in *Lactococcus lactis* and launched on the WCFS website in 2001, was further expanded in 2002 to include the entire glycolytic pathway. Based on detailed experiments, postdoctoral fellow Dr Marcel Hoefnagel optimised and verified the model, which now accurately describes sugar fermentation by *L. lactis* under different conditions. It can also be used for predictive purposes in metabolic engineering.

Hoefnagel, M.H., et al., Microbiology 148: 1003-1013 (2002).

Exopolysaccharide engineering
Exopolysaccharide produced by *Lactococcus lactis* NIZO B40 is composed of a repeating unit containing glucose, galactose and rhamnose. PhD fellow Ingeborg Boels described strategies to increase exopolysaccharide production by metabolic engineering. Several mutants engineered for increased production of the exopolysaccharide precursors (UDP-glucose and UDP-galactose) have been analysed by *in vivo* ^{31}P-NMR for their accumulation of different sugar phosphates during metabolism of lactose, glucose and fructose. This work was done at ITQB, Oeiras (Portugal) as part of a EU collaboration project. The results show that several metabolic intermediates in glycolysis and precursors for exopolysaccharide biosynthesis can be measured with NMR, in most cases even quantitatively, and that exopolysaccharide producers have lower pools of nucleotide sugars.

Boels, I.C., et al., Applied Environmental Microbiology 69: 1129-1135 (2003).

The genes responsible for synthesis of rhamnose nucleotide sugar precursors were cloned, individually and in combination, in *Lactococcus lactis*. A conditional knockout in one of the genes was constructed, which produced altered exopolysaccharide with a lower content of rhamnose. This presents a unique example of how metabolic engineering can lead to production of new exopolysaccharide structures. Finally, overexpression of the whole *eps* gene cluster in *L. lactis*, using a multicopy vector resulted in significant (fourfold) higher production of exopolysaccharide.

Boels, I.C., Metabolic engineering of exopolysaccharide production in Lactococcus lactis*, PhD thesis, Wageningen University (2002).*

Mannitol biosynthesis
Mannitol, which has antioxidant and low-calorie sweetening properties, is generally not produced by lactic acid bacteria, although the pathway for production is written in the genes. Using the metabolic model, several metabolic engineering strategies were designed to induce mannitol production in *Lactococcus lactis*. Work done by PhD fellow Wouter Wisselink led to the overexpression of the gene coding for mannitol phosphate dehydrogenase in *L. lactis*. While this did not lead to mannitol production, significant production of mannitol was observed in combination with a disrupted *ldh* gene or reduced phosphofructokinase activity. The kinetic model now predicts much higher mannitol production with increased activity of mannitol phosphatase.

Wisselink, W., et al., International Dairy Journal 12: 151-161 (2002).

Acetaldehyde production in yoghurt
Acetaldehyde, a major flavour component in yoghurt, is produced in small quantities by yoghurt bacterium *Streptococcus thermophilus* during milk fermentation. Visiting scientist Dr Ana Carolina Chaves has now firmly established that the hydrolysis of threonine through activity of threonine aldolase is the sole biochemical reaction leading to production of acetaldehyde in the yoghurt bacteria. Overproduction of this enzyme resulted in increased production of the flavour compound during milk fermentation.

Chaves, A.C., et al., Applied Environmental Microbiology 68: 5556-5562 (2002).

Folate production in lactic acid bacteria
The essential dietary vitamin, folate is found in fresh green vegetables but can also be produced by different lactic acid bacteria. PhD fellow Wilbert Sybesma studied increased production of folate by the lactic acid bacteria *Lactococcus lactis* and *Lactobacillus plantarum* with the ultimate goal of providing strategies to increase folate levels in fermented food. A HPLC analysis method was developed that can distinguish all different folate-derivatives.

Sybesma, W. et al., in Chemistry and Biology of Pteridines and Folates 623-630 (2002).

ENGINEERING OF MICROBIAL FUNCTIONALITY

A new research project on functional engineering of lactic acid bacteria started in November 2002. Work will focus on what happens to cells when production of crucial metabolites, such as co-factors, is disturbed or changed through specific or random mutation. Use will be made of high-throughput methodologies such as transcriptomics and metabolomics. The co-factors chosen are thiols, such as glutathione, and folates, for which suitable mutants have already been developed. The research project will be incorporated in the work of the newly established Kluyver Centre for Genomics of Industrial Fermentation.

SOLID-STATE FOOD FERMENTATION

WCFS researchers at Wageningen University, TNO and ATO led by Dr Arjen Rinzema are studying the process of solid-state fermentation from the level of the genome and transcriptome to large-scale fermenters. In these studies, *Aspergillus oryzae* is used as the model organism with wheat and soybean as model substrates, and amylases and proteases as model products.

Particularly novel aspects include the modelling of the effects of mixing on the fungal mycelium in solid-state fermentation, studies on the effect of aerial mycelia, and transcriptomics- and proteomics-based approaches to detect genes and proteins specific to solid-state fermentation. Our work on modelling of packed-bed fermentation, modelling of fungal growth and metabolism inside solid particles, and physiology extends previously reported studies to the level of a consolidated approach for process design and operation.

Modelling
WCFS' discrete element models for mixing solid substrate particles were extended to another fermenter geometry. Heat and moisture distributions have been incorporated into the model as well as the effects of inter-particle mycelium bonds in intermittently mixed fermenters. Breaking such bonds has been shown to be important in the solid-state fermentation process. A delay in mixing of only a few hours can adversely affect the fermentation, resulting in the formation of agglomerates and associated loss of process control. The models have now been extended to adequately predict agglomerates remaining after intermittent mixing, based on independent measurements of mycelium strength.

Schutyser, M.A., et al., Biotechnology and Bioengineering 79: 284-294 (2002).

PhD fellow Marisca Hoogschagen has also demonstrated that mass transfer between particles and gas is not rapid enough to reach equilibrium in non-mixed packed bed fermenters. As a consequence, existing process models underestimate the amount of air needed to cool fermenters. We have now adapted our process models accordingly and validation experiments are currently been carried out. The models have also been extended to take account of particle shrinkage due to evaporative cooling.

Inter-particle mycelium bonds present an interesting problem. These bonds need to be broken in order to maintain process control in conventional packed-bed fermenters, but breaking them has a deleterious effect on aerial mycelium. Using a wheat-flour model substrate, PhD fellow Yovita Rahardjo demonstrated that the presence of aerial mycelia could greatly accelerate fungal growth and amylase production. Experiments are now being carried out to establish how these findings can be used to accelerate packed-bed solid-state fermentation.

Rahardjo, Y.S., et al., Biotechnology and Bioengineering 78: 539-544 (2002).

Physiological and genetic aspects of Aspergillus oryzae

Studies on the expression and regulation of glucoamylases and proteases specific for solid-state fermentation have revealed their importance in nutrient availability and penetrative growth. Mutant strains of *A. oryzae* with different hyper-branching characteristics have been isolated using both classical and recombinant DNA approaches. At least one of these mutants shows altered expression levels of genes specific for solid-state fermentation-specific genes. Using transcriptomics-based techniques, a number of new genes were identified specifically induced under solid-state fermentation conditions. These genes are now being further characterised. New genes specific to solid-state fermentation have been identified using cDNA macroarrays/substractive gene libraries and are currently being characterised.

Te Biesebeke, R., et al., FEMS Yeast Research 2 (2002).

BACTERIA RESPOND TO MINIMAL PROCESSING

WCFS researchers at Wageningen University, ATO and TNO led by Dr Tjakko Abee are studying the short-term physiological adaptation and the long-term genetic adaptation response of microorganisms to stress. A major challenge for the food industry is to produce safe foods with the desired functionality using minimal processing technologies, such as the application of ultra high-pressure treatment and natural anti-microbial compounds. However, it is not known how these mild physical and biological preservation methods affect food-borne microorganisms. To apply such methods in a rational way, detailed studies are needed to determine their mode of action and understand the underlying mechanisms in food-borne microorganisms subjected to stress. Cellular targets of selected stress factors are being identified in the food-borne human pathogens *Listeria monocytogenes* and spore-forming *Bacillus cereus*. In addition, the cellular mechanisms that can provoke tolerance to stresses in minimal processing are being unravelled.

The alternative sigma factor σ^B (a secondary subunit of RNA polymerase) is important for survival and growth of a wide range of gram-positive organisms under adverse conditions and plays a crucial role in regulating gene expression in response to environmental stress. Its role in survival of *L. monocytogenes* EGDe at low pH and other stresses has been analysed by PhD fellow Henrike Wemekamp-Kamphuis. In cooperation with Prof. T. Chakraborty and Dr T. Hain, University of Giessen (Germany), the research team constructed an EGD-e σ^B mutant and showed that the σ^B mutant plays a major role in survival at low pH and other relevant food processing conditions.

Wemekamp-Kamphuis, H.H., et al., Applied Environmental Microbiology 68: 456-463 (2002) and 68: 4710-4716 (2002).

Insight has been gained into the role of cold shock proteins and solute transporters in the adaptation and survival of *L. monocytogenes* to low temperature, high osmolarity and high-pressure treatment. Isolation and characterization of a variant resistant to high-pressure treatment showed cross protection against heat, oxidative stress and acid exposure. This variant has a 3-base pair mutation in the *ctsR* gene encoding for the CtsR repressor that controls the expression of heat shock proteins, including clpP.

Karatzas, K.A.G., et al., Applied Environmental Microbiology 68: 3183-3189 (2002).
Karatzas, K.A.G., Listeria monocytogenes *- inactivation by essential oils and high pressure, and contribution of genetic variation to stress resistance, PhD thesis, Wageningen University (2002).*

Bacillus cereus

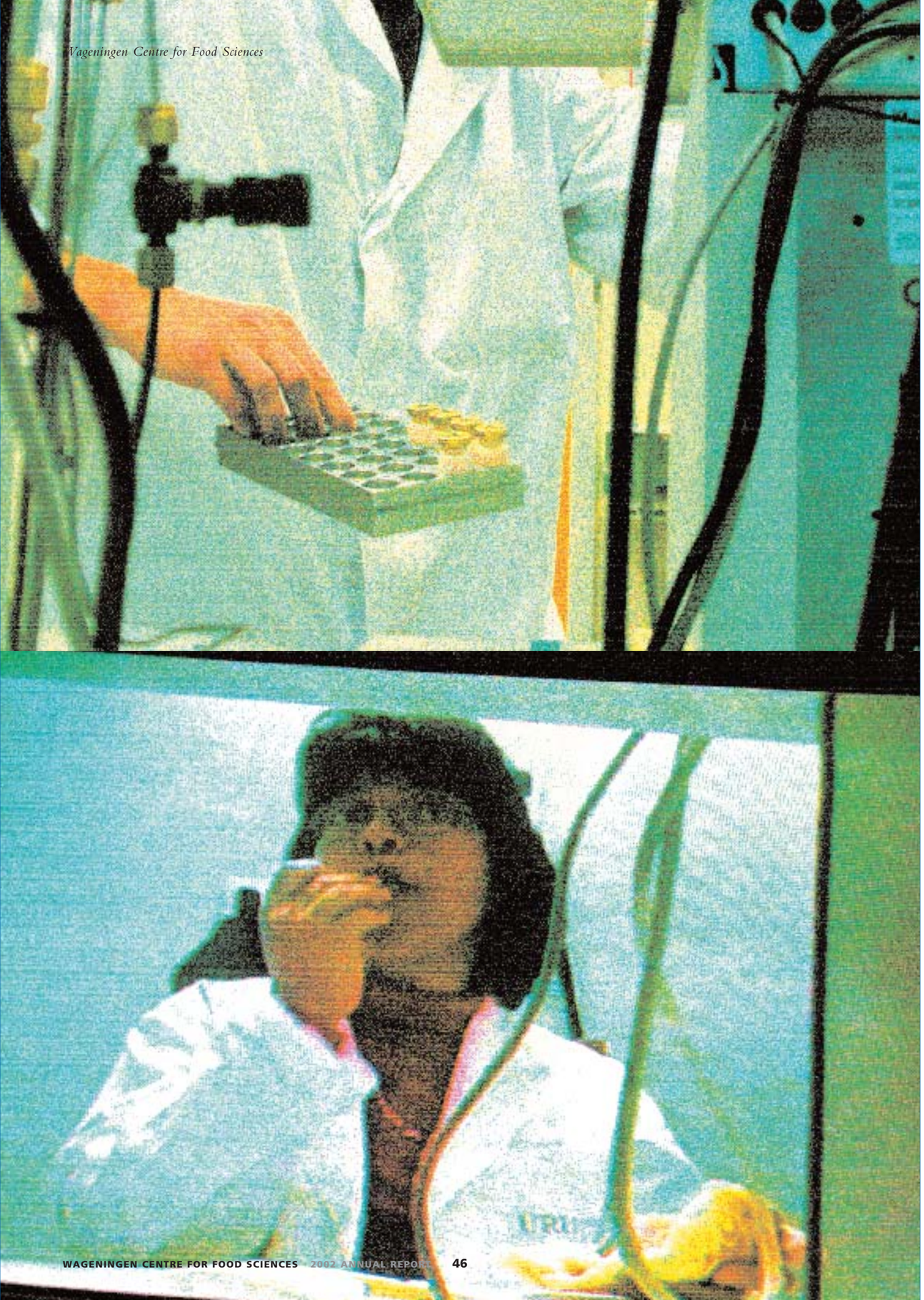
WCFS researchers have been studying the role of sigma factors (RNA-polymerase co-factors essential in fine-tuning DNA transcription) in *B. cereus* as well as the initial events in sporulation and germination. To monitor the ability of *B. cereus* to survive under stress conditions, its heat-adaptive response was determined. During pre-exposure to 42°C, *B. cereus* ATCC14579 adapted to heat exposure at the lethal temperature of 50°C. Other stresses also increased resistance of vegetative cells to exposure at 50°C, most notably exposure to high concentrations of ethanol, NaCl and low pH. This adaptive response required *de novo* protein synthesis. With the aid of two-dimensional gel electrophoresis, 31 stress-induced proteins were identified and the N-terminal sequences determined for 18 of them. One of the proteins induced under stress conditions was RsbV, the anti-sigma factor antagonist of σ^B, suggesting that σ^B does play a role in the stress-response of *B. cereus*.

Periago, P.M., et al., Applied and Environmental Microbiology 68: 3486-3495 (2002).

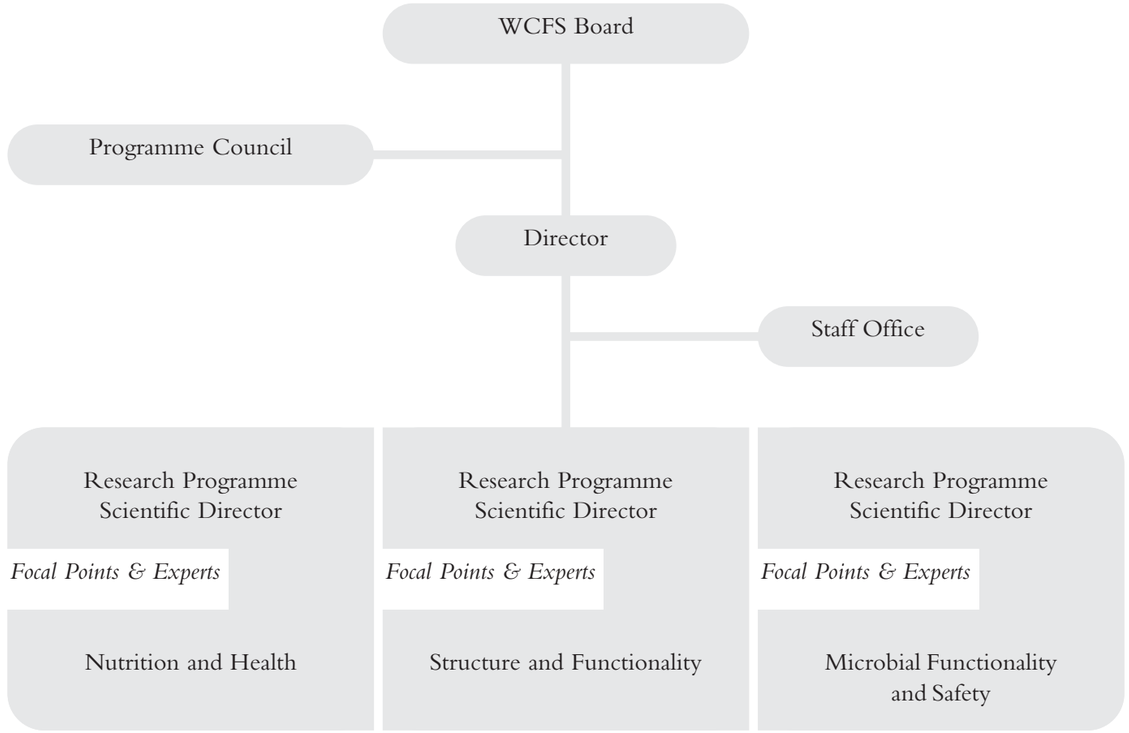
Periago, P.M., et al., International Journal of Food Microbiology 79: 17-26 (2002).

Northern blot analysis with DNA probes specific to σ^B showed a strong expression of σ^B found after a heat shock at 42°C and this corresponds with the upregulation of RsbV in two-dimensional gel electrophoresis. The observation that σ^B is also upregulated under glucose limitation and in the late logarithmic growth suggests that σ^B plays a role under conditions of nutrient starvation. A σ^B-overexpression mutant constructed using the Nisin Inducible Expression (NICE) system resulted in greatly increased tolerance to exposure at 50°C, again indicating the importance of σ^B in the stress response of *B. cereus*. DNA microarray analysis will be carried out on wild type *B. cereus*, σ^B-overexpression and σ^B-deletion mutants in order to identify the σ^B regulon.

Data mining of the *B. cereus* genome has identified seven germination (Ger) operons. Expression of the first gene was determined using RT-PCR. All operons showed expression during sporulation, suggesting a role in germination signalling in *B. cereus*. A random insertion mutant library in *B. cereus* resulted in a mutant blocked in the L-alanine germination pathway, which was analysed at molecular level. Insight into spore germination pathways and signalling may provide tools for the selection of minimal processing that controls the germination of *B. cereus* spores.



WCFS Organisation



WCFS BOARD

The Board of WCFS has executive responsibility and decision-making authority for the Centre's performance. This includes appointing the Director, and approving the WCFS mission, strategies, and financial plans. The Board meets twice a year to approve the research programme strategies and financial plan, and may be convened at other times, as required.

PROGRAMME COUNCIL

The Programme Council advises the Board on the research strategies and the WCFS financial project plans. Its key task is to review the project portfolio regularly and it is thus responsible for the overall direction of the research policy and programmes. All partners in WCFS are represented on the Programme Council, which meets three times a year, and may be convened by the Chairman at the request of partners.

FOCAL POINTS

The Focal Points meet several times a year to discuss with the scientific directors the overall research strategy for the programmes, research progress and to consider proposals for new research topics. The Focal Points have no formal authority over WCFS issues but are in a position to advise WCFS and their Programme Council members on appropriate ways of engaging their organisations in various WCFS activities.

WCFS Organisation

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2003

Discovery cannot wait

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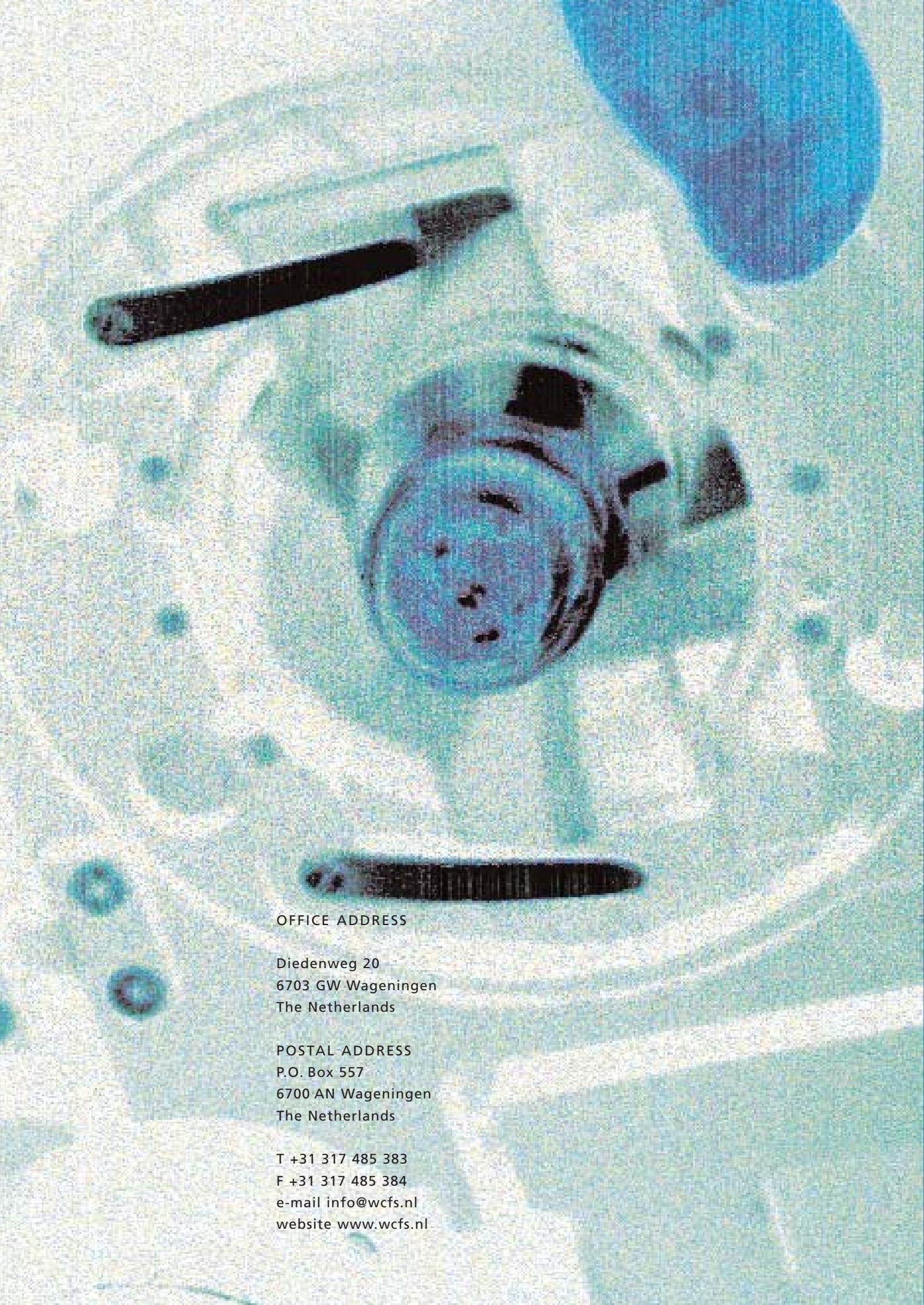

OFFICE ADDRESS

Diedenweg 20
6703 GW Wageningen
The Netherlands

POSTAL ADDRESS
P.O. Box 557
6700 AN Wageningen
The Netherlands

T +31 317 485 383
F +31 317 485 384
e-mail info@wcfs.nl
website www.wcfs.nl