JOURNAL of FOOD, AGRICULTURE & ENVIRONMENT

WFL Publisher
Science and Technology

www.world-food.net

Vol. 1, No. 1 **ISSN: 1459-0255** **January 2003**

General Information

Aims and Scope: The Journal of Food, Agriculture & Environment publishes peer-reviewed original research, critical reviews and short communications on food science and technology, agriculture, animal science and human nutrition, with particular emphasis on interdisciplinary studies that explore the intersection of food, agricultural, and the environmental.The journal publishes news and information about advances in science and technology and their applications in making better use of the environment and its resources products. The journal also considers a limited number of relevant scholarly manuscripts addressing ethical and socioeconomic issues related to modern agricultural and environmental sciences. The Journal offers advertisement space for special announcements from, and employment opportunities within, food, agricultural and environmental organizations in the public and private sectors.

Subscription: orders are only accepted on a prepaid and calendar-year basis. Issues are sent by standard mail (surface within Europe, air delivery outside Europe). Priority rates are available upon request or at:
http://www.world-food.net/ratesorder.php
Please find subscription rates and ordering details in detachable forms included in every Issue of the Journal, or request from the editorial office:
(email:Editorial@world-food.net), or visit our web site: http://www.world-food.net/ratesorder.php and print.

Important Note: If you are not a member of ISFAE yet, please visit our homepage:
http://www.isfae.org/member.php and register to benefit from special rates when purchasing scientific journal (s) or taking part in ISFAE meetings

You may send your registration fee and application form to:
ISFAE, Executive Secretariat
Meri-Rastilantie 3 C, FIN-00980 Helsinki, Finland
For more details, please contact : isfae@isfae.org

Advertising: Inquiries and correspondence regarding advertising should be sent to Advertising Department, World Food RD Ltd. Meri-Rastilantie 3 C, FIN-00980, Helsinki, Finland. Tel: +358 9 323 1768 or +358 50 505 11 35, e-mail: Advertisement@world-food.net

Book Proposal: The WFL Publisher invites scientists and experts to submit book proposal (s). We suggest that books are very focused and of high-level (research and/ or development level). For more details, please contact the WFL Publisher (email: publisher@world-food.net).

Online information: Table of contents, Abstracts, Advisory and Editorial Board listings, and Instructions to Authors regarding manuscript preparation and submission for publication can be accessed free of charge at web site http://www.world-food.net/scientjourn.php.

Published in Finland

Subscription Order

Subscribe Today!

Use the form to subscribe the *Journal of Food, Agriculture & Environment* or to recommend the journal to your **library** or **Department** for acquisition. Thank you so much.

Please fill out all fields in BLOCK PRINT and make sure to include the payment or signed copy of the payment or the check, made payable to World Food RD Ltd/JFAE Editorial Office along with this signed application and return it to the Office Address provided below.

	ISFAE Non-Members		**ISFAE Members**	
	Institution	Individual	Institution	Individual
	Print / online	**Print / online**	**Print / online**	**Print / online**
1. W. Europe; N. America, Australia Japan, Korea, Gulf States, N. Zealand, S.Africa	€ 295 / 195	€ 95 / 80	€ 180 / 145	€ 60 / 50
2. Rest of the world	€ 145 / 90	€ 80 / 65	€ 90 / 70	€ 65 / 45

Please note that these are annual subscription rates **(including postage)**, and issues are sent by standard mail (surface within Europe, air delivery outside Europe). Priority rates are available upon request or at http://www.world-food.net/ratesorder.php

Please make Payment to: World Food RD Ltd / JFAE Editorial Office
Bank Account Number: Okopankki OYJ: 572333-232831
Bank Address: OKOPANKKI OYJ, Pasilan Konttori, Opastinsilta 7, 00520 Helsinki, Finland
Fax: 00 358 9 1290 9756

JOURNAL of FOOD, AGRICULTURE & ENVIRONMENT
Subscription Order

Does your library subscribe the ***Journal of Food, Agriculture & Environment****? If not, please,* complete this form and submit it to your librarian or department head. Thank you so much.

Attention: Librarian/Department Head:

I have examined the *Journal of Food, Agriculture & Environment* and would like to recommend the journal for acquisition.

Signature________________________________Date________________________________

Name____

Library____

Department____

Journal of Food, Agriculture & Environment (JFAE)

Volume 1, 4 Numbers, 2003 (Institution non-ISFAE): € 295 / € 145, (Institution ISFAE) : € 180 / € 90 **(including postage**). Please note that these are annual subscription rates (**including postage**), and issues are sent by standard mail (surface within Europe, air delivery outside Europe). Priority rates are available upon request or at: http://www.world-food.net/ratesorder.php

	ISFAE Non-Members	ISFAE Members
	Institution	Institution
1. W. Europe; N. America, Australia Japan, Korea, Gulf States, N. Zealand, S.Africa	€ 295	€ 180
2. Rest of the world	€ 145	€ 90

WFL Publisher offers **Special Reduced Rates** for Librarian/Department Head from **some developing countries**. Please visit our web site:http://www.world-food.net/ratesorder.php or take contact with the publisher.

Postal Address: World Food RD Ltd.
WFL Publisher
Meri-Rastilantie 3 C,
FIN-00980, Helsinki Finland.
Tel: + 358 50 505 11 35, + 358 9 323 17 68, + 358 9 759 27 75
Email: Publisher@world-food.net
Editorial@world-food.net
Info@world-food.net

Web site: www.world-food.net

International Society for Food, Agriculture and Environment

-ISFAE-

ISFAE Executive Secretariat
Meri-Rastilantie 3 C, 00980 Helsinki, Finland
email:isfae@isfae.org , web site: www.isfae.org

The International Society for Food, Agriculture and Environment–ISFAE- **is a non-profit association**, and its mission is to disseminate news and information and promote science, development and technology all around the world. We invite you to join **ISFAE**.

Aim of ISFAE : The aim of the **ISFAE** is to bring together scientists and professionals, to stimulate, facilitate and coordinate scientific meetings and exhibitions, and to promote research and development and information delivery on an international scale.

Membership and benefits : ISFAE provides its members with a scholarly platform to share knowledge and information, develop information networks with colleagues who have similar interests and concerns. The main benefit of ISFAE membership is the receipt of its newsletters/journal AGRI-FOOD ‘*’Research and News”* as Pdf file or an online journal with a password supplied only after joining. Members of ISFAE will be able to purchase at reduced costs the scientific journal of Food, Agriculture & Environment” (JFAE) (for information, visit our web site: www.world-food.net / section Rates and Orders).

Members of ISFAE who attend any scientific meeting held under the auspices of the ISFAE at reduced costs and receive conference proceeding or resulting books at discounted rates. ISFAE will arrange each year meetings on issues related to ‘’Food, Agriculture or Environment”, and will provide opportunities for private and public firms, companies, industries or institutes to exhibition their products or materials at its conferences.

If you have not joined the ISFAE, now is your opportunity to be updated on current events and news. The annual ISFAE subscription rate is very symbolic, to cover the technical costs. We also welcome donations from individuals or organizations.

The **ISFAE** invites heads of institutions, heads of departments or decision makers to plan local, regional or international scientific meetings or exhibitions. The ISFAE will disseminate the information, invites participants, guides organizers and contributors, and take charge of resulting publication and distribution of the ‘’Agri-Food & Environment” proceeding series, or/and books.

ISFAE online journal accepts: News and information about ongoing R&D projects, findings/achievements, methodology/ technology used in laboratories or fields, popular articles, newsletters, forthcoming specific courses/lectures, news on meeting, conference workshop, important seminar, training programs or exchange visits, opinions and views, nominations, summary of reports, thesis, events, meetings, instructions and recommendations to farmers/growers, job announcements and employment opportunities, advertisements. For more details please contact:isfae@isfae.org

Subscription Rate: Basic fee plus PDF or online journal AGRI-FOOD “*Research and News”* ISSN: 1459-2029, 2003, Volume 1 (4 issues, number of issues will increase).

	Institutions	**Individuals**	**Students***
1. W.Europe; N.America, Australia Japan, Korea, Gulf State, N. Zealand, S.Africa	€ 98	€ 50	€ 25
2. Rest of the world	€ 60	€ 30	€ 18

Students*: Identification is needed when subscribing (to be send by air mail with the signed application form and payment documents).

ISFAE encourages students to join the association. **Special rates for students and members from developing countries are considered**. Please send the payment or signed copy of the payment or the check made payable and the application form to **ISFAE-Executive Secretariat** along with this application.

Payment to: ISFAE - Executive Secretariat
Account Number is: Okopankki OYJ : 572333-236519
Address of the Bank: OKOPANKKI OYJ, Pasilan Konttori, Opastinsilta 7, 00520 Helsinki, Finland
Tel: 00358 9 1290 9561/ 00 358 50 309 3658 / Fax: 00 358 9 1290 9756 / www.oko.fi

ISFAE membership application : Fill out all fields in BLOCK PRINT and PLEASE make sure to include the payment or signed copy of the payment or the check made payable to the ISFAE-Executive Secretariat along with this application and return to: ISFAE-Executive Secretariat, Meri-Rastilantie 3 C, 00980 Helsinki, Finland

Application Form

- Organization/ Institution/ Department Membership ☐

-ISFAE Individual Membership ☐

-ISFAE Student Membership ☐

Last Name ____________________

First Name ____________________

Affiliation ____________________

Address ____________________

City ____________________ Zip code ____________________

State/Province ____________________ Country ____________________

Phone ____________________ Fax ____________________

E-Mail ____________________

Signature ____________________ Date ____________________

JOURNAL of FOOD, AGRICULTURE & ENVIRONMENT

WFL Publisher
Science and Technology *www.world-food.net*

Vol. 1, No. 1 **January 2003**

Preface

Research on Food and Agriculture is progressively moving towards an interdisciplinary study of sustainable food production to meet the demand of growing human populations. However, as more countries industrialize, there is an increase in air and water pollution, soil contamination, as well as elevated levels of global warming and depletion of the ozone layer. This complex scenario challenges researchers to develop and test more appropriate technologies for sustainable agriculture. Research, for example, is being carried out to overcome problems of environmental stress, minimize the use of pesticides, slow post-harvest storage losses, and explore nutrition, animal science and human health, by using conventional and new technologies such as biotechnology, mutation-assisted breeding and molecular biology.

The Journal of Food, Agriculture & Environment (JAFE) is dedicated to meeting the need for a new journal that can encompass this wide range of topics and interdisciplinary approaches. JAFE will publish (in print and on-line versions) peer-reviewed original research, protocols, critical reviews and short communications in food science and technology, and agriculture, with particular emphasis on interdisciplinary studies on food, human nutrition, agricultural, animal science and environment. For advice or more information, please contact the editorial board members.

The members of the International Society for Food, Agriculture and Environment (ISFAE) will receive JFAE at the reduced rate. For more, please visit our website: www.isfae.org or contact the ISFAE executive secretariat.

S. Mohan Jain
Chief-editor

Ramdane Dris
Managing editor

Journal of Food, Agriculture & Environment (JFAE)
Managing Editor
Editorial Office
Meri-Rastilantie 3 C, 00980 Helsinki, Finland
e-mail:editorial@world-food.net or info@world-food.net
Web site: *www.world-food.net*

JFAE: We welcome your contributions including commentary, original research paper or short and detailed reviews for the publication in our journal. You may advertise special announcements from, and employment opportunities.

ISFAE: The ISFAE invites heads of institutions, heads of departments or decision makers to plan local, regional or international scientific meetings or exhibitions. The ISFAE will disseminate the information, invites participants, guides organizers and contributors, and take charge of resulting publication. For more details email to isfae@isfae.org

JOURNAL of FOOD, AGRICULTURE & ENVIRONMENT

WFL Publisher
Science and Technology
www.world-food.net

Vol. 1, No. 1 **CONTENTS** **January 2003**

Commentary

Food and Health

Agriculture

Environment

WFL Publisher
Science and Technology Food, Agriculture & Environment Vol.1(1): 9-11. 2003 *www.world-food.net*

Commentary

Do we need quality assurance and quality control of analytical measurements in R&D laboratories?

Irene Mueller-Harvey
NSRU, Chemistry and Biochemistry Laboratory,Department of Agriculture
The University of Reading, 1, Earley Gate, P O Box 236, Reading RG6 6AT, U.K.
email: i.mueller-harvey@reading.ac.uk

Received 14 October 2002, accepted 5 January 2003.

Introduction

Analysis is of critical importance to a wide range of subject areas, including those relevant to this new journal, i.e. food, agriculture and environment. There is no place within the context of good scientific research for the view that 'analysis is routine, easy and boring, i.e. not worth spending time or money on'. Gillespie[1] put this simply and succinctly: 'rubbish in, rubbish out'. Proper care during the entire analytical process, i.e. from the collection of samples to the analysis in the laboratory, can have far reaching consequences. To mention just one example, the incorporation of a new NIST Standard Reference Material for sulfur content as a tool to improve quality control (QC) during analysis significantly reduced measurement uncertainties, achieved considerable savings for manufacturers, and decreased S emissions to the environment[2].

Inter-Laboratory Variation

The need for comparable, useful data became obvious with the beginning of 'scientific agriculture' in the 19th century[3]. Different methods led to widely varying results and a great deal of confusion. The president of the Association of Official Agricultural Chemists (AOAC), now called the 'Association of Official Analytical Chemists', stated in 1896 'The matter of the analysis of foods and feedstuffs, as shown by the experience of the association, is one of the most difficult questions connected with the work of this organisation'. Numerous inter-laboratory comparisons have demonstrated that the analysis of biological samples tends to be more difficult to standardise between laboratories[3, 4]. This applies especially to some less well-defined, but nutritionally or environmentally important parameters, such as fibre, starch or digestibility of foods and animal feeds or 'available nutrients'in soils for which unacceptably large coefficients of variation have been reported[5-8]. However, other types of analyses are no exception. A report compiled in 1985 on the quality of data relating to Pb and Cd analysis in food laboratories concluded that results were inaccurate and the validity of the data was in doubt[9]. The Global Environmental Monitoring Scheme of the WHO tested the performance of EU laboratories that contribute data on food contamination[10]. This involved 5 proficiency tests and involved 136 laboratories in 21 countries using their own preferred methods for the analysis of trace metals (Pb, Cd, Hg in milk powder), pesticides (organochlorine, organophosphorus, pyrethroid in spinach powder), nitrate in spinach powder and aflatoxins in nut-based animal feeds. Only 60% reported accurate results for trace metals, 41% for pesticides, 43% for nitrate, 88% for aflatoxins and 53% for patulin. Recently, a major UK food manufacturer discovered that only very few laboratories achieved satisfactory results for dioxin analysis (pers. information). Problems with DDT analysis were discovered when only 71% of participants achieved satisfactory z-scores in an international proficiency testing scheme for foods and animal feeds[4]. Similarly, a feed check sample programme operated by the American Association of Feed Control Officials (AAFCO) observed large variation in reported vitamin A results. This variation was investigated further[11] and resulted in several recommendations to reduce errors of Vitamin A in animal feed and pet food analysis. This shows that considerable effort is required in order to achieve reliable data.

Part of the problem seems to be that reports claiming a useful new technique are often based on tests using recently spiked materials, despite the fact that extractions from recently spiked samples are much easier and not as rigorous a test for a robust method than extractions from aged, contaminated matrices. Whilst spiking experiments are suitable for method development, they do not represent a 'real recovery test' for a robust and reliable method as recoveries from weathered soils for example may recover only 50% of the analyte compared to spiked soils[12, 13]. It is surprising, therefore, how many scientific publications do not use CRMs to validate methods or for QC purposes[14]. On the other hand, it has been pointed out[15] that even the incorporation of a performance evaluation standard (PES), as required by many EPA methods, can give a false sense of confidence and may not be sufficient to detect some matrix interferences, as the PES sample may have a different matrix to the real samples being tested. The authors[15] commented that matrix-enhanced GC degradation might cast doubt on the quality of some data relating to DDT degradation in the environment.

Benefits of Quality Assurance Programmes

It has been observed[16] that "most experimentation dealing with analytical methodology in biological sciences has been conducted within a single laboratory. Method validation by other laboratories was considered not only unnecessary but also detrimental because, in the words of one commentator, 'the results are too variable'. Within the last two decades, however, it has become increasingly apparent that a collaborative inter-laboratory study is the only way to estimate the variability characteristics of methods" and to meet the increasing demand for high quality data. The results of the UK Food Analysis Performance Assessment Scheme (FAPAS) have been summarised from 1990-1996[4] as follows: for pig feeds (moisture, ash, oil, protein, fibre, Cu), only 76% of laboratories achieved satisfactory results and for nutritional analysis 80% were satisfactory. However, once laboratories were participating in proficiency tests on a regular basis the average percentage for accurate results increased[4].

How to Obtain Valid Data?

Researchers need to ensure the correct use of blanks, standards, certified reference materials (CRMs) and understand the concepts of traceability in analysis, the purpose of proficiency testing schemes and laboratory accreditation[17, 18]. A case has been made for rigorous Quality Control procedures during routine analysis (although I would prefer to call this 'systematic analysis') and for Quality Assessment in research and development. The UK Department for Trade and Industry launched the Valid Analytical Measurement (VAM, see website in Appendix) initiative in 1994 which incorporates six principles:

1. Analytical measurements should be made to satisfy agreed requirements.
2. Analytical measurements should be made using methods and equipment, which have been tested to ensure they are fit for their purpose.
3. Staff making analytical measurements should be both qualified and competent to undertake the task.
4. There should be a regular independent assessment of the technical performance of a laboratory.
5. Analytical measurements made in one location should be consistent with those elsewhere.
6. Organisations making analytical measurements should have well defined quality control and quality assurance procedures.

These principles require properly validated methods that provide information on the performance of an analytical technique, such as accuracy and precision, ruggedness, operating range, selectivity and limits of detection. It is essential when reporting a measured value to also give its uncertainty. Otherwise, it is not possible for users of the data to know what confidence to place in the data. All Quality Assurance protocols should incorporate certified reference materials (CRMs) to ensure the traceability of measurements. This can be achieved through the use of CRMs, which can be used for:

·Calibration and verification of measurements during systematic analysis
·Internal quality control and quality assurance schemes
·Verification of the correct application of standardised methods
·Development and validation of new methods.

Significant progress has been achieved through international efforts in producing CRMs for elemental composition, pesticides and pollutants in a range of environmental and food matrices (see Appendix for CRM suppliers). Furthermore, a thorough description on how to produce in-house reference materials has been published[19] which are essential if no suitable (i.e. matrix matched) CRMs are available. It is considered good practice to include both CRMs and in-house reference materials into all analytical procedures whenever possible. Independent assessment of a laboratory can be achieved by participating in national and international proficiency testing schemes (PTS). The true concentration of an analyte can be determined by addition of a known amount of analyte to a base material (given the provisos mentioned above) or better still through the use of CRMs with consensus values which have been produced by a group of analysts (see Appendix for proficiency testing schemes). Independent approval of a laboratory's quality assurance arrangements can be obtained by accreditation to a recognised quality standard, such as ISO 90025 or ISO/IEC 17025. Guidelines are available for the statistical evaluation of analytical tests and laboratory performance[5]. Horwitz' group found that the within-laboratory variation was approximately one-half to two-thirds of the between-laboratory variation and can be used as a 'bench mark for judging previously unevaluated methods' [5].

Conclusion - The Need to Report QC Procedures in Publications

A survey[14] of a range of scientific journals found that many authors do not use CRMs appropriately. Jorhem[20] of the National Food Administration in Sweden strongly argued the case that all results presented in published papers should be traceable. Authors can demonstrate this by reporting their results obtained in proficiency testing schemes and from relevant CRMs. This will ensure that research, which has been conducted well, can be recognized by others – and can hopefully also be reproduced by others. It is recommended that papers that are submitted for publication in this Journal report the use of CRMs under a section entitled 'Quality Control' if standard methods have been used or under a section entitled 'Method Validation' for new or adapted methods. It is obvious, that data of reliable analytical quality are needed for evaluating the nutritional value of foods, for improving crops and for better livestock management, for natural resource management, for evaluation of soil and water quality, and for monitoring environmental contamination and land remediation.

References

[1] Gillespie, A., Finnamore, J. and Upton, S. 1999. VAM in the environmental sector. VAM Bulletin **21**: 6-10.

[2] Kuselman, I. 2000. UME-NIST workshop on establishment of chemical and medical metrology laboratories at UME, Gebze, Turkey, 19-20 June 2000. Accred. Qual. Assur **5**: 453- 454.

[3] Midkiff, V. 1984. A century of analytical excellence. The history of feed analysis, as chronicled in the development of AOAC official methods, 1884-1984. J. AOAC **67**: 851-860.

[4] Key, P.E., Patey, A.L., Rowling, S., Wilbourn, A. and Worner, F.M. 1997. International proficiency testing of analytical laboratories for foods and feeds from 1990 to 1996: the experiences of the United Kingdom Food Analysis Performance Assessment Scheme. J. AOAC Internat. **80**: 895-899.

[5] Bailey, S. and Henderson, K. 1990. Consequences of inter-laboratory variation in chemical analysis. In: Wiseman, J. and Cole, D.J.A., (eds.). Feedstuff Evaluation. London: Butterworths. p. 353- 363.

[6] Lanari, D., Pinosa, M., Tibaldi, E. and D'Agaro, E. 1991. Risultati di due ring test condotti sulla composizione chimica di alcuni alimenti e sulla digeribilità in vivo. Zootec. Natr. Anim. **17**: 285-295.

[7] Beever, D.E., Cammell, S.B. and Edmonds, S. 1996. Inter-laboratory variation in feedstuff evaluation of two contrasting maize silage samples. Anim. Feed Sci. **62**: 685-686.

[8] Givens, D.I., Owen, E. and Adesogan, A.T. 2000. Current procedures, future requirements and the need for standardization. In: Givens, D.I., Owen, E., Axford, R.F.E. and Ohmed, H.M. (eds.). Forage Evaluation in Ruminant Nutrition. Wallingford: CABI Publishing. p. 449-474.

[9] Patey, A. 1996. Quality in quantities. Chem. Britain. **32**: 35-37.

[10] Weigert P., Gilbert, J., Patey, A.L., Key, P.E., Wood, R. and Barylko-Pikielna, N. 1997. Analytical quality assurance for the WHO

GEMS/Food EURO programme - results of 1993/94 laboratory proficiency testing. Food Add. Contam. **14**: 399-410.
[11] Thiex, N., Smallidge, R. and Beine, R. 1996. Sources of error in vitamin A analysis. J. AOAC Internat. **79**:1269-1275.
[12] Chiba, M. 1969. Factors affecting the extraction of organochlorine insecticides from soil. Residue Reviews **30**: 63-113.
[13] Dean, J.R. 1998. Extraction Methods for Environmental Analysis. John Wiley & Sons Ltd, Chichester, UK. 225 p.
[14] Jenks, P.J. and Stoeppler, M. 2001. The deplorable state of the description of the use of certified reference materials in the literature. Fresenius J. Anal. Chem. **370**: 64-169.
[15] Foreman, W.T. and Gates, P.M. 1997. Matrix-enhanced degradation of p,p'-DDT during gas chromatographic analysis: a consideration. Env. Sci. Technol. **31**: 905-910.
[16] Horwitz, W. 1993. International coordination and validation of analytical methods. Food Add. Contam. **10**: 61-69.
[17] Prichard, E. 1995. Quality in the Analytical Chemistry Laboratory. John Wiley & Sons, Chichester, UK. 307 p.
[18] Mueller-Harvey, I. and Baker, R.M. 2002. Chemical Analysis in the Laboratory: A Basic Guide. The Royal Society of Chemistry, Cambridge, UK. 112 p.
[19] Walker, R.F. and Brookman, B. 1998. Guidelines for the in-house production of reference materials. LGC/VAM/1998/040. LGC plc, Teddington, UK. 32 p.
[20] Jorhem, L. 2000. Traceability and its role in interlaboratory comparisons (proficiency testing programmes), modeled on trace element determination in biological materials. Accred. Qual. Assur. **5**: 449-450.

Appendix: Useful Websites

http://www.aoac.org/techprog/Intro98.htm;
http://www.european-accreditation.org;
http://ptg.csl.gov.uk/schemes.cfm;
http://www.dfrc.wisc.edu/foragetesting_nfta.html;
http://www.lgc.co.uk/pts.asp;
http://www.lgc.co.uk/ref.asp;
http://www.vam.org.uk;
http://www.ukas.com;
http://ts.nist.gov/ts/htdocs/230/232/232.htm;

Food and Health

Consumer confidence and UK food retailing: why does local food matter?

Phil Lyon [1*], Anne Colquhoun [2], Hille Janhonen-Abruquah [3]
[1] School of Nursing & Midwifery, University of Dundee, [2] Food & Consumer Sciences, University of Abertay Dundee, UK. [3] Kotitalous, Pirkanmaan Ammattikorkeakoulu, Tampere, Finland.
**email: h.p.lyon@dundee.ac.uk*

Received 25 October 2002, accepted 20 December 2002.

Abstract
Over the last 50 years, the UK fresh food market has been increasingly challenged by the growth and domination of large grocery chains. Supermarkets now source supplies on a national and international basis. They have purchasing power to undercut small-scale local production and distribution systems that minimise many of the past advantages of short distances between the producer and the consumer. This alters producer perspectives. Larger-scale production and sales contracts for national distribution are favoured. Smaller producers focusing on supply to local markets have tended to struggle as retail opportunities for their fresh produce diminished. Street markets for fresh food, once the main source for the UK urban population, are now a minor contributor to overall sales. In part this results from a larger population and the limited number of markets operating within traditional constraints of time and location. In part, also, consumer perceptions of quality in street markets declined with the ascent of pre-washed *bimbo* fruit and vegetables along with the disembodied, pre-packed meat favoured by many. Excessive pesticide use, BSE, foot & mouth disease and *E.coli* problems have all recently taken their toll of consumer confidence and have disturbed taken-for-granted urban assumptions about food safety. Furthermore, media attention on factory farming techniques have focussed public disquiet on issues that, until a few years ago, many consumers were happy to ignore. Vegetarianism, organic production methods and attempts to reconnect food producers and local consumers are arguably related strategies emerging from these problematic issues of consumer confidence. By reference to UK experiences over the last 10 years, the paper explores food shopping trend convergence, reduced consumer trust, and attempts to restore confidence.

Key words*:* Local food, organic food, food retail, consumer confidence.

Introduction

Easy access to locally produced food ought not to be a problem in modern societies with long-established agricultural systems. Nevertheless, it is increasingly a problem and this has implications for consumers. Historically, local food was consumed by producers with any surplus distributed to the local community on a trade, exchange or gift basis. Food self-sufficiency was an early, but often precarious, achievement of human social organisation. In northern Europe, year-round supplies were typically secured only through dietary change to reflect seasonal variations in availability and increasingly successful preservation techniques to retain food from periods of glut to those of scarcity[1]. For much of UK history, the focus was on local food production and consumption but this was augmented by development of trade on a national basis so that food consumed in Scotland might well have been produced in southern England, or *vice versa*. Even before extensive urbanisation in the 19th century, there were parts of the UK better suited to the production of particular commodities and developed a specialist reputation - Kent apples, Welsh lamb, Norfolk turkeys, Scotch beef, Lincolnshire potatoes - for example. However, seldom was production undertaken solely in one area and this gave rise to regional variations – butter and cheese for example - and disputed claims for which was best. For most people, augmentation of supplies by importation was a marginal contribution until relatively recent times. In the UK, for example, it was only in the later decades of the 19th century that the British Empire, and an extensive merchant navy, produced substantial dietary benefits for ordinary people. As chronicled in the early 20th century, while imported food had the effect of making basic commodities cheaper, it did not banish hunger among the poorest nor did increases in the variety of food necessarily improve the quality of poor people's diets[2]. Imported foods did, however, support the population redistribution effects of industrialisation and urbanisation. By the start of the last century, 80 per cent of the UK population were living in urban areas and while this amplified demand for local agricultural production, it also stimulated demand from other parts of the country and for foreign trade. Urban grocery chains prospered in these new markets and grew ever more sophisticated in sourcing supplies at the keenest prices and organising regular large volume imports where domestic supplies were inadequate or unavailable[3]. For at least a century, British people have routinely had imported food available. Typically this was known at the point of sale, accepted on the basis of geographical advantage for specialised agricultural production, and sometimes it had a certain caché. Often food was shipped from parts of the British Empire or subsequently from the British Commonwealth – New Zealand cheese and butter, Australian lamb, South African apples, Indian or Ceylon tea – but this did not preclude other sources – Spanish oranges, Danish bacon, Dutch tomatoes or Argentinean beef, for example. Reliance on these imports was usually seen as low risk dependence because sources were reciprocal trading partners, and Britain was a maritime trading nation. Food shortages only occurred when international conflict closed shipping routes, or restricted cargo capacity. There were widespread food shortages in both the 1914-18 and 1939-1945 world wars with partial food rationing persisting until 1954 in the UK. Other than during these periods of crisis, reliance on imports has not been contentious although it has co-existed with fairly extensive government support for British agriculture and periodic campaigns to promote British produce via specific marketing boards. British consumers are thus long-practised in their awareness and acceptance of nationally sourced or imported food. The central concern of this article has little to do with any changes in this aspect of consumer culture. If anything, immigration and tourism in recent decades have meant a wider range of imported commodities is routinely sought. In the last few years, however, there have been extensive consumer concerns about food production methods and related food safety – from salmonella to BSE (Bovine Spongiform Encephalopathy) - that have been translated into reduced confidence and increased scepticism. While food production *per se* is seen to be the source of these problems, arguably they have more to do with the extensive power supermarkets have over food producers and agricultural practices. In response to the sequence of well-publicized crises, there are a number of separate but congruent trends emerging to seek re-imposition of a measure of consumer control over the quality and provenance of the food they purchase.

UK Food Retailing

Writing of the late 1940s and early 1950s, Goldenberg wrote of *Marks & Spencer's* priorities – as retailers - being initially focussed on post-harvest handling of fresh fruit and vegetables. 'There were a number of reasons for this. The most important was that this was where, at the retailing end, we at M&S could really make a worthwhile contribution; we thought that the technology of the growing of fruits and vegetables was pretty well-known and, therefore, for the time being, that our suppliers did not need any help here from us. By-and-large, this view proved to be right in the years to come'[4]. Post-harvest handling and, in particular, presentation at the point of sale has remained an important aspect of the food retail role, but this has not been unproblematic. In the early days of supermarket shopping, 8 out of 10 British housewives saw these new food shops as hygienic and convenient but had reservations about pre-packaging and their suitability for perishable foods[5]. At that time, they were cautious about the quality of supermarket fruit, vegetables, bread, pastries and, especially, fresh meat. They preferred to buy these items at the traditional smaller specialist shops. There was … 'an assumed lack of choice in meat and vegetables compared with the specialist shop and some suspicion that supermarket operators are less knowledgeable about these products'[6]. In part, also, there was a lingering value placed on personal service and a view of supermarket owners as efficient but impersonal. Set against these concerns at the time was the assurance, based on American experience, that the buying power of supermarkets would have a strong positive influence on quality. This was to be based on much earlier interventions with producers and, often, a circumvention of conventional wholesaling arrangements. 'One has only to consider the way the supermarkets in America have raised the standards of meat, and how they have educated the produce growers in the application of new techniques in the gathering and on-the-field preparation of greenstuffs, to recognise the opportunity which lies ahead for the steady process of up-grading which must in the last analysis be the guiding policy behind supermarketing'[7]. An even greater optimism and sense of purpose prevailed in *Marks & Spencer* food retailing later on. In 1966, a senior executive saw their role as central to quality improvement for however mechanized and efficient British farming was, high quality production could not be guaranteed unless: '(1) the farmer starts with the right breed of animal or the right strains of seed, and applies to farming the lessons of modern technological development; (2) the produce is cleaned and graded in modern packing houses, processed in modern factories, and speedily transported to the retailer, so that it is still fresh when it reaches the customer'[8]. Supermarkets were not content to be passive purchasers in the wholesale markets – buying at the quality/price levels available at the time. Rather, they sought to reduce such uncertainties and exploit the advantages of direct relationships with producers to ensure a consistency of supply and quality.

In the UK today, concentration of supermarket purchasing power has produced a very different food retail topography to that which existed even 30 years ago. In 1999, independent grocers comprised 75.2% of the 34,452 shops but shared only 6% of the £67,216 million food retail turnover. Top multiples – *Asda, Iceland, Safeway, Sainsbury's, Somerfield* and *Tesco* - had just 8.6% of the shops, and 71.9% of the turnover[9]. Market domination by a few large firms has undoubtedly brought significant benefits to consumers in terms of the overall product range, convenience and price but it has also distorted the market for food suppliers and has, paradoxically, reduced diversity. To a large extent, supermarket chains – as major or sole purchasers - are now able to specify for suppliers rather more than the food quality and safety characteristics required by legislation, or the latest scientific thinking and production practices. They are increasingly able to determine the variety, size and condition properties of food to meet their commercial requirements as retailers to the extent that these become industry benchmarks. Against the advantages, there are a number of reported disadvantages with the direct linkage of retailers and fresh food producers, most of which are not inherent in the relationship but which are manifest in practice.

-Producers invariably trade greater certainty of high volume sales for reduced prices but ' price pressure has been used particularly on growers of fresh produce, who are uniquely vulnerable, given that their produce is perishable'[10].

-Power in the relationship is asymmetrical. 'Today, in the UK, the retailer has been the dominant force for more than 10 years. A big manufacturer, classically, may find 10 per cent or 15 per cent of his brand selling through a single retailer buying point, which will take a mere 1 per cent of its purchases from this same manufacturer'[11].

-In consequence, retailers determine producer practices. 'Crucial interactions were with the suppliers of fresh foods – meat, vegetables and bakery … who were offered attractive volume contracts but with limited security, and as the market became more competitive, they had to absorb and act upon endless requirements not merely to keep quality standards up, but at the same time to improve efficiencies and lower costs. The supermarkets were in a uniquely strong position to exploit volume strength'[12].

-Being able to dictate low prices for producers has not always been to the benefit of consumers. The gulf between the price paid to producers and that charged for food on the supermarket shelf has been a source of considerable debate in recent years. Against falling 'farm gate' prices, supermarkets justify maintained or increased retail prices by reference to processing costs or by the claim that they are, in fact, losing money on some food products[13].

-Supermarkets are also accused of exploiting producers/ suppliers on a number of specific issues including 'imposing an unfair balance of risk…de-listing producers/growers who are unable to deliver agreed quantities owing to weather conditions … requiring the producer/grower to bear the cost of surplus special packaging ordered by the supermarket chain for a promotion when the sales did not meet expectations … requiring prospective suppliers to contribute to the cost of buyer visits, artwork and packaging design, consumer panels, market research, or to provide hospitality to the supermarket employees requiring suppliers to purchase goods or services from designated companies e.g. hauliers, packaging companies, labellers'[14].

-Supermarket share of the food retail market has a parallel in their dominance of the wholesale markets. With their buying power, it is difficult to avoid selling to their terms especially with reasonable volumes of perishable produce. 'By buying direct from suppliers, the supermarkets had undermined the wholesale markets through which growers had once been able to sell their produce. In 1974 there had been 150 main wholesale markets in Britain; now there were only 37. Of the 11000 growers left … [it is estimated that] … only about 2000 who sell to supermarkets are very profitable. The rest must find markets through farm shops or the declining number of small greengrocers'[15].

-Increasingly, supermarkets want to be supplied by companies 'who can supply all their stores throughout the UK via a small number of dedicated retail distribution depots'[16]. This tends to favour large producers/suppliers at the expense of smaller ones.

-Supermarket power extends also to specification of the products themselves. 'What retailers want is bimbo fruit, all perfect looks and shelf life, with a touch of genetic engineering to help it along the way. So what we eat is determined not by its flavour but the distribution system and retailing strategy of supermarket chains'[17].

Supermarkets have undoubtedly increased the efficiency of food retailing and given price advantages to consumers. However, pressure-group criticism about the role they play has grown in line with the increasing dominance of food retailing and influence in food production by relatively few companies. By the mid-1980s, there was already concern not only about the closure of small specialist shops but the abandonment of main shopping streets in London by supermarket retailers because their older, smaller, premises were deemed uneconomic. 'It is these smaller supermarkets and the people they served, which have proved most vulnerable to large store development in food retailing The closure of 400 Tesco stores over the last ten years has taken place alongside a similar programme of closures of smaller Sainsbury's and Co-operative Society supermarkets'.[18] This pattern of closure for small grocery shops, and then smaller supermarket units, was repeated throughout the UK with the effect that the geography of food shopping has changed, and convenience is largely restricted to those who have cars. The term 'food deserts' - paradoxically existing in urban and rural areas – has been used to describe the residual lack of provision or access difficulties. 'In the cities, food deserts are found in areas where a fall in population has pitched local shops into decline and a doomed struggle against supermarkets. Only corner shops remain, selling a limited and overpriced range of packaged foods. In the country, the closure of hundreds of village shops and poor public transport has left people without cars facing enormous difficulties. The problem is worse for the elderly, the housebound and the poor'[19].

Major supermarket chains control much of the UK food retailing. They profoundly influence variety availability and price; they establish the benchmarks of appearance and packaging. In 50 years they have done much to shape consumer expectations and behaviour but times have changed for supermarkets and shoppers. Many consumer concerns from the 1950s and 1960s have now either turned out better than was feared, or are better tolerated with the eclipse of traditional food shopping expectations[20]. Conversely, there are issues particularly the relationship between supermarket and food producer that echo early consumer anxieties and limit the value of reassurances on food quality. As an extensively urbanised society with a very small agricultural workforce, the producer-retailer relationship warranted little routine consideration. Occasionally animal rights groups would draw attention to particular aspects of industrialised farming but, for most, there was little sustained engagement with such issues. In the main, criticism of the way that supermarkets function – in relation to both consumer and producers - has largely by-passed consumers. Some may bemoan the passing of small food shops, but still collect their groceries – especially their main food shopping - from a suburban retail park.

Food Scares

UK consumers have, however, been confronted with a number of food safety issues in recent years. The media have extensively covered incidents with the effect that, arguably, traditionally widespread trust in the safety of food production practices and regulatory arrangements has been substantially eroded. The UK experience of food safety issues has been both protracted and diverse. From low-key coverage of isolated food poisoning incidents, there has been a changed media stance, arguably in response to the increasing gravity and extent of the problems. Food poisoning, for example, has increased substantially in terms of the number of cases being reported by doctors to the authorities[21]. Salmonella incidents increased substantially in the 1980s and became a political *cause célèbre* with the 1988 resignation of a government health minister for warning that most UK eggs were infected - an inherent risk. Egg sales fell and the government was obliged to support the crippled industry. The spotlight fell on egg production methods and chicken food, genetic selection, possibilities for aerosol transmission via drinking water and environmental contamination, and the difficulties of adequately cleaning poultry houses to eradicate infection may all have had some part in the problem[22]. However, the problem persists and Salmonella cases have continued to increase. Clear identification of the causes of initial infection and subsequent transmission to humans remains elusive. Perhaps necessarily, attention is still drawn to the way that food is sold, handled in the domestic environment and consumed rather than how it is produced. Phillips[23] suggests a combination of the following to explain the increase for Salmonella and Campylobacter.. Changes in shopping habits that mean purchasing larger amounts of food to be stored for longer periods at home; increased numbers of chilled readyprepared meals that have short shelf lives and need carefully controlled storage temperatures and heating procedures; increased consumption of fast foods and 'eating out' generally are factors which sit uneasily with the emergence of new strains of micro-organisms and increased public awareness. Production, and the factors that encourage specific production methods, remain problematic.

In 1986 BSE (Bovine Spongiform Encephalopathy) was identified in British cattle and, eventually, the initial cause was confirmed as contaminated feed. The impact on beef consumption was dramatic, despite government assurances that the problem had been brought under control. Even in the1990s, concern was being expressed about the effectiveness of revised meat processing procedures[24,25,26]. Even though the linkage between this cattle disease and human health was initially tenuous[27], the graphic images of animal distress were sufficient to raise consumer fears. Beef was taken off the menu of many schools[28]. Government assurances became less credible but were still being offered in 1995. Douglas Hogg, the Agriculture Minister said the Government acted on the advice of a panel of experts who had given extremely careful thought to all the issues. "They conclude that BSE is not transmissible to humans and that in any event our controls are effective enough to prevent the infective agent getting into the human food chain"[29].

Despite such official assurances, in 1995 vCJD (variant Creutzfeldt-Jakob Disease) was identified and linked with BSE, bringing into question not only the value of the assurance, but undermining the accuracy of expert advice. The role of Government in consumer protection on food issues had been discredited since the 1988 salmonella problems. Food safety problems were associated with increasingly intensive agricultural production but there were well-publicised problems also with food handling. In 1996, Central Scotland's worst outbreak of *E-coli* food poisoning claimed 18 lives and, at its peak, 127 people were in hospital[30].

Over the years, publicity associated with the way in which food is now produced in the UK has had a damaging effect on consumer confidence. This is expressed to the extent that even when there is no demonstrable problem for humans – for example, genetically modified food, and foot and mouth disease – consumer fears are amplified. The longer-term implications of the UK Government's slaughter policy for foot-and-mouth disease – not just the disease itself - has yet to be seen but it is probable that this will further depress red meat sales. Even now, health problems are in sight for farmed salmon – a food product that many might regard as an acceptable substitute. While Infectious Salmon Anaemia (ISA) is not a direct threat to human health, under European Union legislation infected stocks must be killed[31, 32].

Concern about pesticide use in food production has a long history. *Silent Spring*[33] was first published in 1962 but debate tended to be located within academic or other arena marginal to the lives of most consumers. Ecology and food production may have vied for popular and scientific attention but the latter was clearly of greater weight given widespread belief in the power of science to resolve any undesirable outcomes. Tolerance of the ambiguities of pesticide use is presumably also a function of the perceived degree of risk against benefits for humans.

Scientific reassurance that 'a decision often involves weighing an advantage to human beings against an advantage to wild life'[34] and that any residual pesticides in food usually occur only …'at trace levels, well below the tolerance levels set by the Food and Drug Administration'[35] suggested high levels of knowledge about long-term toxicity in humans. This confidence was reinforced by the idea that any problems of … 'excessive levels - that is those exceeding legal tolerance limits – occur rarely and are normally the result of failure to follow precise recommendations for application'[36]. Carson had already seen the paradox enshrined in setting tolerance levels. 'To establish tolerances is to authorize contamination of public food supplies with poisonous chemicals in order that the farmer and the processor may enjoy the benefits of cheaper production – then to penalize the consumer by taxing him to maintain a policing agency to make certain that he shall not get a lethal dose. But to do the policing job properly would cost money beyond any legislator's courage to appropriate, given the present volume and toxicity of agricultural chemicals. So, in the end the luckless consumer pays his taxes but gets his poisons regardless'[37].

Recently, the Consumers' Association reviewed results of UK Government pesticide residue test data for the previous four years. This analysis concluded that… 'while most fruit and vegetables contain no detectable residues, others consistently contain residues and some exceed the legal limit'[38]. Although specific pesticide residues *per se* are problematic, a different level of concern was expressed in relation to what was being monitored by the testing procedures. 'Pesticide safety evaluations look at individual pesticides and not the effect of combinations of different chemicals. But some foods can contain multiple residues because the crop is treated several times and often with different pesticides. For example, an average lettuce is treated with insecticides 5 times; strawberries are treated about 12 times and dessert apples as many as 16 times with pesticides containing 36 different active ingredients'[39]. In effect, it is suggested that test data may give a false sense of security because the long-term effects of chemical combinations are largely unknown.

Pesticides are only one manifestation of routine reliance on agricultural chemicals. Increasingly, food is also treated to ensure that it reaches supermarket shelves not only blemish-free, but in optimum visual condition[40,41]. While pesticide residue in meat is normally low or absent in tests, assurance of meat safety - and growth promotion - in intensive production systems is often achieved with routine antibiotic (zootechnical feed additives) use[42] . The UK Department of Health reported a significant threat to human health in the growth additives used in food production. 'The Advisory Committee on Microbial Safety of Food which presented the report, warned that unless drastic action were taken several nasty human conditions, from salmonella to *E-coli*, would become wholly resistant to antibiotics. Eat enough carelessly farmed chicken, the report implies, and your immune system will start to pack up'[43]. The problem of chemical contamination emerges in different guises. Recently seven brands of honey – produced in China but sold under UK supermarket own-brand labels – were withdrawn from grocery shelves because they were found to contain streptomycin residue[44]. A month later, several supermarket own-brand frozen prawns were also withdrawn from sale nationally because they were found to contain residues of nitrofurans[5]. Publicity about any one of these food safety issues might have caused a change of consumer behaviour avoidance of specific commodities or revised handling procedures - even if it was both partial and transient. However, the sequence of incidents over a period of years often with substantial overlap between the aftermath of one problem and development of the next has created deep-seated anxieties for consumers. Trust in the systems by which food is produced and tested is hard won and easily lost. In this, the UK is in notable contrast to Norway, for example, where consumer attitudes were reported positive in relation to the way domestic food was grown and the protection provided by the Norwegian Food Control Authority[46]. For many UK consumers, unquestioning belief in scientific reassurance is now less likely and while this appears contradictory to our generally greater reliance on advanced technology, dissonance is more related to the emergent gap between scientific promise and performance in food production than outright rejection of scientific innovation in all domains.

Consumer Reactions

Consumer reactions to information are often difficult to predict. This is true even for shopping behaviour after food scares have been widely publicised. Some will translate anxiety into changed behaviour immediately, while others will not change either though habit or in consequence of their belief in producer, retailer and government reassurances. Given the widespread association of these problems with industrialised food production methods, UK consumer confidence has been undermined. Processing innovations, such as irradiation to extend shelf life, further challenges this[47], as does publicity for older, but little known, storage techniques for apparently fresh food[48] . There is evidence of consumer concern about food safety in recent research undertaken by the UK Food Standards Agency but this is not straightforward. Qualitative research indicated that 'within the context of discussion about shopping for food, it was not common for people to raise issues of food safety spontaneously'[49]. However, discussion group participants who were non-meat eaters, and had already made a decision to change their diet from the norm, were 'much more preoccupied than others with the possibility that some foods might be injurious to health'[50]. When the issue of food safety was prompted, underlying confidence 'quickly evaporates if food safety is raised in the context of scares like BSE, salmonella and GM foods. There is widespread suspicion that science is the servant of people – farmers, processors, distributors and marketeers – who are dedicated to improving profit margins and who may not be sufficiently careful about public health'[51]. So quite clearly, for these respondents, concerns were there even if they were not a major pre-occupation while food shopping. 'There was a widespread feeling that the general public did not know how food production and processing methods were changing, and what the implications of such changes might be. Not knowing produced a sense of powerlessness; and the public had to accept what was available to buy without knowing much about it, and perhaps especially not knowing how pursuit of profit might be affecting the food industry … BSE was widely regarded as a discouraging example of the lengths to which food producers might be going to maximise their returns'[52] . This work for the Food Standards Agency was followed up by quantitative research involving 3153 respondents throughout the UK. This provided confirmation of concern about food safety with 71 per cent of the sample indicating they were 'very' or 'quite concerned' about general food safety. Further, when asked about food issues, BSE and food poisoning were indicated by 61 and 63 per cent respectively but substantial proportions were also concerned with the use of growth hormones, the feed given to livestock, pesticide use, GM foods, additive use, the conditions in which livestock was raised, and antibiotics in meat. Importantly, respondents reported that these concerns 'between two-thirds and three-quarters said it had affected their eating habits*'* [53] but the study did not seek to explore the relationship between this and food shopping behaviour.

Translating Concern into Food Shopping Behaviour

In the UK, environmental concerns and awareness of 'green' issues grew during the 1980s and early 1990s but were never translated into a radical agenda of change. Issues were often adapted by mainstream political parties, consequently losing focus, and were similarly absorbed by food producers and retailers. Arguably, their responses in the form of reassuring labels and packaging were more a public relations exercise than a considered reformulation of products or a rethinking of the way commodities were grown, harvested or acquired. The net effect was a literal 'greening' of supermarket shelves without the underlying process changes[54]. Generic 'green' concerns were thus apparently addressed at the point of sale and relatively few consumers were inclined to pay the additional costs involved in a more substantial response. However, there has been a growing interest in one type of 'green' issue, organically produced food, although there is ambiguity with this as well.

Organically Produced Food

One way to resolve some of the uncertainties is vegetarianism. This avoids problems of industrialised meat or fish production and, perhaps because of the food-related lifestyle change involved, tends to be associated with a renewed emphasis on the 'naturalness' and quality of food. For other consumers, there is an emphasis on purchasing commodities produced by organic methods, and this may include both meat and fish. Organic food production has moved from the margins of agriculture and retail activity to now generate considerable media debate. In a sense the advantages of organic food have long been available but their relevance has been stimulated by perceptions that the problems of conventional agriculture are now sufficiently commonplace to warrant a concerted commercial response. From the shelves of small health food shops since the 1950s, and tentatively by some supermarkets since 1981, organic food has now been added to the range of commodities offered by nearly all large supermarket chains, and over the internet by innovative producer-retailers for urban consumers. However, the most dramatic response was that taken by the UK frozen food company *Iceland*. They had previously moved ahead of competitors by removing 'all artificial colours and flavours from its own-brand products in a move it claims is designed to allay mounting public concern over food safety. The supermarket chain, which was the first to remove genetically modified ingredients from its food, has also said it is planning to sell organic produce at little or no extra cost and introduce…clearer labelling, a reduction in the salt content of its food and the use of identifiable quality cuts of meat in all its processed food'[55]. The organic initiative announced in May 2000, and fully implemented later that year, meant that frozen organic food was readily available at nearly the same price as conventionally produced equivalents. This particular venture failed primarily because profit margins were too low at those prices and sales did not increase enough to offset this[56] . However, this was arguably a tactical failure rather than one of long-term strategy for not only has the former owner of Iceland subsequently opened organic supermarkets, rivals Tesco have recently committed themselves to a stronger emphasis on organic food. Similarly, Co-operative Stores supermarkets imposed an outright ban on 20 pesticides and is asking growers to find alternatives to 30 more'[57]. Moreover, while Iceland's specific retail initiative failed, consumer demand for organic products is growing in terms of the percentage of households who buy this produce but there is still a low frequency of purchases, and the amount spent per household is still low in comparison with overall expenditure on food. However, the market is significant. Sainsbury's, the largest UK supermarket retailer of organic food, anticipates sales of £235 million in 2001[58] . The Institute of Grocery Distribution expects the total UK organic market to exceed £1 billion in 2002-2003. Currently, indications are that consumers purchasing organic food tend to be in the upper socio-economic groups; live in two person households; are aged between 45 and 64 years; and the main shopper is not working[59]. This profile shows congruity with that of consumers most concerned about food safety and other food issues[60]. While such households are limited as a proportion of all food shoppers, they are viewed as an important group of customers. Tesco consumer research indicates that 63 per cent of respondents would buy more organic food if it were available. On this basis, Tesco are increasing their range of organic products and cutting the price to make it more accessible to a broader spectrum of customers[61]. Clearly, this kind of future commitment is important because supermarkets currently account for around 75 per cent of all organic sales.

However, the organic response to food safety concerns is less conclusive that many customers imagine it to be. To reinforce their confidence, there is an apparently strong regulatory framework to limit use of the term 'organic' except where it meets specific production criteria. There are eight organic sector bodies registered with the United Kingdom Register of Organic Food Standards (UKROFS)[62]. Only one -Soil Association Certification Ltd – is accredited by the International Federation of Organic Agricultural Movements (IFOAM). The Organic Products Regulations Act (1992 amended twice) requires that 'any operator who produces, processes, imports, packs or repacks organic food out of sight of the consumer must be licensed by an approved sector body'… [and] … 'to demonstrate that an organic product offered for retail sale has been produced by a certified operator, the product label must display the code of the certification body responsible for the retail packing operation'[63]. In practice, however, there are problems. European and UK legislation allows more than 30 chemical additives and 'although crops may be organic, the term is no guarantee that they are pesticide-free. Organic crops may be contaminated by chemical residues left in land converted for organic farming or from nearby non-organic farms through air or water'[64]. Research for the Food Standards Agency indicated the perception of most respondents was that 'organic' meant 'foodstuffs like fruit and vegetables had been produced as naturally as possible, without the use of pesticides and without incorporating preservatives'[65]. Not only does this run counter to actual permitted practices, there have been claims that organic crops - grown using manure rather than conventional fertilisers - actually brings increased risks of E coli contamination and food poisoning[66]. Equally, the real advantage of organic produce over non-organic may be overstated. Recently reported research on carrots revealed not only that conventionally grown carrots, organic British carrots, foreign organic carrots were all negative when tested for 40 different pesticide residues known to be associated with carrot production[67] . Similarly, 'organic eggs must meet the best free-range standards with flocks of fewer than 500 birds. Beak clipping is not permitted. Yet only 70 per cent of the birds' diet must be organically produced, although it must be free of antibiotics, animal waste and protein, and yolk-colouring dyes'[68]. However, livestock products labelled as organic in the supermarket may quite legally be produced under 'conversion' arrangements. For organic veal or beef, it is only necessary that 'cattle whose progeny is intended for meat production must be managed in accordance with these… [UKROFS] … standards for at least 12 weeks before calving'[69] . Organic poultry might come from chickens that have been kept in conditions compliant with the UKROFS standards for as little as 10 weeks; and organic eggs might come from chickens who have been subject to the standards for as little as six weeks. Claims that organic food is 'healthier' have also been challenged. The Soil Association reviewed a number of published research papers and concluded that analysis suggests 'farming methods can make a significant difference to levels of vitamins, minerals and other nutrients'[70]. However, the UK Food Standards Agency takes the view that there is not enough evidence to support this conclusion. Claims and counterclaims pose an interesting case for media handling of scientific opinion[71] but do little to provide clear guidance for the consumer.

Locally Produced Food

The label – organically produced – has a talismanic quality amidst the uncertainties of food safety and environmental impact. As with other labels – pure, fresh, natural, and traditional – the consumer may seek more reassurance from them than they can provide[72]. While there is more protection against misuse of the word organic than for many others used in the same context, it still has the capacity to beguile consumers. Furthermore, it can be argued that the typical consumer profile – upper socio-economic groups, small households, middle aged – distracts from the importance of cheap, conventionally produced food for less well off consumers. Third World food poverty and, less dramatically, the food poverty that exists in disadvantaged sections of our own European population, is not readily reconciled with an emphasis on lower yield production methods[73]. In this sense, switching to organic production may exacerbate other food-related problems. However, the future of our food might usefully be considered in terms of factors other than organic production *per se*. This does not liberate food producers from the market hegemony of large retailers. Large food retailers are clearly seeking to transpose the power of price, product, supplier and even transportation specification they have in the relationship with conventional, non-organic, food producers to that with organic producers. There is ambiguity here, and many producers would only see the cost and risk of organic conversion acceptable on the basis of secure, large-scale sales prospects. Yet, these are currently available only with supermarket contracts. Arguably, there is a radical agenda to be pursued in the way that food is distributed and sold, not just in the way that it is produced. It is important not to regard organic food production as a proxy for solutions to these problems as well although it is not necessarily inimical.

Coupled with supermarket buying power is an increasing willingness to source supplies nationally or internationally. Distance between producer and store is no longer the primary consideration. This amplifies asymmetry in the producer-retailer relationship by imposing greater competitive pressure on the former while allowing greater margins for the latter. Food now travels further from producer to consumer. The social and economic costs of 'food miles' were highlighted by the SAFE Alliance and, to a large extent, that criticism [74,75] related to energy costs and the distortion of third world agriculture for European needs. One of the key recommendations of these reports was to endorse reconnection of local food production and local consumption. Locally produced food, it was argued, should be a priority for consumers, manufacturers and retailers. Indirectly, this may have an effect on the use of chemicals and processes that ensure food stays fresher in appearance longer, but is not a direct appeal for organic production. To focus on the location of production and consumption, rather than the type of production, offers potential benefits the most straightforward of which is the opportunity to reduce 'food miles', and thereby limit the current trend for long-distance transportation of basic commodities. Moreover, it is arguably not in the interests of consumers if local food producers and manufacturers withdraw from the industry in response to supermarket exploitation of production cost advantages elsewhere in the world. In the medium to long-term, this creates political and economic vulnerability from which recovery is difficult, if not impossible, in the short-term. Food production elsewhere is not necessarily of long-term advantage to the country in which it is located, beyond immediate employment opportunities for some[76]. Even within the UK, the balance of food production/manufacture and consumption between regions, and between rural and urban areas, is arguably important to avoid economic disparities and additional distribution activity. Local production, distribution and consumption promote local employment and increase the chance that employment is sustainable. Furthermore, while it is unlikely that consumers would readily accept a reduction in the current year-round availability of so many commodities, greater reliance on local produce would encourage seasonal food use. Arguably, awareness of seasonal produce has been a casualty of modern distribution practices. However, the real advantages of the reconnection of local production and consumption are in danger of being lost in an assumed congruity of 'local' and 'organic'. Re-emphasising local food need not exclude existing large-scale, conventional growers and manufacturers but, equally, might more readily include small operations whether they are organic or not. Agricultural diversity is clearly the only viable direction in the UK. The organic agenda is important in its own right, but it is also a catalyst for change in the conventional farming methods that predominate. For example, 'the increasing use of precision farming to reduce fertiliser and chemical applications and the targeted inputs methods encouraged by Scottish Natural Heritage and the Farming and Wildlife Advisory Group'[77] are important developments in the context of an on-going demand for food without the organic premium. The sheer scale of demand for food cannot be readily met by the emergent organic sector and, while this is a market to engage with, much of this demand does not benefit UK growers. Currently, *Tesco* imports 80 per cent of its 1000 organic products. Effecting a major change of production methods on a farm, accepting lower production levels in consequence, securing appropriate certification for produce all add to costs and retailer price premiums are, at best, marginally beneficial to producers.

Change in the UK producer-retailer paradigm is probable only if supermarkets change their stance towards locally grown food. This would be assisted by the development of distribution and retail strategies that were genuinely different from those practised by large supermarkets although the latter are still likely to dominate trading practices because of the size of their market share. Alternatives, such as Farmers' Markets and other forms of direct selling, are not new but are revitalised in the context of consumer concerns and the organic food resurgence. Over the last 50 years, there has been something of a retreat from direct selling with an increasing use of intermediaries and, for much of that period, national marketing boards for specific commodities - for example eggs, meat, milk, potatoes – controlled much of the market. Although there were differences in the control exerted by different boards, direct selling was the province of smaller producers or a marginal activity for others. By the 1950s, direct marketing was being described as 'not extensive' … [and applicable only to]… 'products which can be consumed with little or no processing after they have left the farm gate and to the produce of farms situated virtually on the consumers' doorsteps – that is near towns or villages'[78]. Access to consumers has undergone something of a transformation in the recent development of Farmers' Markets and internet-based ordering. In many parts of the UK there had been a tradition of food markets primarily for fruit and vegetable retailing but often including fresh meat and fish sales. Sometimes these involved producers directly, but mostly the retailer was an intermediary. Farmers' Markets reduce reliance on intermediaries and have the advantage of taking produce to centres of population. Farm Shops and 'pick-your-own' enterprises, on the other hand, require consumers to make a specific journey to producers, or rely on passing trade. Promoted as a solution to supermarket hegemony[79], Farmers' Markets offer direct access to fresh food and better profitability for growers. 'It is perhaps ironic that a town which can boast one of the highest ratios of supermarket space per head of population in the country should be one of the few places - so far- in Scotland where farmers have resorted to selling their produce direct to the public in this manner. Stroll among the stalls and you'll find fish farms, nurseries, organic vegetable growers, honey and country wine producers, but it is the livestock farmers who predominate – hill lamb, organic beef, farmed venison and even the exotica of locally reared ostrich and wild boar'[80]. The main advantages for consumers are perceived or real freshness and quality[81] . Price advantage is also sought but, in practice, this may be offset by higher quality purchases and organic production costs. Higher prices are also a feature of the retailing format using the Internet for selection and ordering, with home delivery by post or courier service[82]. The direct link between producer and consumer defines both retailing formats, but the local dimension is stronger for Farmers' Markets. There is little to suggest strict uniformity of approach. Edinburgh Farmers' Market, for example, operates with a 100 miles (160 km) radius rule. The UK National Association of Farmers Markets suggests flexibility for large urban areas but states that locality should be evident in whatever definition is adopted, and an overall area much larger than a 30 mile (48 km) radius is unlikely to retain local characteristics. In the UK, Farmers' Markets and Farm Shops remain small-scale innovations and have a symbolic importance greater than their economic impact. The Foundation for Local Food Initiatives, the National Association of Farmers Markets - and many individual licensing authorities - promote markets on the basis of the freshness and quality of the food. In this there is an implied critique of conventional production although this is not made explicit. 'Producers must produce clear written information about production methods which shall be available to any consumer who requests it. Producers should be encouraged to welcome visitors onto their farm. Markets should for the time being include a policy that no genetically modified organisms are knowingly sold or included in products sold at the market'[83]. Premium prices, infrequent events and a tendency to offer high quality specialist produce, limit the threat to volume sales but, as shown above, supermarkets are not insensitive to the cachet of 'organic' nor, apparently, to the utility of signalling 'local' produce availability. Supermarkets are now indicating willingness to stock local food[84]. Rather than see this change of emphasis as change of heart; one might argue that supermarkets are making a virtue out of necessity. Public opinion is directed towards our ailing countryside, there is a low-key but growing awareness of the 'food miles' debate but there is also the opportunity to purchase cheaply because of the difficulties. As evidenced by the fact that both *Tesco* and *Safeway* have been trying to acquire a placing in an index of ethically sound organisations[85], supermarket chains put a high value on customer opinion. Similarly, supermarkets have sought to reassure consumers with the launch of ethical trading and other environmental initiatives. When their buying practices or products attract criticism, they make a point of being seen to positively respond. It remains to be seen whether local sourcing becomes a significant proportion of all purchasing given its current global dimensions. Furthermore, local sourcing will require change in the increasingly centralised distribution strategy that supermarkets are currently using to claim as a reduction in food miles[86]. If not, it is probable that 'local' will be distorted to signify something more than the prescribed radius of many Farmers' Markets. 'Asda sells Scottish beef in its Scottish stores, Cheshire potatoes in Cheshire, Ayrshire potatoes in Scotland and Puffin potatoes in Kent Supermarkets have been building on a local food strategy for some months. Sainsbury's has already recruited 375 local suppliers who provide over 2000 lines to regional stores'[87]. In usage of this kind, 'local' is a synonym of 'regional' and this detracts from advantages of connection between producer and consumer inherent in a more restricted sense of the word. One abuse of the term has been addressed recently. *Scotch* beef has premium quality associations but until April 2002, this could be applied to cattle that had spent as little as 90 days in Scotland[88].

Furthermore, if 'local' becomes more an adjective in the establishment of premium-priced goods than a reflection of truly local sourcing, then there will have been a similar 'control by adoption' strategy to that deployed for organically-produced food. It is, however, significant that local purchasing is once again being mentioned in supermarket press releases, even if there is ambiguity about the operational implications.

Conclusions

In the wake of the recent Foot and Mouth Disease outbreak in the UK, media attention has been drawn to profound economic difficulties in many rural areas. A July 2001 private sector business initiative called *Rural Action*[89], for example, urged businesses and the community generally to buy their food locally in order to boost farm incomes, stimulate all aspects of rural economic life and reduce rural unemployment. Equally, the UK Government's Countryside Agency is promoting the retention of local buying power based on market research into the multiplier effect of rural business activity. Local businesses retain more consumer spending in the community than do local branches of national or international companies[90]. Similarly, in August 2001 the UK Government appointed a policy commission to 'advise the Government on how we can create a sustainable, competitive and diverse farming and food sector which contributes to a thriving and sustainable rural economy'[91]. One of the many recommendations in the commission's report was that public bodies should not just try to promote healthier eating through their procurement policies but should give serious consideration to the benefits of purchasing locally produced food. 'The development of local food distribution networks is encouraged by the critical mass purchasing that public bodies can deliver, thereby allowing cost effective distribution into other local outlets as well'[92]. The call for this kind of indirect market intervention is an innovative departure in the UK but not uncommon elsewhere in Europe. If supermarkets were to source more commodities and food products locally then, not only would there be benefits in terms of reduced food miles and less distortion of overseas agricultural systems, but there would be an opportunity to limit rural job loss and rural community reoccupation as dormitories for urban economic activity. Already the encroachment of towns has altered the social composition of rural areas[93], but the real damage occurs when this also tends to undermine local economic activity rather than stimulate it. After the sequence of agricultural disasters that have simultaneously reduced consumer confidence in food production, and closed down a number of rural businesses, UK agriculture is vulnerable. Conversion to organic production has appeal because there is increasing demand, but the costs are not readily offset against substantially higher incomes from supermarkets[94].

Paradoxically, the alternative - direct selling - works well on the margins but long- term sustainability needs the demand that can be generated by larger food retailers. Supermarkets hold the key to the future of UK food production but, on present reading, their purchasing power would be better focused on place of production – i.e. local - rather than on the narrower issue of how food is produced – i.e. organic. Both are important, but the diversity of UK agriculture is under threat because producers are not in a position to simultaneously accept higher costs and lower prices on food from lower productivity systems. That said, not only is the Soil Association urging shoppers to 'buy from their local farmers' markets, organic delivery service or farm shop and schools, hospitals, restaurants and other businesses are also being encourage to use local fruit, vegetables and meat whenever possible, instead of relying on imports or food that has travelled across the UK'[95]. Finally, shopping for safe food is increasingly a consumer problem although some consumers will be more able than others to exercise choice in problem resolution. For some, insecurities may be reduced by opting for organic produce wherever possible. However, the advantages are neither clear cut nor cheaply obtained. Strengthened certification rules - such as those launched recently in the UK [96] - might help consumers ensure they are actually buying the safety they perceive in organically labelled food. However, reliance on overseas suppliers inevitably brings uncertainty back into the equation. International standards are hard to agree and even harder to apply. While there is such a high proportion of organic food imported for supermarket sales, domestic certification can have only a limited effect in restoring consumer confidence. While organic food may also be local food, to a large extent it is not. Most supermarket fresh produce is sourced internationally. The real answer to a restoration of confidence would seem, therefore, to depend on a renewed acknowledgement of the importance of local food. Not only would this increase certainty about the standards applied to organic produce, it would produce two major subsidiary benefits.

*Culturally, an urban society that loses touch with its uralhinterland is in danger of dysfunctional *hubris*. The recent UK Foot and Mouth Disease outbreak demonstrated the gap that now exists between people with an urban orientation – even if they live in the countryside - and those who not only live but also work in a rural context. Perhaps belatedly, this is now reflected in recent initiatives from private and public sector. Arguably, selling and consuming food in the locality where it is produced is more exceptional than it should be. Concerted efforts are needed to ensure not absolute local sustainability in food production – that is already beyond the grasp of consumers with raised expectations - but a substantially stronger relationship between town and local food producers even if that production is conventional rather than organic.

* The escalating problem of food miles – the distance food travels to the consumer – is serious and unsustainable in the longer term. While not denying the importance of international trade for all, economic differentials between producer and consumer societies ultimately weaken importer capacity for self-sufficiency. Nowhere does this matter more than for food production. The answer resides not in denial of international trade but in robust stewardship of the domestic capacity to grow and sell food.

The Foot & Mouth Disease outbreak has the potential to be a turning point in relations between UK producers and consumers. Media images of that prolonged tragedy may now be fading in urban minds, but private and public sector initiatives to help rural recovery might yet be longer lasting. Calls for shops to sell locally produced food give symmetry of purpose – if not of fine detail – to the organic lobby, the business sector and the UK Government. The Institute of Grocery Distribution's recent survey of consumer attitudes to food purchasing indicated 59% of respondents were interested in locally-produced foods and that, ... 'local foods offered an additional attractive quality by enabling them to support the local community, but this would always be balanced against the extent to which the product met their needs in all other areas'[97]. Quality, appearance, cost and product availability remained important limiting factors in the translation of this interest into actual shopping behaviour. The auguries for improved sustainability are encouraging especially in the context of continued food quality uncertainties, but it may elude us yet unless consumers are also committed to the change agenda.

References

[1] Colquhoun, A. and Lyon, P., 2001. To everything there was a season: deconstructing UK food availability. Food Service Technology. **1**(2): 93-102.

[2] Colquhoun, A, Lyon, P. and Alexander, E., 2001. Feeding minds and bodies: the Edwardian context of school meals. Nutrition & Food Science, **31**(3):117-124.

[3] Williams, B. 1994. The best butter in the world: a history of Sainsbury's. London: Ebury Press.

[4] Goldenberg, N. 1989. Thought for food: a study of the development of the food division, Marks & Spencer.Orpington: Food Trade Press. 142 p.

[5] British Market Research Bureau (1963).Shopping in suburbia. London: J.Walter Thompson.

[6] *Ibid.* 29 p.

[7] *Ibid.* 44 p.

[8] Rees, G.,1969. St Michael: a history of Marks & Spencer.London: Weidenfeld & Nicolson. 197 p.

[9] AC Neilson, 2000. The retail pocket book. Henley-on-Thames: NTC Publications.

[10] Raven, H. and Lang, T. 1993. Off our trolleys? Food retailing and the hypermarket economy. London: Institute for PublicPolicy Research. 21p.

[11] Seth, A. and Randall, G. 1999. The grocers: the rise and rise of the supermarket chains. London:Kogan Page. 218 p.

[12] *Ibid*. 218 p.

[13] *Ibid*. 283-284 p.

[14] O'Hagan, A. 2001. The end of British farming. London: Profile Books. 73-74 p.

[15] Hawkes, N. 1996. Supermarkets damage British fruit growers, The Times. 5 January. 5 p.

[16] Raven, H. and Lang, T. 1993. *Op. Cit.* 20 p.

[17] Hewison, D. 1996. Bimbo fruit: perfect looks with no taste. The Sunday Times. 17 March. 3/10. 10 p.

[18] Lewis, J. et al. 1985. Food retailing in London: a pilot study of the three largest retailers and Londoners' access to food. Lo ndon: London Food Commission. 56 p.

[19] Elliott, V. 1997. Food deserts threaten health of poor and old. The Times. 5 November. 10 p.

[20] Colquhoun, A., Lyon, P., Kinney, D. and Cockburn, J. 2000. Forgotten shopping: memories of 1950s grocery stores. Education & Ageing. **15**(1): 97-114.

[21] Hornsby, M. 1999. 100000 food poisoning cases reported. The Times. 28 January. 11p.

[22] Advisory Committee on the Microbiological Safety of Food.1993. Report on salmonella in eggs.London: HMSO.

[23] Phillips, C. 1998. Food, bacteria and health: a practical guide. Oxford: Chandos.

[24] Hornsby, M. 1990a. Scientists challenge assurances on the safety of UK beef. The Times.14 June.6 p.

[25] Hornsby, M. 1990b. BSE enquiry leads to new guidelines on meat cutting. The Times. 19 June. 2 p.

[26] Hornsby, M. 1995a. Abattoirs disregard safeguards over BSE. The Times. 28 November. 6 p.

[27] Stuttaford, T. 1990. Has mad cow disease acquired a tragic human face? The Times. 17 May. 20 p.

[28] Hornsby, M. 1990c. Beef will be taken off menu in 1000 schools. The Times. 16 May. 1 p.

[29] Hornsby, M. 1995b. Ministers accuse schools of over-reacting on BSE. The Times. 7 December. 6 p.

[30] The Pennington Group. 1997. Report on the circumstances leading to the 1996 outbreak of infection with E-coli 0157 in Central Scotland, the implications for food safety, and the lessons to be learned. Edinburgh: HMSO.

[31] Ross, J. 2001. Algae puts fish farms at risk. The Scotsman. 22 August, 8 p.

[32] Maxwell, F. 2001. After foot-and-mouth, now it's salmon. The Scotsman. 8 November, 11 p.

[33] Carson, R. 1965. Silent spring. Harmondsworth: Penguin.

[34] Gunn, D. and Stevens, J.(eds.).1976. Pesticides and human welfare. Oxford: Oxford University Press. *v* p.

[35] Rudd, R. 1964. Pesticides and the living landscape. London: Faber and Faber. 153 p.

[36] *Ibid.* 153 p.

[37] Carson, R. 1965. *Op.Cit.* 166 p.

[38] Bristow, A. 2002. Organic processed food. Which? April. 22-25. 26 p.

[39] *Ibid.* 26 p.

[40] SAFE Alliance. 1997. How green are our strawberries? London: SAFE Alliance.
[41] Vaughan, A. 1999a. Salad days: the impact of lettuce production and consumption on people and the environment. London: SUSTAIN (The Alliance for Better Food and Farming).
[42] Vaughan, A. 1999b. Fowl deeds: the impact of chicken production and consumption on people and the environment.London: SUSTAIN (The Alliance for Better Food andFarming).
[43] Walsh, J. 1999. No wonder its feathers are ruffled. The Independent on Sunday. 22 August. 23 p.
[44] Meikle, J. 2002. Stores clear out honey in drug alert. The Guardian. 7 February. 7 p.
[45] The Times. 2002. Prawns taken off shelves. 16 March. 6 p.
[46] Storstad, O. 2001. The impact of consumer trust in the Norwegian food market. In: Tovey, H. and Blanc, M. (eds). Food, nature and society: rural life in late modernity. Aldershot:Ashgate. p.113-133.
[47] Young, R. 2001. Irradiated food being sold by supermarkets. The Times. 7 May. 9 p.
[48] Nuki, P. 2001. Supermarkets selling year-old food as fresh. The Sunday Times. 20 September. 4 p.
[49] Food Standards Agency. 2000. Qualitative research to explore public attitudes to food safety. London: Food Standards Agency/ Cragg Ross Dawson. 19 p.
[50] *Ibid.* 19 p.
[51] *Ibid.* 25/26 p.
[52] *Ibid.* 38/39 p.
[53] Food Standards Agency. 2001. Consumer attitudes to food standards: final report. London: Food Standards Agency/Taylor Nelson Sofres. 55 p.
[54] Harris, F. and O'Brien, L. 1993. The greening of shopping. In: Bromley, R. and Thomas, C.(eds.). Retail change: contemporary issues. London: UCL Press. p.229-246.
[55] Fracassini, C., 1999b. Iceland first to cut out additives in food.The Scotsman. 7 October. 10 p.
[56] Patten, S. 2001. Chill wind freezes Iceland strategists' organic ambitions. The Times. 23 January. 23 p.
[57] Edwards, R. 2002. When a salad is a cocktail of gender bending chemicals and nerve agents. Sunday Herald. 17 February.10-11p.
[58] Patten, S.and Elliott, V. 2001. Tesco sets £1 bn organic target.The Times. 2 November. 29 p.
[59] Whitehead, P.and Nicholson, S. 2001. Organic food: niche or mainstream? Watford: Institute of Grocery Distribution.
[60] Food Standards Agency. 2001. *Op. Cit.*
[61] Pattern, S. and Elliott, V. 2001. *Op. Cit.*
[62] UKROFS (UK Register of Organic Food Standards). 2001. UKROFS standards for organic food production. London: DEFRA (Department for Environment, Food and Rural Affairs).
[63] Troth, J. 2001. Policing the organic field. Food Science and Technology Today. **15**(1): 41-45.
[64] Fracassini, C. 2000. Sold on the organic myth. The Scotsman (Section 2*)*. 8 May. 10 p.
[65] Food Standards Agency. 2000. *Op.Cit.* 47 p.
[66] Fracassini, C. 1999a. Organic food link to E coli deaths. The Scotsman. 18 August. 7 p.
[67] Gray, A. 2000. Organic food no safer or healthier. The Scotsman. 2 September. 6 p.
[68] Young, R. 1999. First read the label, then add a pinch of salt. The Times (Weekend Section). 30 October. 2 p.
[69] UKROFS. 2001. *Op. Cit.* 9 p.
[70] Hawkes, N. 2001. Report proves organic food is healthier, farm group insists. The Times. 7 August. 6 p.
[71] SIRC (Social Issues Research Centre). 2001. Organic (double) standards. http://www.sirc.org/articles/ doublestandards.shtml Accessed 7 August.
[72] Crompton, S. 2001. Pure isn't that simple.The Times. 19 June.12/13 p.
[73] Perkins, A. 2001. Poor need cheap food, says Blair rural aide.The Guardian. 13 August. 9 p.
[74] Paxton, A. 1994. The food miles report: the dangers of long distance food transport. London: SAFE Alliance.
[75] SUSTAIN. 1999. Food miles: still on the road to ruin? London: SUSTAIN (The Alliance for Better Food and Farming).
[76] Pearce, F.1999. Bloom or bust? http://online.guardian.co.uk/ technology/ archive/891442431- bloom.html Accessed 16 August.
[77] Maxwell, F. 2000. Middle ground between GM food and organic is hard to hold. The Scotsman. 14 February. 20 p.
[78] Hallett, G. and James, G. 1963. Farming for consumers. London: Institute of Economic Affairs. 16 p.
[79] Chubb, A. 1998. Farmers' markets: the UK potential. Bristol: Eco Logic Books.
[80] Gilchrist, J. 1999. To market, to market, to buy an organic pig. The Scotsman. 24 August. 13 p.
[81] La Trobe, H. 2001. Farmers' markets: consuming local rural produce. International Journal of Consumer Studies. **25**(3): 181-192.
[82] Beckett, F. 2000. Box of surprises delivered to your door. The Times. 25 November. 8 p.
[83] National Association of Farmers Markets. 2001. Guidance criteria, http://www.farmersmarkets.net/started/guidance/default.htm Accessed 29 November.
[84] Elliott, V. 2001a. Supermarkets promise more produce from local farmers. The Times. 23 July. 4 p.
[85] Gibbs, G. and Finch, J. 2001. Tesco, Safeway clamour for place on ethical index. The Guardian. 10 September. 18.
[86] Hawkes, C. 2000. A Battle in store? A discussion of the social impact of the major UK supermarkets. London: SUSTAIN (The Alliance for Better Food and Farming).
[87] Elliott, V. 2001a. *Op. Cit.* 4 p.
[88] Buglass, D. 2002. NBA questions Scotch rule change. The Scotsman (Business Section*)*. 3 April. 4.
[89]Business in the Community. 2001. Rural action. Bristol: Business in the Community.
[90] The Countryside Agency. 2001. Local products key for rural local economies.http://www.countryside.gov.uk/news/article.asp?NewsItemID=183 Accessed 8 July.
[91] Policy Commission on the Future of Farming and Food. 2002. Farming and food: a sustainable future (The Curry Report). London: The Cabinet Office. 5 p.
[92] *Ibid.* 104 p.
[93] Urquhart, F. 2000. Rural life is at risk as holiday homes spread. The Scotsman. 22 May, 11 p.
[94] Elliott, V. 2001b. Organic farmers miss out on boom. The Times. 20 November. 14 p.
[95] Soil Association. 2001. Shoppers urged to go local. 16 October. http://www.soilassociation.org/sa/saweb.nsf/0055454980256ae000431c78?OpenDocument Accessed 16 May 2002.
[96] Soil Association. 2002. New organic standards launched. 8 May. http://www.soilassociation.org /sa/saweb.nsf/librarytitles/ news08052002.html Accessed 16 May 2002.
[97] IGD (The Institute of Grocery Distribution). 2002. One in three shoppers will look for local food in supermarkets. http://www.igd.com/ViewArticle.asp?AreaID=31&SubAreaID=45&PageID=145&Elem Accessed 18 April.

Molecular approach to citrus flavonoid and limonoid biosynthesis

Takaya Moriguchi[1*], Masayuki Kita[1], Shin Hasegawa[2] and Mitsuo Omura[3]
*[1]Department of Plant, Cell and Environment, National Institute of Fruit Tree Science, Tsukuba, Ibaraki 305-8605, Japan. [2]Western Regional Research Center, United States Department of Agriculture, Agricultural Research Service, 800 Buchanan Street, Albany, CA 94710, USA. [3]Department of Citriculture, National Institute of Fruit Tree Science, Shimizu, Shizuoka 424-0292, Japan. *email:takaya@affrc.go.jp*

Received 5 September 2002, accepted 3 January 2003.

Abstract
Citrus fruits are rich in secondary metabolites such as carotenoids, flavonoids and limonoids. Recent studies show that these secondary metabolites possess potential health benefits for humans, including anti-inflammatory and anti-cancer properties. Due to these properties, biochemical studies of citrus secondary metabolites have been intensively investigated. However, our knowledge on the molecular aspects of the biosynthesis of these compounds has been limited. Some of the genes involved in the flavonoid and limonoid biosynthesis have been isolated from citrus. In this review, we report on the expression of flavonoid- and limonoid-biosynthetic genes in citrus towards pharmacological applications and breeding using a molecular technique.

Key words: Bitterness, citrus, flavonoid, gene expression, limonoid.

Introduction

Citrus fruits are one of the important horticultural crops, with worldwide agricultural production over 100 million metric tons per year. They supply humans with many constituents, such as simple sugars, vitamin C, carotenoids, flavonoids, limonoids, fiber, folic acid and potassium, which have important effects on health. Almost half of the citrus fruits produced are processed into juices, concentrates, jams and other food products. Bitterness has been one of the problems in citrus products because bitterness reduces the quality and value of citrus juices and thus has a significant negative impact on the citrus industry. Bitterness in citrus fruits and juices is mainly caused by two different phytochemical families: flavonoids and limonoids. Limonoid bitterness occurs gradually in certain varieties of winter citrus after juice processing, which is referred to as "delayed bitterness" [21], and in the fruits after freezing or mechanical damage. On the other hand, bitter flavonoids are accumulated in the fruit tissues of species related to pummelo [10]. Pharmacological investigations have shown that certain citrus flavonoids and limonoids have potential health benefits - they possess anti-inflammatory and anti-cancer properties. Flavonols such as rutin accumulate to relatively high levels in citrus leaves and fruits have been shown to be efficient nitric oxide scavengers, a property that ameliorates nitric oxide-induced tissue damage [8]. A recent study indicates that nobiletin, a polymethoxylated-flavone which is accumulated in Shiikuwasha fruits (*C. depressa* Hayata), exhibits anti-carcinogenic activity. Citrus limonoids have been also shown to induce detoxification enzymes such as glutathione S-transferase and quinone reductase and to inhibit the formation of chemically induced neoplasia in the forestomach, small intestine, colon, lung, skin and oral cavity of laboratory animals [18, 22], and of human breast cancer cells in culture [9]. They also exhibit anti-feedant activity against insects [1]. Although many studies on flavonoid and limonoid biosynthesis in citrus have been done from the biochemical point of view [9, 10], molecular studies have been limited in spite of their importance. Within the past few years, the genes encoding the key enzymes required for the biosynthesis of flavonoids and limonoids in citrus have been identified. In this review, we describe recent findings regarding the expressions of genes involved in the biosynthesis of flavonoids and limonoids in citrus fruit.

Citrus Flavonoids

Features of flavonoid pathways in citrus: Chalcone synthase (CHS) is the first enzyme in the biosynthesis of all classes of flavonoids in plants (figure 1). It catalyzes the stepwise condensation of three acetate residues from malonyl CoA with *p*-coumaroyl CoA. The latter *p*-coumaroyl CoA is supplied from the phenylpropanoid pathway, which converts phenylalanine into a myriad of phenolic secondary metabolites in plants. Naringenin chalcone, the product of the CHS reaction, is then converted into a flavanone form by an intra-molecular reaction in which the C-ring is closed by the enzyme chalcone isomerase (CHI). These two forms of naringenin -- the chalcone form and the flavanone form -- appear to be the precursors for all the myriad compounds produced by plants with this related structure. By 3ß-hydroxylation, flavanone 3ß-hydroxylase (F3H) catalyzes the conversion of (*2S*)-flavanones to (*2R*, *3R*)-dihydroflavonols, which are intermediates in the biosynthesis of flavonols, anthocyanidins, catechins and proanthocyanidins. Citrus species are of great interest because they accumulate large amounts of flavonoids, especially flavanone glycosides[15]. Flavanone is probably modified in a stepwise fashion to the various derivatives by hydroxylation, methylation, glucosylation and then rhamonosylation [20]. The most common glycosidic group attached to the flavonoids in citrus is rhamnose-glucose diglycoside. This sugar group is present in two isomeric forms, neohesperidose and rutinose. The isomers differ only in the position at which rhamnose is attached to glucose [10]. Flavanone neohesperidosides are accumulated (sometimes along with the rutinosides) in citrus species related to the pummelo, such as grapefruit, sour orange and natsudaidai, and they give a bitter taste to citrus fruits [15, 27]. Major flavanone neohesperidosides detected in these species are naringin, poncirin, neoeriocitrin and neohesperidin. Other species related to citron and mandarin

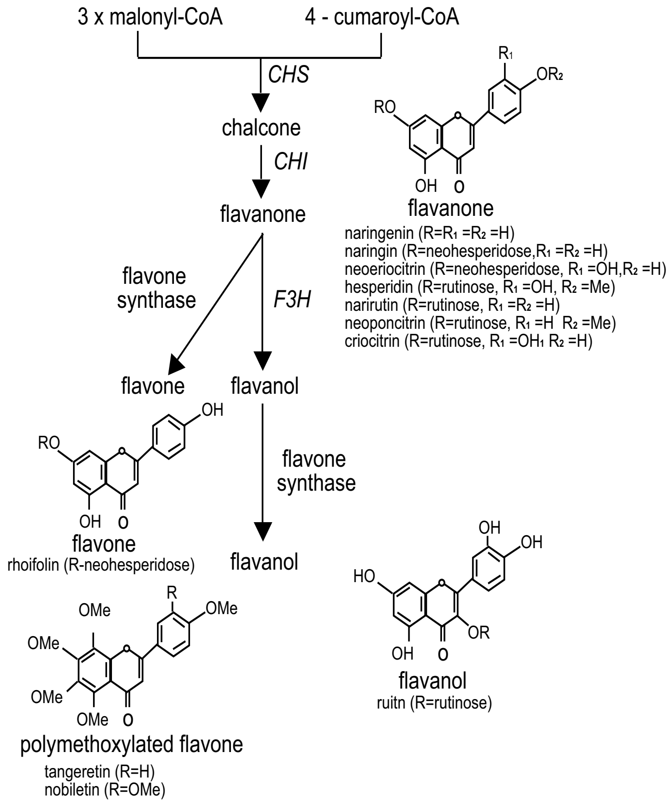

Figure 1. Biosynthesis of flavonoids in citrus (partially modified from Moriguchi et al.,[24]).Major flavonoids accumulated in *Citrus unshiu* are also indicated.

orange accumulate the tasteless flavanone rutinosides narirutin and hesperidin [25]. Interestingly, the major flavonoids accumulated differ according to the tissues. For example, fruits of satsuma mandarin (*Citrus unshiu* Marc.) intensively accumulate hesperidin and narirutin, whereas leaves accumulate hesperidin and rutin instead of narirutin [25].

***Biochemical studies of flavonoid biosynthesis**:* The flavonoid pigments of plants have been intensively investigated, and flavonoid pigments provide good taxonomic markers because of their diversification from species to species. In addition to a taxonomic interest, bitterness is a major problem for the citrus industry due to the lower market value of bitter juice. Biochemical investigations of the accumulation patterns of flavonoids have been done. High levels of the flavanone glycoside naringin are associated with very young tissue, and lower levels are found in older tissues in grapefruit (*C. paradisi* Macf., cv 'Duncan') [15]. Radioactively labeled acetate and phenylalanine fed to detached immature grapefruit fruits resulted in the accumulation of labeled naringin [4]. Similar experiments using intact and detached tissues of grapefruit seedlings showed that naringin is synthesized in young, rapidly growing leaves and then is transported to other parts of the plants. The radiolabeled naringin is not synthesized in detached stems and roots when fed labeled acetate [5]. Lewinsohn et al. [19] showed that grapefruit suspension cells are able to specifically *O*-glucosylate exogenous naringenin and hesperitin at position 7. They also proved that flavanones can be hydroxylated, methylated, glucosylated and then rhamnosylated in a stepwise fashion in cell-free extracts from citrus [20]. Collectively, these biochemical studies indicate that the biosynthesis of flavanone glycosides occurs constitutively during the cell stages of cell division and differentiation and not during periods of cell elongation and subsequent maturation. This constitutive biosynthetic pathway is highly regulated in citrus. Another interest lies in identifying the genes expressed with the induction of somatic embryogenesis and the regulation of flavonoid production in citrus cell cultures. It has been generally reported that grapefruit suspension cells [19] and undifferentiated calli of grapefruit and orange [2] lose the ability to produce flavonoids. Lime (*C. aurantifolia* (Christm.) Swing.) cultures are an exception [3]. For the relationship between enzyme activities and anthocyanin production in carrot suspension-cell cultures, Gleitz and Seitz [7] reported two CHS forms with different molecular subunit weights and different isoelectric points, and they showed the differential accumulation of these proteins during the course of anthocyanin synthesis. Ozeki et al. [26] showed that CHS mRNA in carrot cells was induced when anthocyanin synthesis was initiated. In contrast, the amount of enzyme protein and the amount of mRNA for CHS were below detectable levels when anthocyanin synthesis was repressed [26]. These reports imply that the production of anthocyanin in carrot-cultured cells is regulated by CHS genes.

***Expression analysis of chalcone synthase, chalcone isomerase and flavanone 3ß-hydroxylase during embryogenesis in citrus cell cultures or during citrus fruit development**:* Clones of two CHS (CitCHS1 and CitCHS2), one CHI (CitCHI) and one F3H (CitF3H) were isolated from the cDNA library of Valencia orange (*C. sinensis* Osbeck) seeds [23, 24]. During citrus somatic embryogenesis [23], both transcripts corresponding to CitCHS1 and CitCHS2 mRNAs were hardly detectable in the undifferentiated calli. When the calli were transferred onto the medium for the induction of somatic embryogenesis, the accumulation of transcripts corresponding to CitCHS2 and CitCHI mRNAs was induced and continued up to the formation of small green plantlets. Unlike CitCHS2 and CitCHI, the transcript corresponding to CitCHS1 mRNA hardly accumulated in either proliferation or the somatic embryogenesis process. Interestingly, the transcript for CitF3H was detectable even in the undifferentiated calli. Flavonoid contents were below the detection level in the undifferentiated citrus calli and were detected in greenish embryoids [23]. The major flavonoids detected in the greenish embryoids and in the leaf were neodiosmin and hesperidin, respectively, indicating the changes in the flavonoid metabolic pathway during morphogenesis. Thorpe et al. [28] reported that phenylalanine ammonia-lyase (PAL) activity is detected in grapefruit callus cultures. They also showed that although the callus possesses the ability to convert phenylalanine to cinnamic acid, there is no detectable formation of naringenin and its glycosides, unlike in intact grapefruit [28]. These results indicate that CitCHS2 may be a primary key enzyme of the flavonoid biosynthetic pathway for flavonoid accumulation in citrus cell cultures. Expression profiles in *C. unshiu* have been investigated using Northern blot analysis of a preparation of the total RNA of the albedo (inner spongy part of the rind), flavedo (outer part of the rind) and juice sacs/segment epidermis (edible part) from various stages of development, as well as from flowers (balloon stage) and young and mature leaves [24]. The transcript levels of CitCHS1, CitCHS2, CitCHI and CitF3H were high in young active tissues such as young leaves and fruitlets at 26 days after flowering (DAF), decreasing and disappearing in senescent tissues and/or towards fruit development. Major flavanone glycosides were narirutin and hesperidin in the flowers, albedo, juice sacs/segment epidermis and flavedo of satsuma mandarin, accounting for more than 91% of the total flavonoids [24]. In both young and mature leaves, hesperidin and the flavonol rutin, instead of narirutin, mainly accumulated. The flavonoid contents in the flavedo and juice sacs/segment epidermis were highest in the young tissues, decreasing with ripening [24]. Therefore, taking into consideration the patterns of gene expression and flavonoid accumulation, flavonoids are synthesized in the early developmental stage of citrus as indicated by the previous biochemical studies.

Citrus Limonoids

***Features of limonoid biosynthesis in citrus*:** Limonoids are highly oxygenated triterpenes present in Rutaceae and other limited plants such as Meliaceae. Significant progress has been made in understanding where, when and how limonoids and limonoid glucosides are biosynthesized and accumulated. The citrus limonoids occur as limonoid aglycones and limonoid glucosides. So far, 36 limonoid aglycones and 17 limonoid glucosides have been isolated from citrus and its closely related genera [12]. These aglycones are classified into four taxonomic groups: the *Citrus* group (19 limonoids), the *Fortunella* group (12 limonoids), the *Papedocitrus* group (one limonoid) and the *Poncirus* group (four limonoids). The biosynthetic pathways of each of these groups have been elucidated based on redioactive tracer work, and the details have been reviewed recently [9]. Nomilin is most likely the initial precursor of all the known limonoids in citrus. Nomilin has been shown to be biosynthesized from acetate, mevalonate and/or farnesyl pyrophosphate in the phloem region of stems. This precursor then migrates to other tissues such as leaves, fruit tissues and seeds, where other limonoids are biosynthesized from this compound independently. Limonoid aglycones are converted to non-bitter 17ß-D-glucopyraonoside derivatives such as limonin 17ß-D-glucopyranoside (LG) during maturation. This natural debittering process is catalyzed by the enzyme UDP-D-glucose: limonoid glucosyltransferase (limonoid glucosyltransferase) (figure 2). A single enyme appears to be responsible for the glucosidation of all the limonoid aglycones to their respective glucosides. Limonoid glucosides are major secondary metabolites and accumulate in the fruit tissues and seeds in significant quantities. Limonoid glucosyltransferase (GTase) has been identified and chosen for possible genetic manipulation to create transgenic citrus fruit trees that have fruit free from limonin bitterness.

***Molecular cloning and expression analysis of limonoid glucosyltransferase*:** Since debitterness is catalyzed by limonoid GTase [13], the isolation of limonoid GTase is crucial for creating transgenic citrus free from limonoid bitterness as well as for increasing specific limonoid glucoside molecules having anti-cancer properties. As the first step, Kita et al. [17] isolated limonoid GTase based on the purified protein sequences of N-terminal and internal regions [13] and confirmed that the isolated limonoid GTase (CitLGT) possesses limonoid GTase activity using the recombinant fusion protein. They also showed that CitLGT is a single copy without introns by Southern blot analysis and the genomic sequence amplified by PCR. Using the cloned CitLGT, Kita et al. [17] showed that CitLGT is transcribed strongly in the juice sacs/segment epidermis and albedo at the later developmental stage of navel orange fruit. In navel orange juice, the limonoid bitterness decreases as the fruit matures [11]. LARL is converted to nonbitter LG, which increases sharply in the juice sacs at the late stage of maturation [14]. Since the increase of the CitLGT transcript level is parallel to fruit maturation, the conversion of LARL to LG and mRNA accumulation could probably take place simultaneously in the juice sacs/segment epidermis. Furthermore, in albedo, the onset and increase of transcription occur simultaneously with LG production. No evidence of translocation of LG has been found in fruit tissues from other organs and the site of the metabolization of limonoid aglycon to glucosides is limited to the seed and mature fruit [6,14]. Therefore, the close correlation between mRNA accumulation and LG content in these tissues suggests that the transcription level of the CitLGT gene regulates the LG accumulation during citrus fruit maturation.

Future Prospects

Bitterness is an important determining factor for the quality and values of citrus fruits and their processed products. Particularly, delayed bitterness due to limonin is still a major problem in the citrus industry worldwide. An Ongoing molecular biology study aimed at the creation of transgenic citrus fruit certainly provides a way to eliminate the bitterness problems. Significant progress has been made in understanding the biological activities of citrus flavonoids and limonoids in animal systems. Further comprehensive research to define the

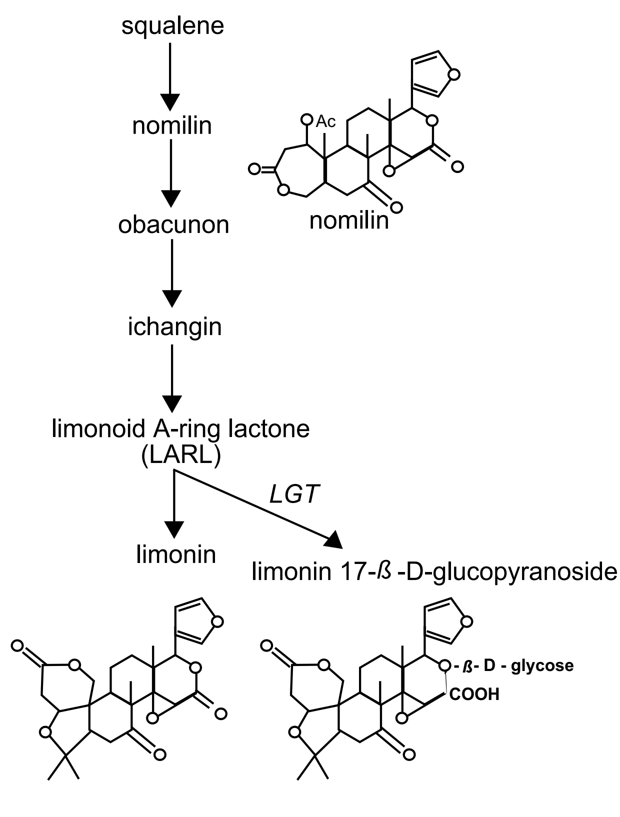

Figure 2. Biosynthesis of limonoids in citrus with structures of the major limonoids.

role of these compounds in human health is necessary. Nonetheless, there already has been commercial interest in utilization of these compounds. Since the by-products of citrus juice processing contain high concentrations of these compounds, these by-products may represent new profitable commercial products for the citrus industry. Using the isolated genes, the mass production of the useful flavonoids and limonoids in *E. coli* or citrus cell culture systems could be expected. Overall, these genes would contribute not only to citrus breeding but also to pharmacological applications.

References

[1] Alford, A.R., and Murray, K.D. 2000. Prospects for citrus limonoids in insect pest management. In: Berhow, M.A. et al. (eds.). Citrus Limonoids. Functional Chemicals in Agriculture and Foods. ACS (American Chemical Society) Symposium Series 758. Washington, DC, Oxford University Press. p. 201-211.

[2] Barthe, G.A., Jourdan, P.S., McIntosh, C.A., and Mansell, R.L. 1987. Naringin and limonin production in callus cultures and regenerated shoots from citrus sp. J. Plant Physiol. **127**: 55-65.

[3] Berhow, M.A., Bennett R.D., Poling, S.M., Vannier, S., Hidaka, T., and Omura, M. 1994. Acylated flavonoids in callus cultures of *Citrus aurantifolia*. Phytochemistry **36**: 1225-1227.

[4] Berhow, M.A. and Vandercook, C.E. 1989. Biosynthesis of naringin and prunin in detached grapefruit. Phytochemistry 28: 1627-1630.

[5] Berhow, M.A., and Vandercook, C.E. 1991. Sites of naringin biosynthesis in grapefruit seedlings. J. Plant Physiol. **138**: 176-179.

[6] Fong, C.H., Hasegawa, S., Coggins Jr. C.W., Atkin, D.R., and Miyake, M. 1992. Contents of limonoids and limonin 17-ß-D-glucopyranoside in fruit tissue of Valencia orange during fruit growth and maturation. J. Agric. Food Chem. **40**: 1178-1181.

[7] Gleitz, J., and Seitz, H.U. 1989. Induction of chalcone synthase in cell suspension cultures of carrot (*Daucus carota* L. ssp. *sativus*) by ultraviolet light: evidence for two different forms of chalcone synthase. Planta **179**: 323-330.

[8] Haenen, G.R.M.M., Paquary, J.B.G., Korthouwer, R.E.M., and Bast, A. 1997. Peroxynitrite scavenging by flavonoids. Biochem. Biophys. Res. Commun**.** **236**: 591-593.

[9] Hasegawa, S. 2000. Biochemistry of limonoids in *Citrus*. In: Berhow, M.A. (eds.). Citrus Limonoids. Functional Chemicals in Agriculture and Foods. ACS (American Chemical Society) Symposium Series 758. Washington, DC, Oxford University Press p. 9-30.

[10] Hasegawa, S., Berhow, M.A., and Fong, C.H. 1996. Analysis of bitter principles in *Citrus*. In: Linskens, H.-F. and Jackson, J.F. (eds.). Modern methods of plant analysis Vol 18. Berlin, Springer-Verlag. p. 59-80.

[11] Hasegawa, S., Ou, P., Fong, C.H., Herman, Z., Coggins Jr. C.W., and Atokin D.R. 1991. Changes in the limonate A-ring lactone and limonoid 17-ß-glucopyranoside content of navel oranges during fruit growth and maturation. J. Agric. Food Chem. **39**: 262-265.

[12] Hasegawa, S., and Miyake, M. 1996. Biochemistry and biological functions of citrus limonoids. Food Rev. Int. **12**: 413-435.

[13] Hasegawa, S., Suhayda, C.G., Hsu, W.-J. and Robertson, G.H. 1997. Purification of limonoid glucosyltransferase from navel orange albedo tissues. Phytochemistry **46**: 33-37.

[14] Herman, Z., Fong, C.H., and Hasegawa, S. 1991. Biosynthesis of limonoid glucosides in navel orange. Phytochemistry **30**: 1487-1488.

[15] Horowitz, R.M. 1964. Relations between the taste and structure of some phenolic glycosides. In: Harbone, J.B. (ed.). Biochemistry of phenolic compounds. New York, Academic Press. p. 545-571.

[16] Jourdan, P.S., McIntosh, C.A., and Mansell, R.L. 1985. Naringin levels in citrus tissues. quantitative distribution of naringin in *Citrus paradisi* Macfad. Plant Physiol. **77**: 903-908.

[17] Kita, M., Hirata, Y., Moriguchi, T., Endo-Inagaki, T., Matsumoto, R., Hasegawa, S., Suhayda, C.G., and Omura, M. 2000. Molecular cloning and characterization of a novel gene encoding limonoid UDP-glucosyltransferase in *Citrus*. FEBS Lett. **469**: 173-178.

[18] Lam, L.K.T., Zhang, J., and Hasegawa, S. 1994. Citrus limonoid reduction of chemically induced tumorigenesis. Bitter compounds found in citrus fruits may be useful cancer-preventive agents. Food Technol. **48**: 104-109.

[19] Lewinsohn, E., Berman, E., Mazur, Y., and Gressel, J. 1986. Glucosylation of exogenous flavanones by grapefruit (*Citrus paradisi*) cell cultures. Phytochemistry **25**: 2531-2535.

[20] Lewinsohn, E., Britsch, L., Mazur, Y., and Gressel, J. 1989. Flavanone glycoside biosynthesis in *Citrus*: chalcone synthase, UDP-glucose:flavanone-7-O-glucosyl-transferase and rhamnosyltransferase activities in cell-free extracts. Plant Physiol. **91**: 1323-1328.

[21] Maier, V.P., and Beverly, G.D. 1968. Limonin monolactone, the nonbitter precursor responsible for delayed bitterness in certain citrus juices. J. Food Sci. **33**: 488-492.

[22] Miller, E.G., Gonzales-Sanders, A.P., Couvillon, A.M., Binnie, W.H., Hasegawa, S., Lam, L.K.T. 1994. Citrus limonoids as inhibitors of oral carcinogenesis. seven citrus compounds were tested for their cancer-preventive activity. Food Technol. **48**: 110-114.

[23] Moriguchi, T., Kita, M., Tomono, Y., Endo-Inagaki, T., and Omura, M. 1999. One type of chalcone synthase gene expressed during embryogenesis regulates the flavonoid accumulation in citrus cell cultures. Plant Cell Physiol. **40:** 651-655.

[24] Moriguchi, T., Kita, M., Tomono, Y., Endo-Inagaki, T., and Omura, M. 2001. Gene expression in flavonoid biosynthesis: Correlation with flavonoid accumulation in developing citrus fruit. Physiol. Plant. **111**: 66-74.

[25] Nishiura, M., Kamiya, S., and Esaki, S. 1971. Flavonoids in citrus and related genera. Part III. Flavonoid pattern and citrus taxonomy. Agric. Biol. Chem. **35**: 1691-1706.

[26] Ozeki, Y., Komamine, A., and Tanaka, Y. 1990. Induction and repression of phenylalanine-lyase and chalcone synthase proteins and mRNAs in carrot cell suspension cultures regulated by 2,4-D. Physiol. Plant. **78**: 400-408.

[27] Rouseff, R.L. 1980. Flavonoids and *Citrus* quality. In: Nagy, S. and Attaway, J. (eds.). *Citrus* nutrition and quality. ACS (American Chemical Society) Symposium Series 143. Washington, DC, Oxford University Press. p. 83-108.

[28] Thorpe, T.A., Maier, V.P., and Hasegawa, S. 1971. Phenylalanine ammonia-lyase activity in citrus fruit tissue cultured *in vitro*. Phytochemistry **10:** 711-718.

WFL Publisher
Science and Technology Food, Agriculture & Environment Vol.1(1):26-29. 2003 *www.world-food.net*

Insulin resistance, diet and cardiovascular disease: a review

Rebecca L. Jones
Prince Henry's Institute of Medical Research, P.O. Box 5152, Clayton, VIC 3168, Australia. email:Rebecca.Jones@med.monash.edu.au

Received 5 September 2002, accepted 21 December 2003.

Abstract
Obesity and cardiovascular disease (CVD) are two of the leading causes of morbidity and mortality in developed countries. Direct influences of diet on obesity are obvious, however equally strong is the evidence that CVD is caused by and preventable by diet. A common link between these two conditions is insulin resistance (IR), characterised by elevated insulin levels due to an insensitivity to insulin's action postprandally. Recent research has highlighted the strong connection between IR and incidence and severity of CVD. IR is associated with the generation of very small dense low density lipoprotein (LDL) which confers a high risk for cardiovascular disease, more so than any previously identified risk factor for CVD. Given the clear correlation with IR, dietary approaches may be considered to be highly effective as a treatment strategy or preventative measure. This review concentrates on this newly discovered link between IR and CVD, and presents a balanced view of dietary measures which could contribute to corrections in insulin imbalances, responsiveness to insulin, and therefore reduce CVD incidence and obesity.

Key words: Cardiovascular disease, obesity, insulin resistance, diet.

Introduction

Obesity is a well-recognised problem in modern western society and is associated with increased risk of a number of chronic diseases including diabetes, hypertension and cardiovascular disease (CVD)[1]. Further, accumulation of fat in the abdominal region particularly increases risk of obesity related diseases. This is of primary concern as the prevalence of such diseases is increasing dramatically, and CVD is the single most common cause of death in developed countries.

Insulin and CVD

There are a number of risk factors for CVD, including lack of physical exercise, smoking and consumption of saturated or oxidized polyunsaturated fats[2] of both CVD and obesity. Insulin, released from the cells of the pancreas in response to rising blood glucose levels, acts to facilitate the transport of glucose across cell membranes to clear blood glucose levels. Rapidly absorbed sugars, such as those from refined carbohydrates with a high glycaemic index (GI), are rapidly removed from the circulation under the control of insulin and preferentially enter the lipogenic pathway to be stored as fats when glycogen stores are replete. Insulin also regulates plasma levels of triglycerides in the postprandial period, by reducing the metabolism and release of fats into the blood stream from adipose tissue by inhibiting the action of lipases. This ensures that plasma triglycerides levels are not excessively raised while the newly consumed fats are being absorbed, metabolised and/or stored. Insulin activates adipocyte lipoprotein lipase (LPL), which acts to clear lipids being transported in chylomicrons following absorption. Therefore the action of insulin is very important for regulating blood lipids post-prandially, and any disorders in its action impact plasma concentration of both glucose and fats.

Insulin Resistance

Insulin resistance is caused by a relative insensitivity of target tissues to insulin, which may be caused by a number of defects in the insulin signalling pathway, including decreased binding to insulin receptors or defective post-receptor signalling[3]. The pancreas responds by producing elevated levels of insulin in order to obtain an adequate response, resulting in hyper-insulinemia. Basal levels of insulin rise with age but are also significantly elevated in obese subjects, and in particular in subjects with abdominal (visceral) obesity[4]. The insensitivity of adipose cells and other target tissues to insulin results in dysregulation of enzymes such as lipoprotein lipase[5], resulting in elevated and extended postprandial lipaemia due to the failure to rapidly clear plasma triglycerides.

Lipids and CVD

Elevated circulating lipids are a risk factor for cardiovascular disease, as they can contribute to and exacerbate the atherosclerotic build up in blood arteries. Atherosclerosis is initiated by injury (e.g. oxidative and/or inflammatory damage) to the endothelial cell lining of the arteries, allowing the attachment or accumulation of cholesterol, which is the basis for atherosclerotic plaques. LDL (low density lipoprotein) cholesterol is readily oxidised by free radicals, which increases its adherence to the artery walls and uptake into the inner layers of the artery wall, the intima, where further oxidation takes place. Oxidized LDL is phagocytosed by infiltrating macrophages, which stimulates their differentiation into foam cells and promotes plaque formation by the release of chemotactic agents, stimulating further macrophage infiltration. This results in the generation of a large plaque, which becomes encapsulated by a fibrous cap, and blood lipids are deposited, further enlarging the blockage. Rupture of atherosclerotic plaques results in thrombus formation and occlusion of the artery lumen, which can cause reduced blood supply to the heart, leading to myocardial infarction. Elevated LDL cholesterol levels are a predictor of elevated CVD risk, and particularly the ratio of LDL or total cholesterol to HDL (high density lipoprotein). HDL is responsible for transporting cholesterol to the liver to be metabolized and is not as important in the pathogenesis of atherosclerosis, as it is less susceptible to oxidation than LDL and less adherent to arterial endothelial cells. Further, HDL is extremely protective against CVD. Recent epidemiological and prospective studies, such as the Quebec Cardiovascular Study, have described heterogeneity in the size and type of LDL and the implications for predicting CVD risk[6, 7]. In particular, the presence of very low density small LDL appears to increase risk of CVD three-fold, independent of other risk factors such as triglyceride levels and total cholesterol levels. Small dense LDL are produced by an elevation in the triglyceride content of the LDL, which occurs in the presence of elevated plasma triglycerides [8], such as those seen in insulin resistance. Triglyceride rich LDL is acted on by hepatic lipases in the liver, which produce the small dense LDL. Due to its smaller size and increased adhesiveness to the extracellular matrix of the endothelium, small dense LDL has higher affinity for the intima of the artery and is more readily oxidised, accelerating the formation of atherosclerotic plaques. Oxidation of the small dense LDL may further promote arterial blockage by promoting activation of prothrombin and other procoagulant factors [9]. This elevation in small dense LDL, which frequently occurs in the absence of elevated total cholesterol levels, is a key element in the syndrome known as atherogenic lipoprotein phenotype (ALP) and is also associated with elevated triglycerides, reduced HDL and elevated serum lipoproteins A and B (protein constituents of LDL)[10] . The pathogenesis of ALP is very strongly correlated to, and is likely to be caused by, obesity and insulin resistance. This explains, at least in part, the major problem of CVD in patients with type II diabetes[11].

Insulin Resistance and Evolution

From an evolutionary perspective, predisposition to insulin resistance would have been beneficial, in order to cope with cycles of feast and famine. This was recently reviewed by Brand-Miller and Colagiuri[12]. Insulin resistance protects the brain and placenta in an environment characterised by low carbohydrate, intermittent high protein, high physical activity – i.e. our evolutionary origins. As a consequence, insulin resistance promotes fat storage during times of high carbohydrate intake, ensuring that energy demands can be met in times of food shortage. With the onset of the agricultural revolution food not only became more plentiful, but the balance of macronutrients altered from a low carbohydrate / high protein diet to a carbohydrate rich diet. Further, the refinement of carbohydrates by removal of fibre and production of ground wheat flour has produced carbohydrates that are more readily digested and absorbed and therefore stimulate a more rapid and amplified insulin response. It is easy to see how in these modern times of plentiful, energy-rich foods, and high intake of refined, rapidly absorbed, high GI carbohydrates, this evolutionary measure to promote fat storage has lead to rising obesity and exacerbated insulin resistance. This theory is reinforced by the sharp increase in type II diabetes with the introduction to Britain in the 17^{th} century of refined flour and potatoes and the prevalence of such conditions in populations that have more recently adopted a western diet and lifestyle, such as the Australian Aborigines [12].

Dietary Strategies

To reduce serum cholesterol levels to the normal range, therapeutic low saturated fat, low cholesterol diets are recommended for patients at risk from coronary heat disease. However, the recent association between insulin resistance and CVD may lead to a number of different dietary strategies. Conventional low fat/low cholesterol diets tend to be rich in carbohydrates, leading to elevated blood glucose levels which may exacerbate symptoms of insulin resistance. The key to reducing CVD risk and obesity, which can predispose to insulin resistance, is to use dietary approaches to reduce circulating insulin levels or improve the insulin – glucose uptake mechanism, to restore the balance in blood triglycerides, cholesterol and glucose levels.

Lowering the intake of carbohydrates or altering the balance of macronutrients can have significant effects on parameters of ALP. A further dietary approach is to not only adjust the total intake of carbohydrates, but to emphasize carbohydrates which have a reduced impact on blood sugar levels and therefore on insulin production. Low glycaemic index carbohydrates are more slowly digested and absorbed, leading to a controlled rise in blood glucose levels. This results in a reduced insulin response, as lower concentrations of insulin are required to bring blood glucose levels back to basal levels. The glycaemic index of a carbohydrate food source is determined by the balance of different starches (amylose, amylopectin, resistant starch) and the amount of proteins, fats, and soluble and insoluble fibre present. Digestion is slowed down by the presence of both soluble and insoluble fibre, such as from grains and fruit, which reduces the speed of glucose absorption from the intestine by increasing intra-luminal viscosity. Proteins and fats are slower to digest than carbohydrates and therefore have a similar effect in reducing glucose absorption if consumed as a constituent, or with, carbohydrate sources. Preferential consumption of carbohydrates with a low glycaemic index accompanied by sufficient protein and fibre, reduces serum glucose and insulin levels, which in turn has beneficial effects on plasma LDL and triglyceride levels [13]. Additionally, ensuring adequate intake of dietary micronutrients that are essential for insulin action (e.g. chromium) or glucose and fatty acid metabolism (e.g. vitamins B1, B2, B3 and B6) is essential for correct energy utilization / storage. In addition to their actions on slowing glucose entry into the blood, dietary protein, fats and fibre have been shown to have independent actions on blood lipid and cholesterol levels. Increased fibre intake improves the ratio of HDL to total cholesterol by the preferential reduction of LDL levels[13,15]. Similarly, elevated intake of vegetable protein (both wheat- and soy- derived) has been demonstrated to decrease the ratio of total cholesterol to HDL by decreasing LDL concentrations, decrease the proportion of oxidized LDL and reduce levels of serum triglycerides [14,17]. This beneficial effect may be in part attributable to soy-derived isoflavones and wheat-derived phenolics exerting direct effects on cardiovascular tissues. These recent data suggest that increased intake of protein from vegetable sources and fibre can assist in restoration of normal serum triglycerides and lipoproteins.

Beneficial Effects of Lipids

Previous studies have shown that substitution of monounsaturated fat (e.g. oleic acid) for carbohydrate reduces triglyceride levels and improves glycaemic control [18]. Mono- and poly-unsaturated fatty acids from plant oils also have well-promoted roles in increasing HDL, which again affects the ratio of LDL to HDL. These data suggest that the currently recommended therapeutic diet could be modified to emphasize fats from vegetable sources, to increase the effectiveness of correcting blood lipid / cholesterol profiles and reducing insulin levels. This is supported by a trial conducted by Dumesnil and colleagues [19] where a low fat / low GI carbohydrate-rich diet, with a high intake of protein not only reduced plasma triglyceride levels, but also had other beneficial effects - lower cholesterol and increased diameter of LDL particles. Furthermore, energy intake was significantly reduced on the modified diet, which would assist in weight management. Several recent clinical trials have investigated the effect of essential fatty acids on the serum cholesterol and lipid levels. Supplementation with omega 3 series fatty acids derived from fish oils was shown to significantly reduce plasma triglyceride levels [20,22] and attenuate the postprandial lipid response, resulting in a reduction in the proportion of small dense LDL. One possible mechanism is through the stimulation of LPL in adipose tissue, leading to rapid clearance of newly absorbed fats from the circulation[20]. Omega 3 fatty acids may have further beneficial effects with respect to cardiovascular health due to the anti-thrombotic influence through the production of thromboxanes with low blood clotting potential.

Other Considerations

A number of micronutrient imbalances may also be directly associated with CHD risk, independent of the effects of insulin resistance. An example of this is the recently discovered link between raised homocysteine levels and mortality from CHD (reviewed [23,24]). Homocysteine is an intermediate product during the enzymatic conversion of the amino acid methionine to cysteine. Elevated levels of homocysteine have been shown to be pro-oxidative and it has been proposed that these may increase oxidative damage to vascular endothelium and therefore increase the likelihood of oxidised LDL accumulation and plaque formation. Generally homocysteine levels are low, due to its efficient conversion to cysteine or back to methionine, but these processes are dependent on vitamins B6, B12 and folic acid. Deficiencies in any or all of these micronutrients may therefore increase the damage to the cardiovascular system and predispose to heart disease, especially when dietary protein is high. Similarly, deficiencies in nutrients essential for normal functioning of the heart or blood vessels, such as calcium, magnesium and potassium may have detrimental effects on cardiovascular health. Finally, as the initial step in atherosclerotic plaque formation is oxidative damage to the endothelium, reducing the oxidative status of blood vessels by enhancing intrinsic antioxidant systems would reduce the risk of plaque build-up. Plant foods contain a wide range of phytochemicals, including the flavonoids, isoflavones and carotenoids, all of which exhibit potent antioxidant effects, as do vitamins A, C and E, acting locally to terminate the chain reaction of free radical damage in blood vessels and other tissues. A well balanced, varied, diet rich in sources of these micronutrients and phytochemicals would therefore assist in maintenance of cardiovascular health.

Conclusions

In summary, a number of previously unknown risk factors for CHD and agents involved in the pathogenesis of atherosclerosis have recently come to light and these mean that a number of novel dietary strategies may be used to lower risk of CHD and associated mortality. The description of the atherogenic lipid phenotype and its close association with insulin resistance and type II diabetes highlights the need for alteration of the conventional low fat therapeutic diet for CHD, which may exacerbate this condition by emphasizing intake of carbohydrates. Lowering insulin levels and thereby correcting imbalances in circulating glucose and fat concentrations is also the key to successful weight loss. It is clear that any dietary strategy to improve cardiovascular health would aim to readjust the imbalances in blood lipid profiles, reduce the oxidative potential of LDL and improve responsiveness to insulin.

References

[1] Abate, N. 2000. Obesity and cardiovascular disease. Pathogenetic role of the metabolic syndrome and therapeutic implications. J. Diabetes Complications. **14**: 154-174.

[2] Frohlich, J., Dobiasova, M., Lear, S., and Lee, K.W. 2001. The role of risk factors in the development of atherosclerosis. Crit Rev.Clin. Lab Sci. **38**: 401-440.

[3] Yki-Jarvinen, H. 1994. Pathogenesis of non-insulin-dependent diabetes mellitus. Lancet. **343**: 91-95.

[4] Despres, J.P. 2001. Health consequences of visceral obesity. Ann.Med. **33**: 534-541.

[5] Panarotto, D., Remillard, P., Bouffard, L., and Maheux, P. 2002. Insulin resistance affects the regulation of lipoprotein lipase in the postprandial period and in an adipose tissue-specific manner. Eur. J. Clin. Invest. **32**: 84-92.

[6] Lamarche, B., Tchernof, A., Moorjani, S., Cantin, B., Dagenais, G.R., Lupien, P.J., and Despres, J.P. 1997. Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation. **95**: 69-75.

[7] Griffin, B.A. 1999. Lipoprotein atherogenicity: an overview of current mechanisms. Proc Nutr. Soc. **58**: 163-169.

[8] Griffin, B.A., Freeman, D.J., Tait, G.W., Thomson, J., Caslake, M.J., Packard, C.J., and Shepherd, J. 1994. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. Atherosclerosis. **106**: 241-253.

[9] Griffin, J.H., Fernandez, J.A., and Deguchi, H. 2001. Plasma lipoproteins, hemostasis and thrombosis. Thromb. Haemost. **86**: 386-394.

[10] Despres, J.P., Lemieux, I., Dagenais, G.R., Cantin, B., and Lamarche, B. 2001. Evaluation and management of atherogenic dyslipidemia: beyond low-density lipoprotein cholesterol.CMAJ. **165**: 1331-1333.

[11] Nesto, R. 2001. CHD: a major burden in type 2 diabetes. Acta Diabetol. 38 Suppl 1: S3-S8.

[12] Brand-Miller, J.C. and Colagiuri, S. 1999. Evolutionary aspects of diet and insulin resistance. World Rev. Nutr. Diet. **84**: 74-105.

[13] Jenkins, D.J., Kendall, C.W., Axelsen, M., Augustin, L.S., and Vuksan, V. 2000. Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease. Curr. Opin. Lipidol. **11**: 49-56.

[14] Jenkins, D.J., Kendall, C.W., Mehling, C.C., Parker, T., Rao, A.V., Agarwal, S., Novokmet, R., Jones, P.J., Raeini, M., Story, J.A., Furumoto, E., Vidgen, E., Griffin, L.C., Cunnane, S.C., Ryan, M.A., and Connelly, P.W. 1999. Combined effect of vegetable protein (soy) and soluble fiber added to a standard cholesterol- lowering diet. Metabolism. **48**: 809-816.

[15] Jenkins, D.J., Kendall, C.W., Popovich, D.G., Vidgen, E.,Mehling, C.C., Vuksan, V., Ransom, T.P., Rao, A.V., Rosenberg-Zand, R., Tariq, N., Corey, P., Jones, P.J., Raeini, M., Story, J.A., Furumoto, E.J., Illingworth, D.R., Pappu, A.S., and Connelly, P.W. 2001. Effect of a very-high-fiber vegetable, fruit, and nut diet on serum lipids and colonic function. Metabolism. **50**: 494-503.

[16] Jenkins, D.J., Kendall, C.W., Vidgen, E., Vuksan, V., Jackson,C.J., Augustin, L.S., Lee, B., Garsetti, M., Agarwal, S., Rao, A.V., Cagampang, G.B., and Fulgoni, V., III 2000. Effect of soy-based breakfast cereal on blood lipids and oxidized low-density lipoprotein. Metabolism. **49**: 1496-1500.

[17] Jenkins, D.J., Kendall, C.W., Vidgen, E., Augustin, L.S., van Erk, M., Geelen, A., Parker, T., Faulkner, D., Vuksan, V., Josse, R.G., Leiter, L.A., and Connelly, P.W. 2001. High-protein diets in hyperlipidemia: effect of wheat gluten on serum lipids, uric acid,

and renal function. Am. J. Clin. Nutr. **74**: 57-63.

[18] Griffin, M.E., Dimitriadis, E., Lenehan, K., Owens, D., Collins, P., Johnson, A., and Tomkin, G.H. 1996. Non-insulin-dependent diabetes mellitus: dietary monounsaturated fatty acids and low-density lipoprotein composition and function. QJM. **89**: 211-216.

[19] Dumesnil, J.G., Turgeon, J., Tremblay, A., Poirier, P., Gilbert, M., Gagnon, L., St Pierre, S., Garneau, C., Lemieux, I., Pascot, A., Bergeron, J., and Despres, J.P. 2001. Effect of a low-glycaemic index—low-fat—high protein diet on the atherogenic metabolic risk profile of abdominally obese men. Br. J. Nutr. **86:** 557-568.

[20] Khan, S., Minihane, A.M., Talmud, P.J., Wright, J.W., Murphy, M.C., Williams, C.M., and Griffin, B.A. 2002. Dietary long- chain n-3 PUFAs increase LPL gene expression in adipose tissue of subjects with an atherogenic lipoprotein phenotype. J. Lipid Res. **43**: 979-985.

[21] Minihane, A.M., Khan, S., Leigh-Firbank, E.C., Talmud, P., Wright, J.W., Murphy, M.C., Griffin, B.A., and Williams, C.M.2000. ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. Arterioscler. Thromb. Vasc. Biol. **20**: 1990-1997.

[22] Griffin, B.A. 2001. The effect of n-3 fatty acids on low density lipoprotein subfractions. Lipids. 36 Suppl: S91-S97.

[23] Terrass, S. 2001. Homocysteine - the new cholesterol. Nutr. Pract. **3**: 5-7.

[24] Hunnisett, A. 2001. Homocysteine - basic biochemistry. Nutr. Pract. **3**: 8-9.

WFL Publisher
Science and Technology
Food, Agriculture & Environment Vol.1(1):30-34. 2003
www.world-food.net

Consumer attitudes to high pressure food processing

Peter Butz[1*], Eric C. Needs[4], Alain Baron,[2] Otto Bayer[1], Bärbel Geisel[1], Bharat Gupta[3], Ulrich Oltersdorf[1] and Bernhard Tauscher[1]

[1]Federal Research Centre for Nutrition, Haid-und-Neu-Straße 9, D-76131Karlsruhe, Germany. [2] INRA, Le Rheu, France, [3]St Ivel Ltd, Wooton Basset, UK, [4]Institute of Food Research, Reading, UK.
**email:Peter.butz@bfe.uni-karlsruhe.de*

Received 12 October 2002, accepted 20 December 2002.

Abstract

As part of an EU-funded research project a representative survey of consumer attitudes concerning high pressure processing (HPP) of foods was carried out. 3000 adults aged 14 years and over, in France, Germany and the UK were interviewed in face-to-face computer assisted personal interviews (CAPI) and asked to indicate their opinion by evaluating 35 positive and 25 negative statements about the new technique, to compare the new technique with the conventional techniques, and they were asked if they would buy products preserved using High Pressure Pasteurization. The concept used for the data analysis was that of a market segmentation model using sociodemographical, geographical and psychographical attributes. The average acceptability rate is discussed with respect to the MAYA threshold value (Most Advanced Yet Acceptable), a pragmatic market research threshold value.

Key words: High pressure, food processing, consumer, survey.

Introduction

HP-treatment is a new preservation method without high temperatures, avoiding undesirable alterations caused by thermal treatment of foods such as vitamin loss, reduced bioavailability of essential amino acids, f1avour loss, modification of taste and colour, etc.. Biological effects of high pressure like inactivation of micro-organisms or changing functional properties of food biopolymers, are known for decades, but only in the last ten years foods preserved by high pressure became commercial reality. First products were fruit jams in Japan, now there are a number of products available mainly in America and Japan including fruit juices, guacamole, sauces, oysters and packaged cured ham. However, in Europe the method is more or less unknown for the consumer and food manufacturers face impediments due to the EU novel food regulation. EU, on the other hand, in the last ten years funded a number of research projects on high pressure treatment of food, including this work which partly dealt with consumer attitudes to high pressure food processing.

Methods

Qualitative research was conducted in two workshops of 7-12 participants organized by Adriant, Nantes, France. The two workshops had discussions from different starting points; one form from the quality of food the other from technology and high pressure. The aim was to discern the reservations or motivations of consumers in relation to the use of high pressure in order to construct a questionnaire. It should also provide some of the terms and vocabulary used in the survey. A series of questions were devised and the questionnaire constructed. After reading the introductory show card (Fig. 1), the interviewees were asked a series of questions on seven topics (Fig. 2).

Questions B1 and B2 deal with the feelings the consumers have regarding the process. Question B3 compared high pressure with other preservation methods. Questions B4 and B5 deal with the product, about advantages and disadvantages that the new process might have (effect on taste, quality, price etc). Question B6 was about personal advantages and benefits for the consumer. In all, the questionnaire required the interviewees to evaluate 35 positive and 25 negative statements about the new technique. The last topic (Question B7) concerned the interviewee's willingness to buy high pressure treated food products. Under each question were a series of statements and the interviewees were asked to indicate whether they agreed or disagreed with each statement, or for some questions to indicate the statement which best answered the question. There was a total of 80 statements to be evaluated. The survey was conducted by GFM Getas (Hamburg, Germany). Face to face computer assisted personal interviews (CAPI) were conducted with 1000 people in each of France, Germany and UK. Data was analyzed using SPSS Answer Tree (Answer TreeTM 2.0 by SPSS Inc. Chicago, Illinois 60606) with the method of CHAID (Chi-squared Automatic Interactions Detector). This technique is a highly efficient process for data segmentation and the construction of Decision Trees. CHAID uses the significance of statistical tests as criteria to evaluate all the values of a potential predictor variable. It merges values that are judged statistically homogeneous with respect to the target variable and maintains all other values as heterogeneous. It then selects the best predictor value to form the first branch of the Decision Tree. As the process is repeated so the tree grows.

> Conventional methods of preserving food by heating to reduce the number of bacteria and activity of enzymes often produce a number of undesirable changes in foods, such as loss of colour, flavour and nutritional quality. This can be avoided by using alternative minimal processing strategies.
>
> One of these, already being commercialized on a small scale by food industry, is high pressure processing (HPP) in which foods are compressed in the range of 1000 to 5000 bar for a few minutes. (These are pressures comparable to those found in the depth of oceans.).
>
> The process can be used to extend the shelf-live of a range of products, such as juices.

Figure 1. Showcard B0 shown to interviewees at the start of the interview.

B 0	Information showcard presented by interviewer
B 1	What feeling do you have in general terms with regard to this new process?
B 2	Can you tell me how you feel about this technique? Which of the comments on this showcard do you agree with?
B 3	I'm going to read out various techniques for preserving food wich are currently available. Can you tell me, how you regard this new technique?
B 4	I'm going to read out some advantages and disadvantages that this new process might have. Please tell me for each of these statements how much you agree or disagree.
B 5	In your view: would high pressure preservation of foods results in more expensive products, better tasting andquality, both or none of these?
B 6	Would you say, that for you this new treatment has more advantages or disadvantages? Which statement on this showcards meets your expectations best?
B 7	Would you buy these products?

Figure 2. Questionnaire "Preserving foods". Interviewees were asked to consider seven topics and answer a series of questions about each

Results and Discussion

Following the qualitative study it was agreed that the large scale survey should contain certain key elements: (1) An outline of high pressure processing which should present the interviewee with basic information in a neutral format. (2) Determine views about high pressure technology and how it compares with existing treatments. (3) Perceptions of advantages and disadvantages to the consumer. (4) Indication of the requirements of consumers to become buyers of high pressure treated foods. Figure 3 shows the distribution of interviewees between the buying and non-buying groups.
Non-buyers included those who were uncertain and those who would not buy at all.

All the collected data were used to build a predictive model which examined perceptions about high pressure in relation to the consumers willingness to buy high pressure treated foods. In market research an acceptability threshold is set for new technologies described as the Most Advanced Yet Acceptable (MAYA) threshold, experience has set the value at 60%. From the survey an acceptability value was calculated as the sum of the conditional and unconditional sub-groups of buyers (see Fig. 3). These were found to be 74% in Germany, 71% in France and 55% in UK, with an overall average value of 67%. This suggests that, without personal experience and based largely on information provided on the statement card, high pressure processing was acceptable to the majority, with some reservations in the UK.

The majority of the potential buyers were conditional buyers i.e. they will buy if there are advantages (or no disadvantages perhaps) for them. The data suggest which conditions are most important (Fig. 4). There was similar behaviour of the British and the Germans. For both it was most important that the products are not more expensive than for conventional products

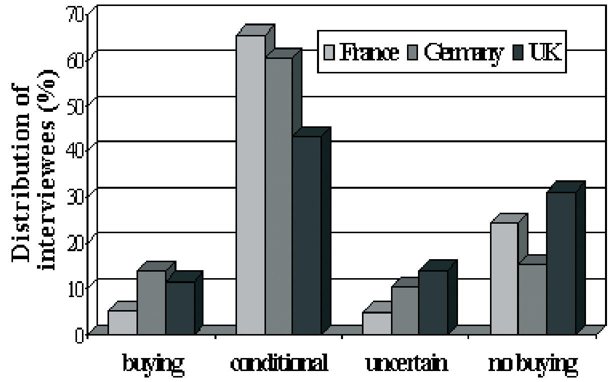

Figure 3. Interviewees grouped as buyers or non-buyers of high pressure treated foods.Buyers were subdevided into unconditional or conditional (will buy if...)

and that there is a health benefit. The French were more prepared to pay a bit more for the products, while both the Germans and the British were more reluctant to pay more. For the gastronomically aware French, quality was clearly important (a condition for 50% compared to less than 10% in Germany and the UK). Increased shelf-life was also a French concern. Buyers were allowed to choose more than one condition To investigate the data further for predictions of consumer behaviour, a factorial analysis was performed on the data of the public opinion poll using SPSS software. In this case we wanted to detect differences between the non-buyer group (including the undecided) and the buyer group (including conditional buyers). The data (Fig. 5) was subdivided into the same three factors for both groups: The upper part of the table deals with the positive properties of the process The middle deals with negative properties and fears. The bottom compares the process with other food preservation technologies.
The table shows the importance of single variables given by the values in the right hand row. Such a ranking could be used directly to determine advertising strategies.

The result suggests that both the buyers and non-buyers perceive high pressure to be similar to sterilisation, pasteurisation and UHT treatments. Also both groups were aware of the positive properties, like retention of vitamins, real taste and natural quality. However, the non-buyers expressed more fears and concerns. For example: „I would be concerned" is loading with 0.64 instead of 0.49 for the buyers.

To determine consumer attitudes a hypothesis was formulated: Consumer acceptance of high pressure processing is likely to depend on whether individuals perceive that the benefit to them (e.g. reduced risk of microbial contamination) outweighs any negative perceptions (e.g. loss of quality or perceptions of dangers inherent in the process). Acceptance of novel technologies are dependent upon perceptions of advantage or need as well as risk. These perceptions are important if predictions regarding consumer acceptance are to be made. Further statistical analysis, using SPSS software was applied to develop AnswerTrees. This was used to determine the best predictor for the buying behaviour in the three countries (Fig. 5). In the first node of the table all information is given about all the participants (2120 or 66.6% potential buyers and 1063 or 33.4% potential non-buyers. At the next level AnswerTree searches for the best predictor variable to form the first branch in the decision tree, which was the variable 72 from Question

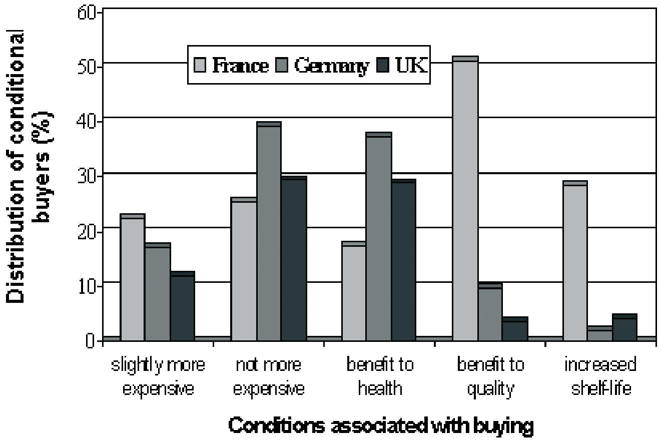

Figure 4. Conditional buyers grouped by condition and country.

B6 ("For me this new treatment has more advantages") (fig.6). The chi-square value of 973 was very high. Dividing the sample between those who agreed with this statement and those that disagreed produced groups of roughly similar size.

*The group of the people who saw more personal advantages contained over 90 % potential buyers. There is no need for a further split.

* The group of the people who do not see more personal advantages contained nearly 60% non-buyers.

The answers to question B7 were correlated with other responses throughout the questionnaire. The statement that "For me, this treatment has more advantage" was found to be the best predictor of buying habit.

The group that did not agree that the process offered them more advantage were further split (Fig. 7). The first node is again that of the 1561 people who did not see more personal advantages**.** AnswerTree found the best predictor variable to form the first branch of this decision tree was the variable 43 from Question B2 with a high chi-square value of 157. Variable 43 was "High Pressure Processing (HPP) sounds like an environmentally-friendly process". This question splits the sample into one large and one small group:

* Those that agree with this statement form the smaller group and nearly of them were 80 % potential buyers. In a further split this percentage rises to 87% for people who think the process preserves the real taste (B4_59).

v In the larger group of people, who do not see the process as environmentally friendly over 65% were non-buyers. In a further split this percentage rose to 71% for people who did not think the process preserves vitamins (B4_59).

* These analyses help to show perceptions and misconceptions about novel technology. The other important consideration required to improve the appeal of pressure treated products, was the target group. What kind of people were the non-buyers? What were the socio-demographic differences between the group of non-buyers and buyers? Age was a key factor, 22% of non-buyers were under 30 years while 28% of buyers were in that age group. Conversely 27% of non-buyers were over 59 but 20% of buyers were in that age group. The remaining age group (30 to 59) were evenly divided, 50 and 51% non-buyers and buyers respectively. Education was another important factor, people in the buyers group generally had a

non buyers + undecided		buyers + conditional buyers	
B4_61: vitamin	0.71	B4_59: real taste	0.72
B4_60: nutritional qualities	0.71	B4_61: vitamin	0.71
B4_63: natural qualities	0.65	B4_60: nutritional qualities	0.71
B4_59: real taste	0.62	B4_63: natural qualities	0.70
B4_62: freshness	0.50	B4_67: fewer additives	0.55
B4_67: fewer additives	0.44	B4_62: freshness	0.50
B2_43: friendly process	0.42	B5_69: better tasting	0.50
B6_72: more advantages	0.41	B6_72: more advantages	0.44
B4_65: risk of damage	0.66	B2_47: it changes the products	0.58
B2_46: I would be concerned	0.64	B4_65: risk of damage	0.55
B2_47: it changes the products	0.60	B2_46: I would be concerned	0.49
B2_51: do not know	-0.53	B1_29: quality must damage	0.38
B1_40: no answer	-0.51	B2_42: comfortable	0.33
B4_64: more processing	0.49	B2_44: environm. risk	0.32
B4_66: limited shelf-life	0.42	B1_27: high temperatures	0.29
B1_25: unnatural technique	0.40	B4_66: limited shelf-life	0.28
B2_44: environm. risk	0.38	B1_25: unnatural technique	0.27
B1_29: quality must damage	0.31	B1_31: benefits	0.24
B3_53: pasteurisation	0.73	B3_55: sterilisation	0.69
B3_55: sterilisation	0.68	B3_53: pasteurisation	0.68
B3_58: ultra high temp.	0.68	B3_58: ultra high temp.	0.65
B3_54: canning	0.61	B3_54: canning	0.47
B3_52: deep freezing	0.57	B3_57: irradiation	0.46
B3_57: irradiation	0.53	B3_52: deep freezing	0.42

Figure 5. Factorial analysis (SPSS) of public opinion poll. Participants form two groups: buyers and non-buyers. The table shows the relative importance of factors grouped according to 1. the positive properties of the process; 2. negative properties and fears 3. comparison of the process with other food preservation methods. Numbers (e.g. B4_61) refers to the question within the questionnaire. Rotation method: varimax with Kaiser-Normalizing; Extraction method: Principal Component Analysis; Rotation converged in 5 iterations.

higher level of education or better qualifications than those in the non-buyer group.

Conclusions

High pressure processing was acceptable to the majority of consumers interviewed in France and Germany, UK produced an acceptability value below the market research threshold. The overall average value of 67% suggests that, without personal experience and based largely on information provided on the statement card, high pressure processing was acceptable to the majority, with some reservations in the UK. The majority of the potential buyers were conditional buyers. Concerning which conditions are most important there was similar behaviour of the British and the Germans. For both it was most important that the products are not more expensive than for conventional products and that there is a health benefit. The French were more prepared to pay a bit more for the products, while both the Germans and the British were more reluctant to pay more. For the gastronomically aware French, quality was clearly important (a condition for 50% compared to less than 10% in Germany and the UK). Increased shelf-life was also a French concern. Those who perceived the greatest personal advantage from the technology were most likely to buy the products. This group tended to include a higher proportion of young educated people.

Acknowledgement

This work was supported by the European Community (EC) Framework Programme for Research and Technological Development, Contract: FAIR-CT96-1113.

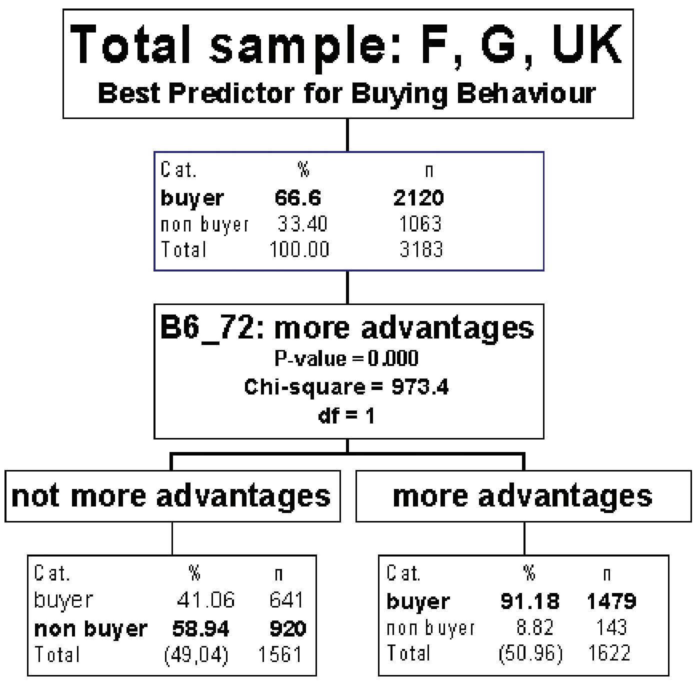

Figure 6. AnswerTree derived from SPSS analysis of data from international survey of public perception of high pressure food processing. To determine the best predictor of buying behaviour.

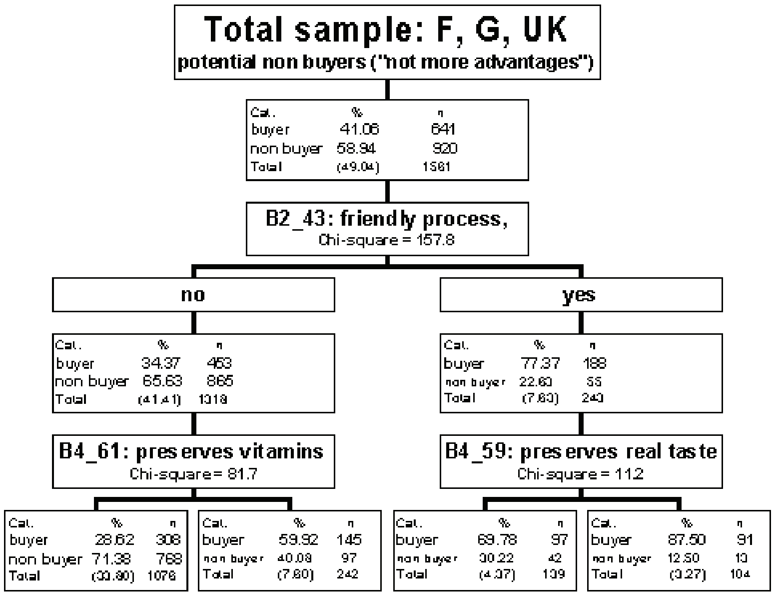

Figure 7. AnswerTree derived from SPSS analysis of data from international survey of public perception of high pressure food processing. Sub-division of the group who saw no personal advantage in high pressure processing.

WFL Publisher
Science and Technology
Food, Agriculture & Environment Vol.1(1): 35- 41. 2003
www.world-food.net

Effect of ripening stage and storage temperature on postharvest quality of pepino (*Solanum muricatum* Ait.)

Heru Prono-Widayat[1], Monika Schreiner[2], Susanne Huyskens-Keil[1*] and Peter Lüdders[3]
[1]Humboldt University Berlin, Institute of Horticultural Sciences, Section Quality Dynamics/Postharvest Physiology, Lentzeallee 75, 14195 Berlin, Germany. [2] Institute of Vegetable and Ornamental Crops Großbeeren/Erfurt e.V., Theodor-Echtermeyer-Weg 1, 14979 Großbeeren, Germany.[3] Humboldt University Berlin, Institute of Horticultural Sciences, Department Fruit Science, Albrecht-Thaer-Weg 3, 14195 Berlin, Germany.
**email:susanne.huyskens@agrar.hu-berlin.de*

Received 2 November 2002, accepted 7 January 2003.

Abstract

Pepino fruits cv. 'Golden Globe' were harvested at three ripening stages, premature (28-42 days after pollination), mature (38-57 days after pollination), and ripe (53-72 days after pollination), and stored at temperatures of 5°C and 18°C with a relative air humidity of 95% and 75% respectively, for up to 21 days. The results indicate that the metabolic activities leading to the overall fruit quality changes in pepino during ripening were more and predominantly affected by the genetically determined ripening process in comparison to storage temperatures in the range of 5°C to 18°C. However, the results revealed that pigments and carbohydrates, i.e. chlorophylls, pectic substances, monosaccharides and disaccharides, belong to those fruit quality attributes which vary to some extent depending on the storage temperature, specifically at high temperatures of 18°C. This indicates a pronounced temperature effect on the dynamics of fruit quality. Storing pepinos at lower temperature (5°C) with a high relative air humidity (95 %) appeared to limit the loss of most of the fruit quality attributes without resulting in chilling injuries.

Key words: Pepino, ripening stages, storage temperature, carbohydrates, ß-carotene, titratable acidity.

Introduction

In recent times pepino (*Solanum muricatum* Ait.), also called pepino dulce or melon pear, has raised an increasing market interest due to its aromatic, sweet, juicy, and nutritive value on the exotic fruit markets of New Zealand, Australia, Israel, Japan, Europe and North America[1,3]. Due to long distance transportation, pepino quality in terms of sensory and nutritional attributes and shelf life is often insufficient when arriving at the wholesale or retail market and does not correspond with consumer requests[4]. Recommendations are still required for choosing the optimum ripening stage at harvest in combination with fruit stage adequate storage temperatures in order to prolong the marketing period with a consumer oriented high sensory, nutritional, and functional quality. Moreover, investigations on storage conditions for pepino of different ripening stages are contradictious[5-7]. The objective of the present study was to achieve basic knowledge about the effects of different ripening stages and storage temperatures on characteristic external and internal quality attributes as well as the storability of pepino fruits.

Materials and Methods

Pepino plants cv. 'Golden Globe' which originated from Columbia were grown from January to October 1998 in a greenhouse located at the experimental station of Humboldt University in Berlin, Germany. One plant per pot (5 L) was thinned to three stems supported individually by vertical strings. All lateral shoots were removed. The plants were daily drip irrigated and after five weeks of planting fertigated weekly with 200 L of 0.2% nutrient solution of 'Combiflor Fertilizer' (ADB GmbH, Germany) which contained of N, P_2O_5, K_2O, and micronutrients (B, Cu, Fe, Mn, Mo, and Zn) in ratio of 8%, 8%, 6%, and 0.06%, respectively. When the plants produced flowers, the amount of the soluble fertilizer applied was reduced to one-third for improving fruit set. Pepino fruits at three ripening stages, premature (28-42 days after pollination), mature (38-57 days after pollination), and ripe (53-72 days after pollination) were harvested based on the fruit characteristics as described by Huyskens-Keil et al.[4] . Further harvesting criteria were the uniformity in colour, shape and size (250-350 g in weight and 60-90 mm in diameter) for each ripening stage and freedom of defects and injuries. At each ripening stage, 60 fruits were harvested randomly and divided into five groups (12 fruits each) for analytical measurements. One group of pepinos (12 fruits) was used as initial (without storage treatment) for which analysis measurements was carried out directly after harvest. Three groups of fruits were stored at temperature of 5°C and 18°C with relative air humidity of 95% and 75% respectively for up to 21 days. The 5°C-treatment was chosen in order to examine the chilling sensitivity of pepinos, whereas the high temperature of 18°C was applied to simulate typical climate conditions during marketing. After storage period of 14 and 21 days, twelve fruits per treatment were taken out from storage for analysis purposes and for colour measurement. Different fruit quality characteristics, such as dry weight, chlorophylls and ß-carotene, pectic substances, mono- and disaccharides, titratable acidity and the skin ground colour were examined directly after harvest and after 14 and 21 days of storage in order to comprehensively describe the changes in the postharvest dynamics of pepino during storage. For analysis of the compounds three fruits per treatment were flushed with liquid nitrogen and kept at -30°C.

Fruit skin colour: Fruit colour was measured with a Minolta LR 321 colourimeter (Minolta Camera Co., Osaka) using a standardized light type D65. Calibration was conducted using a white standard tile. Colour measurements were expressed in the L*a*b* scale, where L* indicates the luminescence, a* represents the green-red colour axis and b* the blue-yellow axis. Colour was recorded on each of six fruits per treatment. Eight colour measurements were made equatorial for each fruit and the function for chroma was computed from the recorded a* and b* values as follows[8]:

Chroma $C = [(a^*)^2+(b^*)^2]^{1/2}$

Pigments: Pigments like chlorophylls and carotenoids were determined according to the method developed by Goodwin[9]. The average content of chlorophyll a, chlorophyll b and ß-carotene per treatment were computed on two replicates for each experiment.

Pectic substances: Cell wall extraction of pepino fruits was conducted as described by McComb and McCready[10] and modified by Huyskens[12]. The alcohol-insoluble solids (AIS) was fractionated into three pectin fractions, the water-soluble pectin fraction (WSP), the EDTA-soluble pectin fraction (EDTA-SP), the insoluble pectin fraction (ISP) and the total pectic substances (TSP) according to the method described by Blumenkrantz and Asboe-Hansen[11]. The colourimetric determination of the pectin fractions was conducted using metahydroxybiphenyl (MHDP, Sigma H 6527) as a colour reagent and following the method described by McComb and McReady[10] . The amount of galacturonic acid was measured in each fraction photometrically at 520 nm. Analyses were performed in two replicates for each treatment.

Dry weight: The determination of dry weight was conducted according to the method described by Maier[13] where samples were dried at a temperature of 103°C until they reach constant weight. The measurement of dry weight was performed on three fruits per treatment with two replicates. The results were expressed as percent of fresh weight.

Storage and transport carbohydrates: Transport and storage carbohydrates like glucose, fructose and sucrose were analysed enzymatically[14] in freeze-dried fruit tissues. Analyses were performed in two replicates per treatment.

Titratable acidity: Titratable acidity was determined by titrating the juice samples of six fruits with 0.1 N NaOH up to pH 8.2[15] and expressed as mg g FW^{-1}. The procedures per treatment were conducted with two replicates.

Statistical analysis: Analyses of variance (ANOVA) of the main effects and the standard error were obtained using SPSS (Version 7.5). Presented data are the means of six fruits per treatment. Chemical assays were performed in duplicate, except pectin determination. Samples for pectin analysis were measured in triplicates. Each colour data point represents 48 measurements. Since the third week of the experimental storage period showed no significant quality changes in pepino fruits, the results after the 14-day storage will be represented and discussed in the following.

Results and Discussion

Colour is the most obvious change that occurs in many fruits and is often the major criterion used by consumers to determined whether the fruit is ripe or unripe[16]. As ripening progressed, skin ground colour of pepino changed from green to dark yellow. The yellowing of the skin ground colour during pepino ripening was indicated by increases in chroma. As ripening progressed, the chroma of pepino fruits increased significantly, mainly from the premature to the mature stage (Fig. 1). The effects of storage temperatures on increase in chroma were found in fruits of the premature and the mature stage, at which the 18°C-treatment showed tendentiously higher chroma values than at the 5°C-treatment and the initial. At the ripe stage, the fruits stored at 18°C showed tendentiously lower chroma than those stored at 5°C, having almost at the same value as fruits of the initial. The changes in skin ground colour of pepinos were associated with changes in chlorophylls and carotenoids of the fruits. During ripening pepino fruits showed the degradation of chlorophyll a (blue-green) and an increase of chlorophyll b (yellow-green) (Table 1) leading to a rising chlorophyll ratio (chl b/chl a) promoting the colour change from green to yellow (Fig. 2). Following the degradation of chlorophyll, the carotenoid pigments become visible. One of the most abundance and important carotenoid in most fruits including pepino is ß-carotene. As the ripening progressed from the premature to the mature stage, the contents of ß-carotene increased significantly and remained constant as the fruits reached the ripe stage (Fig. 3). The tendentiously increasing content of ß-carotene at 18° C after 14 days storage is a typical characteristic of the progressive ripening process[17-19]. Pepino fruits stored at 18°C performed tendentiously higher chlorophyll ratios than those stored at lower storage temperature of 5°C (Fig. 2) and higher contents of chlorophyll a and b than the initial (Table 1). During the short-term storage at 5°C chlorophyll a and b showed no degradation or accumulation (Table 1). Similar results have been reported for other crops[19-26]. This effect might have been caused by the temperature induced activity reduction of the synthesising enzymes for the formation of g-aminoevulinate as precurser of chlorophyll[27] and degrading enzymes such as chlorophyllase and Mg-dechelatase[17, 19]. In contrast, at the 5° C short-term storage the ß-carotene content increased (Fig. 3). This indicates that the activity of enzymes catalyzing the ß-carotene biosynthesis - phytoene synthase and ß-carotene synthase[28] – are not suppressed by low temperatures particularly in fruits in the premature and mature stage. The tendentiously higher chlorophyll ratio and ß-carotene contents at 18°C demonstrate that temperature only slightly effects the pigment synthesis in comparison to the genetically determined ripening process.

Softening is one of the most significant quality alterations in pepino[29-31]. The content of total pectic substances in pepino fruits remained unchanged during ripening (data not shown), however, changes in total pectic substances were not associated with changes in physical measured textural properties, i.e. fruit firmness ($r^2 > 0.50$) as it was also found in other crops[23, 33]. However, the pectic ratio as ratio of the water-soluble pectin to the insoluble pectin, however, showed a strong correlation to pepino fruit firmness ($r^2 = 0.87$). Fruit softening characterized by reduction in fruit firmness is reported to be associated with the breakdown of pectic substances and hemicelluloses in the middle lamella which weakens cell walls and reduces the cohesive forces binding cells together[17]. The degradation of pectic substances is influenced highly by the activities of some cell-wall degrading enzymes such as pectinmethylesterase and polygalacturonase. Activities of both enzymes increase during fruit ripening including pepino[30]. As ripening progresses, insoluble pectin in the cell walls is converted into soluble pectin by the action of cell wall hydrolases, indicated by the decrease in the content of insoluble pectin and the increase in water-soluble pectin. The results showed that changes in pectic substances, characterized by an increase of the pectic ratio, were evident during ripening (Fig. 4). The increase in water-soluble pectin was more pronounced in ripe fruits than that in less mature fruits. This suggested that pepino fruit at a progressed ripening stage may contain a higher activity of cell wall degrading enzymes contributing to tissue softening processes. Almost similar observations have been reported by O'Donoghue et al.,[34]. During 14 days of storage a rising pectic ratio was found at 18°C, assumingly due to the temperature promoting effect on the pectolytic enzyme activity[35]. At 5°C the ratio remained unchanged in comparison to the initial values at harvest (Fig. 4). Both storage temperatures showed no difference in the loss of dry weight. However, as pepino fruits ripened the dry weight decreased continuously and ripe pepino fruits were marked by a lower dry weight than premature pepinos (Fig. 5). This indicates that the metabolic activities were mainly dominated by genetic factors determining the ripening process than by the storage temperature. The main storage carbohydrate in pepino at the premature stage was fructose (Table 2). The content of glucose and fructose declined during ripening, whereas sucrose showed a sharp increase as ripening progressed (Table 2), thus leading to a higher ratio of disaccharides to monosaccharides in ripe pepino fruits in comparison to physiological younger fruits (Fig. 6). It is assumed that the increase in sucrose content is caused by a reduction in acid invertase activity associated with normal levels of sucrose synthase. A similar fact was reported for some wild tomato species and muskmelon[36]. Moreover, it is assumed that monosaccharides in young pepino fruits, mainly glucose, might have been already used as primary respiration substrates. Therefore, the ratio of disaccarides to monosaccarides might be a good indicator of physiological processes during ripening. The characteristic changes in storage carbohydrates during fruit development indicates that pepino can be considered as a sucrose accumulator fruit[37, 38]. Storage temperature of 18°C led tendentiously to an increased ratio of disaccharides to monosaccharides in comparison to the 5°C storage, revealing a temperature promoting effect on ripening processes as it was found in other fruits[39, 40]. The results of our study indicate that the metabolism of storage carbohydrates is predominantly determined by genetic parameters. The main organic acids in pepino fruits are citric, malic and tartaric acid[4,41]. Titratable acidity decreased significantly as pepino fruits ripened from the premature to the mature stage and remained unchanged until it reached the ripe stage (Fig. 7). These results are unlike other fruits, e.g. tomato, banana and pineapple[18, 42, 43]. Except for the premature stage, the fruits stored at 18°C tended to have lower titratable acidity compared to the other treatments (the initial and the 5°C storage) indicating a temperature induced senescence effect. Significant differences in titratable acidity were found only between the initial and the 18°C storage of fruits in the premature and mature ripening stage (Fig. 7). This is due to the increase of malic acid while citric acid content decreased[4].

Conclusions

The results indicate that the metabolic activities and hence the quality changes in pepino fruits during ripening were more affected by genetic parameters rather than by storage temperature in the range of 5°C to 18°C. The results revealed that chlorophylls as well as cell wall, transport and storage carbohydrates belong to those fruit quality attributes that vary to some extent due to storage temperature, especially at high temperatures of 18°C, indicating temperature effects on fruit physiological processes. Storing pepinos at lower temperature (5°C) with high relative air humidity (95 %) appearently limit the loss of most fruit quality attributes without showing chilling injuries. These results also demonstrate that during storage, even at low temperatures, pepino fruits still continue to synthesize carotenoids and thus yellowing of the skin colour. Similar results had been also reported in loquat fruits by Ding et al.,[44].

References

[1] Burge, G.K., 1989. Fruit set in the pepino (*Solanum muricatum* Ait.). Sci. Hort. **41**:63-68.

[2] Prohens, J., Ruiz, J., and Nuez, F. 1996. The pepino (*Solanum muricatum*, Solanaceae): A "new" crop with a history. Econ. Bot. **50**:355-368.

[3] Gonzalez, M., Cámara, M., Prohens, J., Ruiz, J., Torija, E., and Nuez, F. 2000. Colour and composition of improved pepino cultivars at three ripening stages. Gartenbauwiss. **65**:83-87.

[4] Huyskens-Keil, S., Widayat, H.P., Lüdders, P., Schreiner, M., and Peters, P. 2000. Physiological changes in pepino fruits during ripening. Acta Hort. **531**:251-255.

[5] Lizana,L.A., and Levano,B. 1977. Caracterizacion y comportamiento de post-cosecha del pepino dulce *Solanum muricatum* Ait. Proc. Reg. Am. Soc. Hortic. Sci. **21**:11-15.

[6] Dennis, D.J., Burge, G.K., and Lill, R.1985. Pepinos: cultural techniques. Information Services, Ministry of Agriculture, Wellington, N.Z.2 p.

[7] Ahumada, M., and Cantwell, M. 1996. Postharvest studies on pepino dulce (*Solanum muricatum* Ait.): maturity at harvest and storage behavior. Postharvest Biol. Technol. **7**:129-136.

[8] McGuire, R. 1992: Reporting of objective colour measurements. HortSci. **27**:1254-1255.

[9] Goodwin, T.W. 1976. Chemistry and Biochemistry of Plant Pigments. 2nd ed. London, New York, San Francisco: Academic Press. pp. 67-72.

[10] McComb, E., and McCready, R. 1952. Colourimetric determination of pectic substances. Anal. Biochem. **24**:1630-1632.

[11] Blumenkrantz, N., and Asboe-Hansen, G. 1973. New method for quantitative determination of uronic acids. Anal. Biochem. **54**:484-489.

[12] Huyskens, S. 1991. Morphological, physiological and biochemical aspects in the cultivation of two pantropical cucurbits: *Luffa acutangula* L., Roxb. and *Momordica charantia* L. Thesis, Bonn, Germany.

[13] Maier, H.G. 1990. Lebensmittel- und Umweltanalytik - Methoden und Anwendungen. Darmstadt: Steinkopf Verlag. 329 p.

[14] Boehringer Mannheim. 1986. Methoden der enzymatischen Lebensmittelanalytik. Boehringer Mannheim GmbH Mannheim, Germany.

[15] AOAC 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

[16] Kays, S. 1999. Preharvest factors affecting appearance. Postharvest Biol. Technol**.** **15**:233-247.

[17] Wills, R., Lee, T., Graham, D., McGlasson, W., and Hall, E. 1998. Postharvest, an introduction to the physiology and handling of fruit and vegetables. Sidney: UNSW Press Australia. 262 p.

[18] Kays, S. 1991. Postharvest physiology and handling of perishable

plant products. New York: Van Nostrand-Reinhold. 532 p.
[19] Gross, J, 1991. Pigments in vegetables. New York: Van Nostrand-Reinhold. 351 p.
[20] Goodenough, P., and Thomas, T. 1980. Comparative physiology of field-grown tomatoes during ripening on the plant or retarded ripening in controlled atmospheres. Ann. Appl. Biol. **94**:445.
[21] Yamauchi, N., and Watada, A.E. 1991. Regulated chlorophyll degradation in spinach leaves during storage. J. Amer. Soc. Hort. Sci. **116**:58-62.
[22] Zagory, D., and Kader, A.A. 1993. Modified atmospheres – an indexed reference list with emphasis on horticultural commodities. Supplement 6 University of California, Davis. Postharvest Hort Series 7.
[23] Herregods, M, 1995. Preservation of the quality and nutritional value of fruit and vegetables by CA storage. Acta Hort. **379**:321-328.
[24] Salveit, M. 1997. A summary of CA and MA requirements and recommendations for harvested vegetables. CA'97 Proceedings Volume 4: Vegetables and ornamentals. University of California, Davis. Postharvest Hort Series **18**:98-117.
[25] Kader, A.A. 1997. Biological bases of O_2 and CO_2 effects on postharvest-life of horticultural perishables. In: Mitcham, E.J. (ed.). Seventh International Controlled Atmosphere Research Conference. Volume 2: Apples and Pears. University of California, Davis: Postharvest Horticulture Series 16. p. 160-163.
[26] Adamicki, F. 1997. Effect of ultra low oxygen on the storage and quality of some vegetables. In: Saltveit, M. (ed.). Seventh International Controlled Atmosphere Research Conference. Volume 4: Vegetables and Ornamentals. University of California, Davis: Postharvest Horticulture Series 18. p. 26-33.
[27] Ignatov, N.V., and Litvin, F.F. 2001. Biosynthesis of chlorophyll of photosystem II reaction centers in plant leaves in early stages of etiolation. Biochem Moscow **66**:195-204.
[28] Fraser, D., Romer, S., Shipton, C.A., Mills, P.B., Kiano, J.W., Misawa, N., Drake, R.G., Schuch, W., and Bramley, P.M. 2002. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. PNAS **99**: 1092-1097.
[29] El-Zeftawi, B.M., Brohier, L., Dooley, L., Goubran, F.H., Holmes, R., and Scott, B. 1988. Some maturity indices for tamarillo and pepino fruits. J. Hort. Sci. **63**: 163-169.
[30] Heyes, J.A., Blaikie, F.M., Downs, C.G., and Sealey, D.F. 1994. Textural and physiological changes during pepino (*Solanum muricatum* Ait.) ripening. Sci. Hort. **58**: 1-15.
[31] Lopez, S., Maroto, J.V., San Bautista, A., Pascual, B., and Alagarda, J. 2000. Qualitative changes in pepino fruits following preharvest applications of ethephon. Sci. Hort. **83**: 157-164.
[32] Huyskens-Keil, S., Schreiner, M., and Widell, S. 1998. Qualitätssicherung bei Radies. TASPO-Gartenbaumagazin **2**: 40-41.
[33] McGarry, A. 1995. Cellular basis of tissue toughness in carrots (*Daucus carota* L.) storage roots. An. Bot. **75**:157-163.
[34] O'Donoghue, E., Somerfield, S.D., de Vré, L.A., and Heyes, J.A. 1997. Developmental and ripening-related effects on the cell wall of pepino (*Solanum muricatum*) fruit. J. Sci. Food Agric. **73**: 455-463.
[35] Tijskens, L., and van Dijk, C. 2000. Enzyme activity and firmness in tomatoes. In: Florkowski, W. et al. (eds.). Fruit and vegetable quality: an integrated view. Lancaster: Technomic Publishing INC. p. 73-80.
[36] Klann, E., Yelle, S., and Bennett, A.B. 1992. Tomato fruit acid invertase complementary DNA. Plant Physio**l. 99**:351-353.
[37] Schaffer, A.A., Rykski, I., and Fogelman, M. 1989. Carbohydrate content and sucrose metabolism in developing *Solanum muricatum* fruits. Phytochem. **28**:737-739.
[38] Sánchez, M., Cámara, M., Prohens, J., Ruiz, J., Torija, E., and Nuez, F. 2000. Colour characteristics and composition of improved pepino cultivars at three ripening stages. Gartenbauwiss. **65**:83-87.
[39] Nunes, M., Brecht, J., Morais, A., and Sargent, S. 1995. Physical and chemical quality characteristics of strawberries after storage are reduced by a short delay to cooling. Postharvest Biol. Technol. 6: 17-28.
[40] Kader, A.A. 2002. Postharvest Technology of Horticultural Crops. University of California, Division of Agriculture and Natural Resources, Publication No. 3311.535 p.
[41] Herrmann, K. 1994. Über die Inhaltsstoffe und die Verwendung wichtiger exotischer Obstarten. Die industrielle Obst- und Gemüseverwertung **6**:202-206.
[42] Böttcher, H. 1996. Frischhaltung und Lagerung von Gemüse. Stuttgart: Ulmer. 252 p.
[43] Wills, R., Lim, J., and Greenfield, H. 1984. Changes in biochemical composition of 'Cavendish' banana (*Musa acuminata*) during ripening. J. Food Biochem. **8**:69-77.
[44] Ding, C.K., Chanchin, K., and Ueda, Y. 1997. Effects of polyethylene bag packaging and low-temperature storage on the physical and chemical characteristics of loquat fruits. In: Kader, A.A. (ed.). Seventh International Controlled Atmosphere Research Conference. Volume 3: Fruits Other Than Apples and Pears. University of California, Davis: Postharvest Horticulture Series 17. p.177-184.

Table 1. Chlorophyll a and b of pepino fruits as affected by ripening stage and storage temperature (n ± SE)

Treatment	Chlorophyll a (mg g DW-1)	Chlorophyll a (mg g DW-1)
Premature, Initial	1.26 ± 0.23	1.14 ± 0.50
Mature, Initial	0.83 ± 0.41	1.34 ± 0.27
Ripe, Initial	0.82 ± 0.42	1.49 ± 0.31
Premature, 5°C	1.30 ± 0.25	1.23 ± 0.24
Mature, 5°C	1.32 ± 0.26	1.47 ± 0.22
Ripe, 5°C	1.42 ± 0.09	1.65 ± 0.17
Premature, 18°C	1.32 ± 0.26	1.41 ± 0.15
Mature, 18°C	1.52 ± 0.28	1.78 ± 0.28
Ripe, 18°C	1.45 ± 0.10	1.83 ± 0.32

Table 2. Storage and transport carbohydrate contents of pepino fruits as affected by ripening stage and storage temperature (n ± SE).

Treatment	Fructose (mg g DW-[1])	Glucose (mg g DW-[1])	Sucrose (mg g DW-[1])
Premature, Initial	243.69 ± 2.21	103.49 ± 1.60	240.93 ± 10.51
Mature, Initial	209.69 ± 1.58	111.14 ± 2.65	357.76 ± 9.42
Ripe, Initial	197.02 ± 2.91	116.82 ± 3.50	420.24 ± 5.34
Premature, 5°C	250.16 ± 5.03	108.31 ± 5.10	265.85 ± 10.71
Mature, 5°C	221.74 ± 8.73	118.01 ± 5.86	416.48 ± 3.50
Ripe, 5°C	206.31 ± 5.58	121.87 ± 1.42	458.75 ± 12.80
Premature, 18°C	263.62 ± 0.60	119.42 ± 6.08	305.34 ± 3.12
Mature, 18°C	240.64 ± 6.53	131.39 ± 5.03	478.41 ± 18.73
Ripe, 18°C	224.69 ± 11.01	139.67 ± 6.23	538.91 ± 3.69

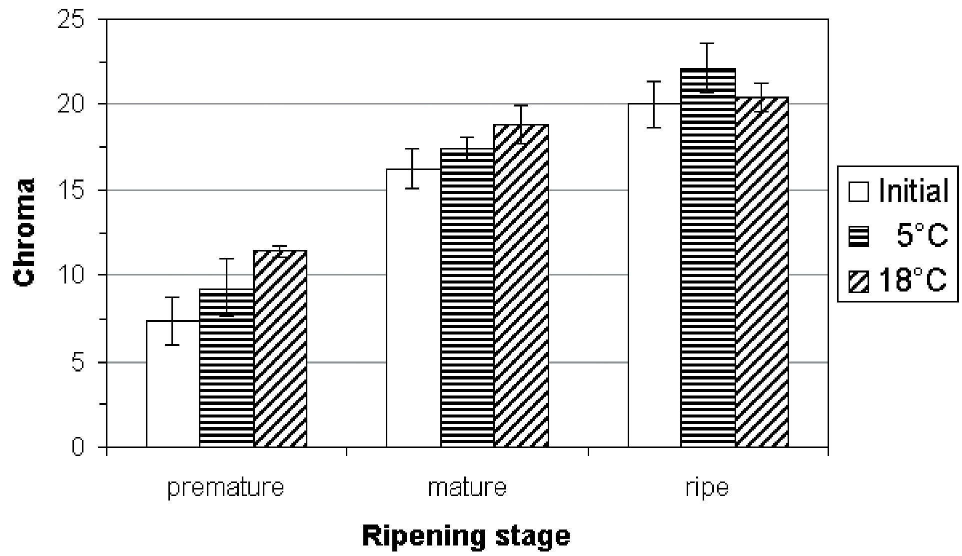

Figure 1. Chroma of pepino fruits as affected by ripening stage and storage temperature.

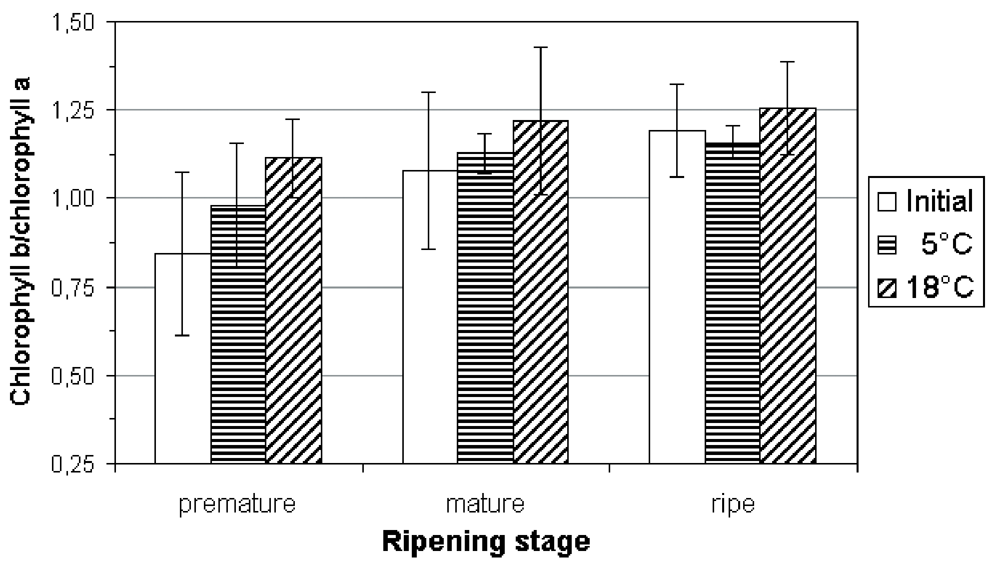

Figure 2. Chlorophyll ratio of pepino fruits as affected by ripening stage and storage temperature.

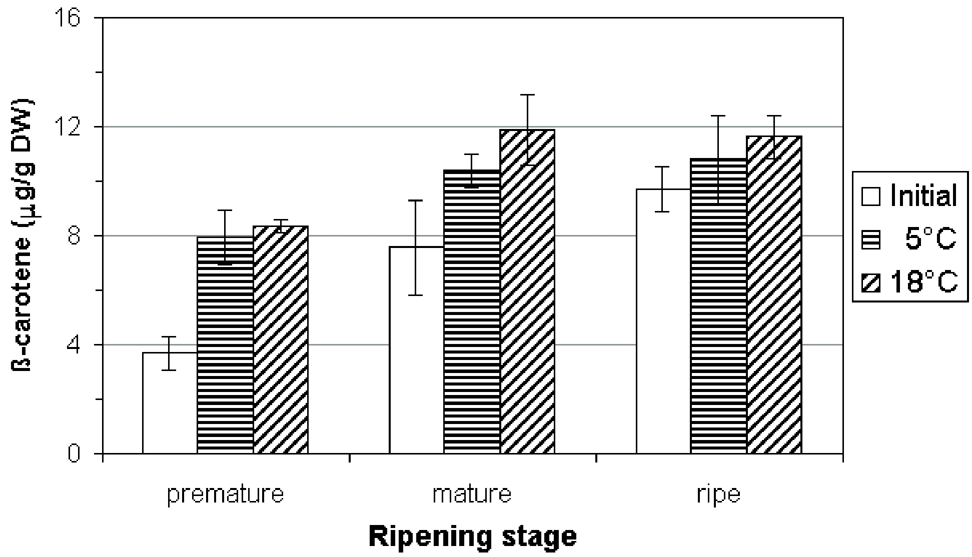

Figure 3. ß-carotene content of pepino fruits as affected by ripening stage and storage temperature.

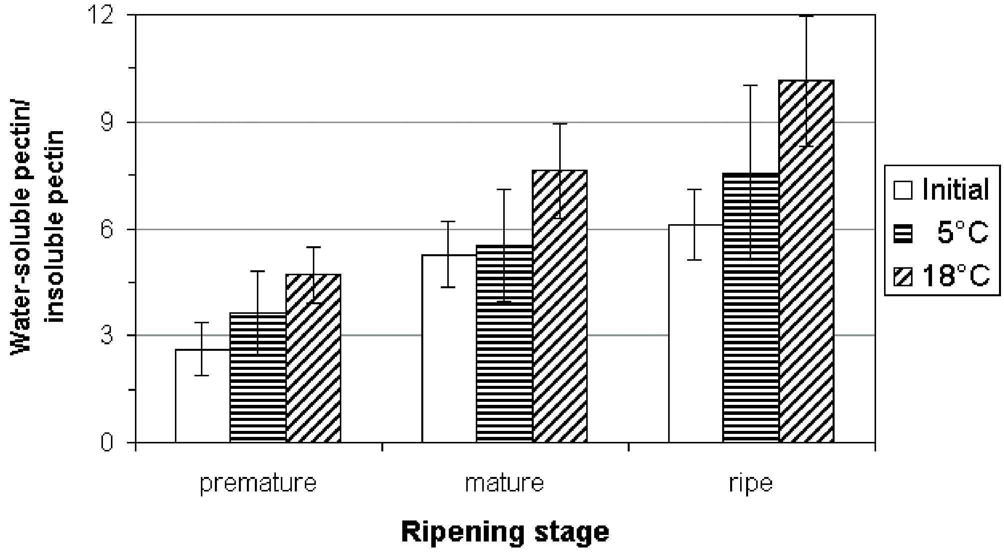

Figure 4. Pectic ratio of pepino fruits as affected by ripening stage and storage temperature.

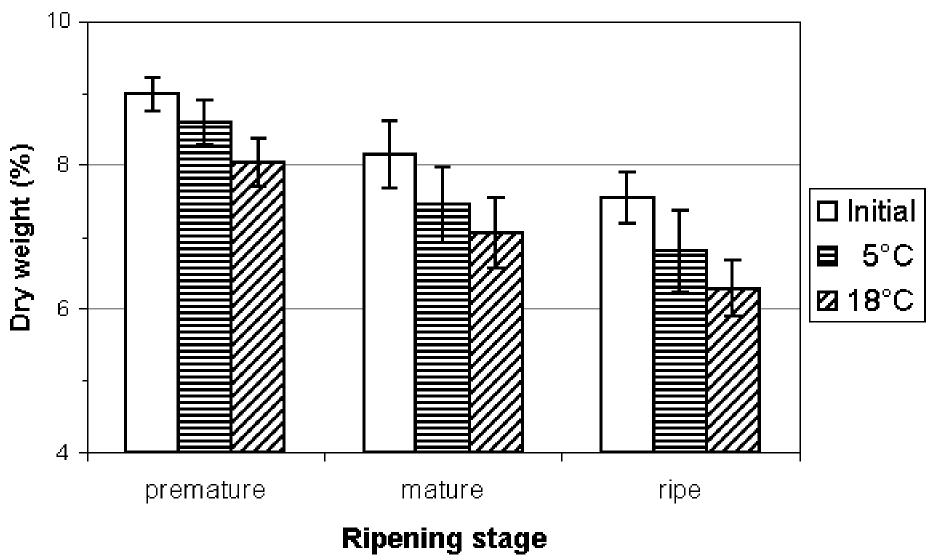

Figure 5. Dry weight of pepino fruits as affected by ripening stage and storage temperature.

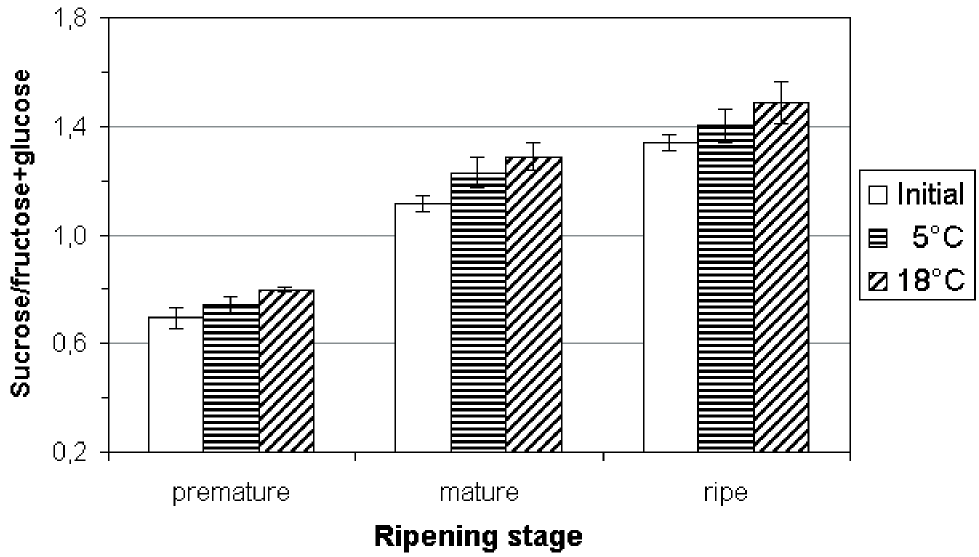

Figure 6. Sugar ratio of pepino fruits as affected by ripening stage and storage temperature.

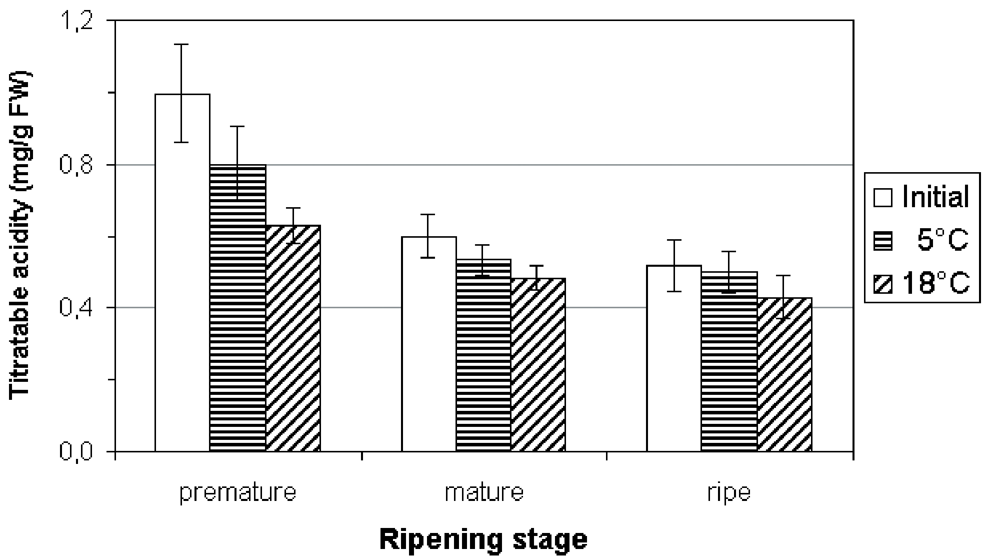

Figure 7. Titratable acidity of pepino fruits as affected by ripening stage and storage temperature.

GM food: The risk- assessment of immune hypersensitivity reactions covers more than allergenicity

Alexander G. Haslberger
Institute for Microbiology and Genetics, University of Vienna, Austria, c.o. Department of food safety, WHO; Geneva , Switzerland. email: alexander.haslberger@univie.ac.at

Received 25 September 2002, accepted 20 December 2002.

Abstract

Allergenicity of genetically modified food (GM food) has become a public concern and international expert panels e.g. WHO/ FAO have depicted decision trees for a rigorous assessment and testing for GM foods, especially where no history of safe use is available. The way to use patient sera for the assessment of allergenicity is still under discussion in cases of proteins where stability and protein sequences may not be conclusive or for potential new allergies. The risk assessment of immune hypersensitivity reactions induced potentially by GM food needs also to consider effects on other type of immune responses e.g. activation of specific immune cell populations. The role of antigen presenting cells of the gut is now understood to direct immune responses resulting in humoral, cellular or IgE predominant characteristics. For GM microorganisms potential effects on the immune system need to be assessed.

Key words: GM food, allergenicity, hypersensitivity, immune cells.

Introduction

No specific international regulatory systems for GM foods safety or GMO environmental safety are currently in place. In the field of environmental safety the Cartagena Protocol on Biosafety may enter into force soon and Codex Alimentarius principles on human health risk analysis are expected to be adopted in 2003. The premise of these Codex principles dictates a premarket assessment, performed on a case-by-case basis and including an evaluation of both direct effects (from the inserted gene) and unintended effects (that may arise as a consequence of insertion of the new gene). The safety assessment of GM foods investigates:

(a) direct health effects (toxicity)
(b) tendency to provoke allergic reactions (allergenicity)
(c) specific components thought to have nutritional or toxic properties
(d) the stability of the inserted gene
(e) nutritional effects associated with the specific genetic modification
(f) any unintended effects which could result from the gene insertion[1].

When new foods are developed by natural methods, some of the existing characteristics of foods can be altered unintentionally, either in a positive or a negative way. New plants developed through traditional breeding techniques may not be evaluated rigorously using risk assessment techniques[2]. In contrast to traditionally developed foods which are not generally tested for allergenicity before market introduction, protocols for testing detrimental immune responses, especially the allergenic potential of GM foods have been established by international expert panels[2].

The Role of the Gut Immune System and Hypersensitivity Responses

Food allergies and other food sensitivities are individualistic adverse reactions to foods because they affect only a few people in the population. Within the different types of reactions involved in adverse reactions to foods non-immunological intolerances (such as reactions to increased contents of histamins or intolerances against lactose) and reactions involving components of the immune system need to be differentiated[3]. In hypersensitivity reactions involving elements of the immune-system it became evident that these reactions are mainly caused by a lack of the induction of a tolerance against components of the foods in specific individuals. While research has delivered a very good understanding for the structural specificities of the protein food components which are often the cause for allergenic reactions, basic mechanisms underlying the reactions are at the focus of present research: Genetic and environmental factors are believed to influence antigen presenting cells, especially dendritic cells and T cell subsets which, using different sets of immune mediators, differentially regulate both, the synthesis of Immunoglobulin E which is the basis for humoral, immediate (or real, Type I) allergic hypersensitivity reactions and cellular reactions involving sensitized or self reactive T cells (delayed type, hypersensitivity reactions). Antigen presenting cells belong to the gut associated lymphoid tissue (GALT). Immature dendritic cells reside in the epithelia also of the gut and have the potential to sense foreign antigens. Following recognition and uptake of Ag, mature dendritic cells provide signals which polarize Th0 cells into Th1 or Th2 cells, the basis for humoral or cellular immune-responses as well as decisions for the production of enhanced IgE[4]. Systemic immune responses to soluble oral antigens are most likely induced by gut-conditioned dendritic cells that function both to initiate the gut-oriented response and to impart the characteristic features that discriminate it from responses induced parenterally[5]. Also the differential stimulation of cytokines effecting immune responses and activation of the immune system was shown in intestinal epithelial immune cells using non-pathogenic *E. coli* and *Lactobacilli*[6]. Specific microbes in the gut microflora and sporadic infections are so thought to be important in allergy prevention. The gastrointestinal microflora promotes potentially antiallergenic processes such as TH1-type immunity, suppression of TH2-induced allergic inflammation, induction of oral tolerance and IgA production. The gut microflora might therefore be a major postnatal counter-regulator of the universal TH2-skewed immune system in fetuses and neonates[7]. Because of its role to serve as a barrier to pathogenic bacteria and to enable an immune surveillance of the antigenic environment the local mucosal immunity of the gut is of a central importance for health. Antigens, primarily associated with intestinal microbes and dietary antigens, can stimulate production of IgA in the intestine resulting in local protective immunity. Because of its role for a stimulation and regulation of immune responses the gut has become a favourite system for developments and techniques to interfere with modified or functional foods or vaccines including DNA vaccines [8,9,10].

GM Food and Hypersensitivity

Molecular biology and biochemistry have significantly increased the knowledge of the nature of allergens. However, only limited information about specific properties of food allergens is presently available. The majority of known plant food allergens belong to seed storage proteins, protease and amylase-inhibitors, profilins or pathogenesis-related (PR) proteins. Less variety is found among allergenic proteins derived from animal sources. For allergic reactions it became clear that plant food allergens belong almost exclusively to one of two structurally related protein superfamilies, which share remarkable stability to processes such as heating (being stable to temperatures between 75-95ºC, compared with 45-50ºC for most proteins), and the extremes of pH and the proteolytic processing environment found in the digestive tract. These proteins mainly come from foods or food groups often referred to as "The Big Eight" which account for more than 90-percent of all Type I allergic reactions worldwide. These Big Eight are; milk, eggs, fish, crustacean shellfish, peanuts, soybeans, tree nuts and wheat [11]. In response to pathogens, plants synthesize and accumulate a variety of proteins which are part of a plants defence system. As plant protection against bacteria, fungi, viruses and insects is a major challenge to agriculture world-wide, over-expression of such proteins in transgenic plants has been applied to increase the defense potential. Some of these proteins which are considered for use in the production of GMOs to increase the resistance to microbial and insectal attack include proteins with allergenic potential e.g. chitinases providing protection against fungal attack or insecticidal proteins including protease inhibitors[12] .

Risk Assessment of Allergenicity

An assessment of the potential allergenicity of GM foods typically follows the generally well known decision-tree process which depends from the source of the genes transferred as outlined by international expert panels [3, 13, 14, 15]. The most difficult assessment occurs when genes are obtained from sources with no history of allergenicity, such as viruses, bacteria or non food plants. The likelyhood that the proteins derived from such sources of DNA will be allergens is not very high, since most proteins in nature are not allergens. The key features of the allergenicity assessment for such foods than again involves a comparison of the amino acid sequence of the introduced protein with the amino acid sequences of known allergens and the digestive stability of the introduced protein. While the combination of these two criteria provides reasonable assurance that the introduced protein has limited allergenic potential, the ideal approaches to the application of these two criteria have been debated, and the desirability of adding other criteria for the allergenicity assessment of such products and additional testing has been advocated [14]. The development of additional criteria and additional tests to use in the assessment of the allergenicity of GM foods would be advantageous in cases where the gene is obtained from sources with no history of allergenicity. The level of expression of the introduced protein and the functional category of the introduced protein could be used as additional criteria. In addition, the development of suitable animal models for the prediction of the allergenic potential of the introduced proteins is anticipated in the future. While several animal models appear to be promising , none has been sufficiently validated for its routine use in the assessment of the allergenicity of GM foods. It must also be realised that the absence of sequence similarity with allergenic protein-epitopes and a missing stability against digestion does not necessarily prove for a missing allergenicity as examples are known which contradict to the general rules: Highly homologous sequences with allergens in case of allergen-isoforms have been shown without any allergenicity. Furthermore, proteins with a low stability have been shown to exhibit a significant potency to induce allergenicity or to sensitize for allergic reactions[17-19]. The use of patients sera for the testing of allergenicity is therefore recommended[20] .There is also some discussion if the generally agreed system is sensitive enough to detect upcoming new allergies in time. It is likely that the first manifestations of a new allergy will occur in pre-existing adult allergic individuals and could occur as a consequence of cross-reactivity. A screening programme may be desirable to predict such cross-reactivities by employing patients sera, however, the number of sera that would need to be screened may need to be much larger than that hitherto recommended in international documents [21].

Risk Assessment of Cell Mediated Reactions and Microbial Impact on the Immune System

Although the well characterised interactions which lead to allergic immediate hypersensitivities may comprise the fare most important food derived hypersensitivity problems, the role of other type of responses, and their relevance for food hypersensitivity in general and for the safety assessment for foods from GM organisms specifically remain more unclear. Adverse food reactions are discussed and also need to be taken in mind in the assessment of GM-foods[22]. Such reactions could comprise delayed type hypersensitivity reactions which have been characterised to develop slowly, reaching a peak at approximately 48 hours and then slowly subsiding over 72-96 hours. They are known to involve cell mediated responses without important IgE involvement. Also reactions to cow milk, soy proteins, eggs etc known in infants and children, Celiac disease and Crohns disease show missing tolerance and mislead activation of T cells[23]. Potential immune-stimulatory or immune-modulatory effects of GM microorganisms (GMMs) used as or in foods are a specific area of a risk assessment which evaluates immune responses to GM organisms. GMMs may establish themselves within the GI tract and exert influences on the immune system via interactions with the gut immune system. Even non-viable microorganisms are known to retain functional properties (i.e. cell adhesion, binding of chemicals, immunomodulating activities), which can have direct or indirect effects on both microflora- and host associated functions[24]. Gut-associated lymphoid tissue (GALT) has important interactions with the immune system and it is well established that microbial stimuli are the main antigenic forces in the development and maintenance of GALT and acquired immunity[24]. Stimulation of antigen presenting, dendritic cells influencing immune type responses was shown for bacterial cell walls before[25] Potential safety relevant consequences from rare, but possible uptake of recombinant DNA from GM food by cells of the immune system remain to be investigated [26-28].

Conclusions

In general, it seems that the present discussion on GM food safety, especially in the field of hypersensitivity reactions does not so much point towards a significantly increased safety problem of GM foods compared with conventional foods, but it reflects increased regulatory demands as well as perceived needs for a higher safety level. Conventional foods often have not been subject of hypersensitivity assessment. Public awareness as well as genuine scientific considerations in the field of GM foods has resulted in general guidelines being elaborated for allergenicity assessment of such foods. These internationally agreed guidelines are specifically important for foods which are traded globally. Standards established for the assessment of GM foods may then turn out to be a paragon for the testing of conventional foods. The detailed analysis of immune mechanisms involved in the stimulation of different type of immune responses has revealed complex ways and some of these ways are still poorly understood, such as pathways resulting in cell mediated hypersensitivity reactions to food. An improved investigation of activation pathways including antigen presenting- and T cells will not only contribute to a better understanding of these reactions but may also result in improved testing methods for allergenicity, where the possibilities for testing, especially of whole foods, in animal models are still limited.

References

1 Codex, 2001. Consideration of proposed draft general principles for the risk analysis of foods derived from modern methods of biotechnology. http://www.who.int/fsf/ GMfood/bt01_05e.pdf

2 WHO, 2002. 20 Questions on GM Foods. http:// www.who.int/ fsf/ GMfood/q&a.pdf

3 IFT. 2001. Food Allergies and other Food sensitivities, expert panel on food safety and nutrition: Institute of food technologists. http://www.ift.org/publications/sss/allergens.pdf

4 Lambrecht B.N. 2001. Allergen uptake and presentation by dendritic cells : Curr Opin Allergy Clin Immunol **1**:51-59

5 Alpan O., Rudomen G., Matzinger P. 2001. The role of dendritic cells, B cells, and M cells in gut-oriented immune responses. J. Immunol **166**:4843-4852

6 Haller D., Bode C., Hammes W.P., Pfeifer A.M., Schiffrin E.J., Blum S. 2000. Non-pathogenic bacteria elicit a difcytokine response by intestinal epithelial cell/leucocyte co-cultures. Gut 47:79-87

7 Kalliomaki M., Salminen S., Arvilommi H., Kero P., Koskinen P., Isolauri E. 2001. Probiotics in primary prevention of atopic disease: a randomised placebo- controlled trial. Lancet 357:1076-1079

8 Johnson I.T.,2001. New food components and gastrointestinal health. Proc Nutr Soc.6:481-488. Review.

9 Bouvet JP., Decroix N., Pamonsinlapatham P. 2002. Stimulation of local antibody production: parenteral or mucosal vaccination? Trends Immunol. **23**:209-213. Review.

10 Howard K.A., Alpar H.O. 2002. The development of polyplex-based DNA vaccines. J. Drug Target.**10**:143-151

11 Institute of food research, 2002. Food allergy.http://www.ifr.bbsrc.ac.uk/Diet/Immunology.html

12 Bindslev-Jensen C., Ebner C., Madsen C., Mäkinen-Kiljunen S., Peltre G., Poulsen L.K., van Ree R., Viets S. 2000. Genetically modified foods and allergenicity., preliminary Position Paper. European Academy for Allergology and Clinical.Immunology, http://www.ig-food.org/seiten/position2k.htm

13 Metcalfe, D.D., Astwood, J.D., Townsend, R., Sampson,H.A., Taylor, S.L., and Fuchs, R.L. 1996. Assessment of the allergenic potential of food derived from genetically engineered crop plants. Crit. Rev. Food Sci. Nutr. **36**: 165-186

14 WHO/FAO 2000. Second Joint WHO/FAO Expert Consultation on Foods Derived from Biotechnology, Safety aspects of genetically modified foods of plant originhttp://www.who.int/fsf/GMfood/FAO-2000.pdf

15 WHO/FAO 2001. Expert Consultation on Allergenicity of Foods Derived from Biotechnology, http://www.who.int/fsf/Documents/Biotech_Consult_Jan2001/ report20

16 IFT 2000. Expert Report on Biotechnology and Foods:Human Food safety Evaluation of rDNA Biotechnology- derived Foods, Food technology **54**: 15-23 http:// www.ift.org/publications/ docs hop/ft_shop/09-00/ 09_00_pdfs/09-00-bio-safety.pdf

17 Ferreira F., Hirtenlehner K., Jilek A., Godnik-Cvar J., Breiteneder H., Grimm R., Hoffmann-Sommergruber K., Scheiner O., Kraft D., Breitenbach M., Rheinerger H. J., Ebner C. 1996. Dissection of immunoglobulin E and T lymphocyte reactivity of isoforms of the major birch pollen allergen Bet v 1: potential use of hypoallergenic isoforms for immunotherapy. J. Exp.Med.**183**: 599-609.

18 Heiss S., Fischer S., Muller W D., Weber B., Hirschwehr R., Spitzauer S., Kraft D., Valenta R. 1996. Identification of a 60d cross-reactive allergen in pollen and plant-derived food. J. Allergy Clin . Immunol. **98**:938-947.

19 Vrtala S., Fischer S., Grote M., Vangelista L., Pastore A., Sperr W R ., Valent P., Reichelt R., Kraft D., Valenta R . 1999. Molecular, immunological, and structural characterization of Phl p 6, a major allergen and P-particle-associated protein from Timothy grass (*Phleum pratense*) pollen. J. Immunol.**163**:5489- 5496.

20 Valenta R.2002. p. comm.

21 Warner J. 2002. p. comm.

22 Baldwin J.L. 1997. Pharmacologic food reactions. In: Metcalfe DD, Sampson HA, Simon RA. Food Allergy: Adverse reactions to foods and food additives 2nd ed. Blackwell science, p. 419-429.

23 Janeway C.A.2001.Immunobiology, New York : Garland; Edinburgh : Churchill Livingstone, Edition 5th ed., Biology Publications. http://www.bmb.leeds.ac.uk/illingworth/icu3/ lecture/09/

24 WHO/FAO expert consultation, 2001.Safety assessment of foods derived from genetically modified microorganisms. Report of a Joint WHO/FAO expert consultation. http://www.who.int/fsf/Documents/GMMConsult_Final_.pdf

25 Haslberger A.G., Kohl G., Felnerova D., Mayr U.B., Fuerst- Ladani S., Lubitz W. 2000. Activation,stimulation and uptake of bacterial ghosts in antigen presenting cells. J. Biotechnol. **83**: 57-66

26 Schubbert, R., Renz, D., Schmidt, B. and Dorfler, W.1997. Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen and liver via the intestinal wall mucosa and can be

covalently linked to mouse DNA. Proceedings of the National Academy of Sciences, USA **94**: 961–966.

[27] Hohlweg, U. and Doerfler, W. 2001. On the fate of plant or other foreign genes upon the uptake of food or after intramuscular injection in mice. Molecular Genetics and Genomics **265**: 225–233.

[28] Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser, R.,Schwägele, F., Jahreis, G., Flachowsky, G. 2001. The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material.European Food Research and Technology **212**: 129–134.