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Advancing analytical microbiology in the dairy industry

Jing Geng and Mickaël Boyer, Danone Nutricia Research

Beer flavour stability

Patricia Aron, MillerCoors

NIRS to detect contaminants in food and feed

Vincent Baeten, Quality Department of Agricultural Products, CRA-W

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Quality matters

Today's consumer expectations are greater than ever before – they expect their food to be safe, of high quality and of reasonable value. And with technology and research accelerating at an unprecedented rate, it's an exciting time for the food and beverage industry. I am delighted to have joined *New Food* at such an important time and will continue to bring you in-depth coverage of the issues that are shaping the sector.

Quality control is one of the key themes of this issue, and in our supplement starting on page 15, we look at two principal methods of detection. Vincent Baeten of CRA-W in Belgium examines the use of NIRS to detect contaminants and foreign bodies in food and feed, while Monika Hohmann of the Bavarian Heath and Food Safety Authority looks at quantitative determination of taurine in energy drinks using ^{1}H NMR spectroscopy. Authentication plays a crucial role in the food and beverage manufacturing process, and with continuous improvements in spectroscopic techniques, identification is becoming quicker and more cost-effective than using wet chemistry alone. It will be interesting to see what new developments arise in the coming months.

Elsewhere in the issue, Mickaël Boyer and Jing Geng of Danone Nutricia Research provide an interesting article looking at advancing analytical microbiology in the dairy industry. The study of fermenting microorganisms is an essential element of product manufacturing, and their article, starting on page 59, looks at innovations in this area.

If you have any comments or would like to contribute an end-user article to *New Food*, please contact me directly via the email address below. Don't forget to also bookmark our website (www.newfoodmagazine.com) and join our LinkedIn and Twitter groups – details are opposite.

Anne-Marie McKenna
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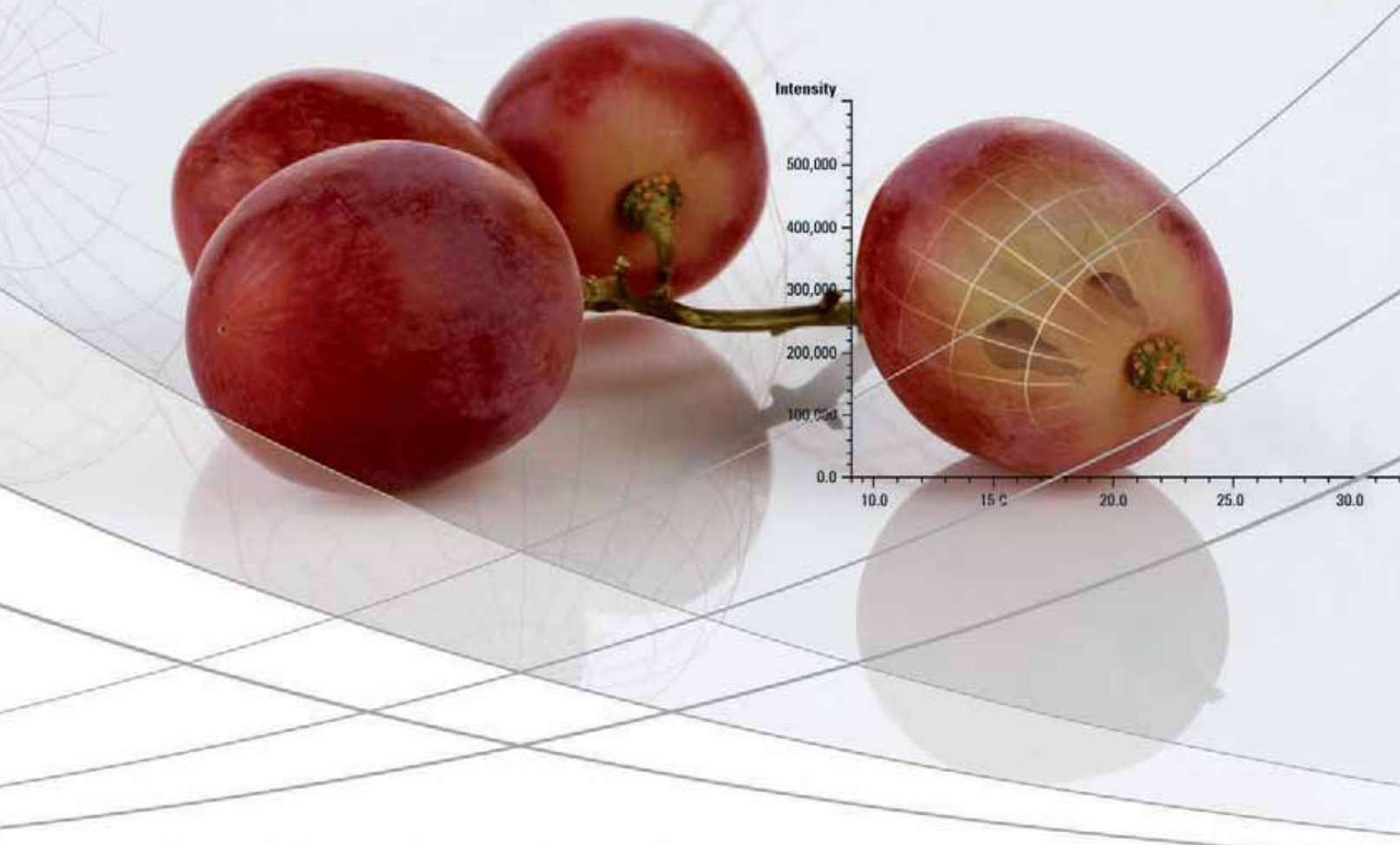

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Coming up in the next issue of *New Food:*

- Food Grade Lubricants and Rapid Methods Supplements
- Chocolate conching
- Lowering the fat content in cheese
- Food authenticity: a new approach by LC/MS

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Events

JULY 2014

LC/MS/MS Workshop on Environmental Applications and Food Safety
Date: 1 – 3 July
Location: Barcelona, Spain
e: mpetrovic@icra.cat
w: www.idaea.csic.es/barcelona/home.htm

Global Food & Packaging Global Summit
Date: 16 – 17 July
Location: Chicago, ILL, USA
e: conferencesusa@ubm.com
w: www.fbpackaging.com

FoodAfrica 2014
Date: 16 – 18 July
Location: Nairobi, Kenya
e: foodafrica@foodtradeafrica.com
w: www.foodtradeafrica.com

Food Processing & Technology
Date: 21 – 23 July
Location: Las Vegas, USA
e: contact@omicsgroup.com
w: www.foodtechnology2014.conferenceseries.net

AUGUST 2014

IAFP 2014
Date: 3 – 6 August
Location: Indiana, USA
e: info@foodprotection.org
w: www.foodprotection.org/annualmeeting

International FoodTec Brasil
Date: 5 – 7 August
Location: Curitiba, Brazil
e: j.kurzke@koelnmesse.de
w: www.foodtecbrasil.com/en/iftb/home/index.php

IUFost 17th World Congress
Date: 17 – 21 August
Location: Montreal, Canada
e: carole@iseventsolutions.com
w: www.iufost2014.org

60th International Congress of Meat Science & Technology
Date: 17 – 22 August
Location: Punta del Este, Uraguay
e: info@icomst2014.org
w: www.icomst2014.org

biocat 2014
Date: 31 August – 4 September
Location: Hamburg, Germany
e: loebkens@tutech.de
w: www.biocatconference.de

SEPTEMBER 2014

Food Micro 2014
Date: 1 – 4 September
Location: Nantes, France
t: +33 (0) 29 67 86 130
w: www.foodmicro2014.org

MSACL-EU: Clinical Mass Spectrometry
Date: 2 – 5 September
Location: Salzburg, Austria
e: chris.herold@msacl.org
w: www.msacl.org

INDC 2014
Date: 2 – 5 September
Location: Prague, Czech Republic
e: asistentka@radanal.cz
w: www.indc.cz

7th International Whey Conference
Date: 7 – 9 September
Location: Rotterdam, The Netherlands
e: t.faulkner@elsevier.com
w: www.iwc2014.com

Worldwide Distilled Spirits
Date: 8 – 11 September
Location: Glasgow, UK
e: info@wdsc2014.org
w: www.wdsc2014.org

IMTS 2014
Date: 8 – 13 September
Location: Chicago, USA
e: imts2014@xpressreg.net
w: www.imts.org

12th EuroFed Lipid congress
Date: 14 – 17 September
Location: Montpellier, France
e: info@eurofedlipid.org
w: www.eurofedlipid.org/meetings/montpellier2014/index.htm

InterCool
Date: 21 – 23 September
Location: Dusseldorf, Germany
e: intercool@messe-duesseldorf.de
w: www.intercool-tradefair.com

Fi Global Summit
Date: 23 – 25 September
Location: London, UK
e: jennifer.knight@ubm.com
w: www.foodingredientsglobal.com

Benefiq 2014
Date: 23 – 25 September
Location: Quebec, Canada
e: helene.marceau@benefiq.ca
w: www.benefiq.ca

PPMA Show 2014
Date: 30 September – 2 October
Location: NEC Birmingham, UK
e: tom.fisher@ppma.co.uk
w: www.ppmashow.co.uk

POWTECH
Date: 30 September – 2 October
Location: Nuremberg, Germany
t: +49 (0) 9 11.86 06-83 55
w: www.powtech.de

World Dairy Expo 2014
Date: 30 September – 4 October
Location: Wisconsin, USA
e: wde@wdexpo.com
w: www.worlddairyexpo.com

OCTOBER 2014

2014 NFRA Convention
Date: 11 – 14 October
Location: Orlando, USA
e: info@nfraweb.org
w: www.nfraweb.org/meetings/nfrf-convention

Global Cheese Technology Forum
Date: 21 – 23 October
Location: Reno, USA
e: ljacobso@calpoly.edu
w: www.globalcheesetechnologyforum.org

Food Analysis Congress
Date: 29 – 30 October
Location: Barcelona, Spain
e: enquiries@selectbio.com
w: www.selectbiosciences.com/fac2014

If you have a diary event you wish to publicise, send details to Martine Shirtcliff at: mshirtcliff@russellpublishing.com

Tyson Foods submits $8.55bn offer to acquire Hillshire Brands

Tyson Foods announced on 10 June 2014 that it had submitted a unilaterally binding offer to acquire packaged food company The Hillshire Brands Company for $63 per share. The all cash transaction values the company at approximately $8.55 billion, including Hillshire Brands' outstanding net debt.

The proposal was higher than Pilgrim's Pride's offer of $55 per share, which valued the company at $7.7 billion. According to Tyson Foods, the offer is subject to Hillshire Brands being released from its existing agreement to acquire Pinnacle Foods Inc.

The board of directors at Tyson Foods have unanimously approved the offer, which will remain in effect until 12 December 2014, which is the final termination date of the Hillshire Brands/Pinnacle Foods agreement.

"The Hillshire Brands acquisition would represent a defining moment for Tyson Foods," said Donnie Smith, Tyson's President and CEO. "Our strategy has been to grow our prepared foods business, and it has been our aspiration to be a leader in retail prepared foods just as we are in chicken. Now we will have those iconic #1 and #2 brands in numerous categories."

The combination of Tyson Foods and Hillshire Brands will reposition Tyson as a clear leader in the retail sale of prepared foods, with a complementary portfolio of well-recognised brands including Tyson®, Wright Brand®, Jimmy Dean®, Ball Park®, State Fair® and Hillshire Farm®.

"After a disciplined process to identify ways of growing our prepared foods segment, we are convinced that combining Tyson and Hillshire Brands would make strategic, financial and operational sense and would stabilise earnings by increasing return on sales and de-commoditising our business," Smith revealed.

Chairman of Tyson's board, John Tyson, commented: "Tyson Foods has a history of growing through strategic acquisition. It is the view of the board of directors that this is truly a transformational opportunity and one that best fits with our strategic plan while enhancing our margins and creating long-term shareholder value."

www.tysonfoods.com

Hi Europe & Ni 2014: The right ingredient for innovation and sourcing

Health ingredients & Natural ingredients Europe 2014 is the world's most important gathering of ingredients suppliers, distributors and buyers of the food and beverage industry. Taking place from 2-4 December 2014 in Amsterdam, the event is now open for registration.

Over 500 exhibitors and 8,000 attendees are expected at Hi Europe & Ni 2014, where visitors will have the opportunity to source innovative ingredients, grow their market share, nurture their business network and keep up-to-date with the latest market developments. There will be numerous on-site features, exhibitor seminars and an on-site conference, so whether you are looking at controlling costs, reformulating existing products or further developing the products in your pipeline, this is the event for you.

Hi Europe & Ni is a truly global meeting place for nutritional food and beverage innovation that will provide a complete perspective of the nutritional and wellness industry. You can be part of this powerhouse exhibition, and join the other market leaders and key industry players of the health and natural ingredients industries under one roof in Amsterdam.

Hi Europe & Ni is brought to you by the organisers of Fi Europe. Registration is free and can be done via the website.

www.hieurope.com/nf

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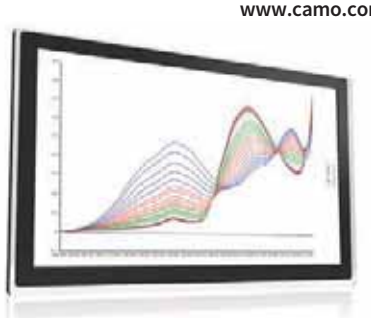

Nestlé UK & Ireland on track to achieve 250 calories or less for all single serve confectionery

Nestlé UK & Ireland has supported the Government's Public Health Responsibility Deal since its launch in 2011, and is a signatory to all the relevant Food Pledges including the calorie reduction pledge which supports Nestlé's commitment to enabling customers to eat and drink fewer calories.

As a result of the programme of calorie reduction, Nestlé UK & Ireland has announced that it is on track to lead the market by having no single serve confectionery products over 250 calories by the end of 2014. Currently 89 per cent of our confectionery products contain 250 calories or less per serving. Whilst in total 62 per cent of Nestlé's confectionery now contains fewer than 110 calories per serving.

Nestlé continually reformulates its products to improve them nutritionally and aims to provide its consumers with the information they need to make informed food choices.

Nestlé has also adopted the Government's new labelling scheme in the UK, recognising its importance to the public health agenda. For example in 2013, Nestlé UK & Ireland announced it had reformulated its iconic KitKat bar, resulting in the removal of 3,800 tonnes of saturated fat form the public's diet and improving the nutritional profile of the product.

www.nestle.co.uk

Gian Paolo Rossini Università di Modena e Reggio Emilia

Old and new challenges in seafood safety

Fishery and aquaculture have been increasing in already exploited parts of the sea, as well as new areas, following the strong demand of food on global and regional scales. The possible contamination of fishery products due to toxins of microalgal origin is a matter of concern for fishery and aquaculture worldwide, and food safety has been a major drive for the development and implementation of procedures to protect consumers. The advancements of knowledge on toxic microalgae and microalgal toxins have played a fundamental role for developments in the last 10 years. Increasing awareness regarding the complexity of issues to be approached in this field has been providing better theoretical and operational tools for future progress in the risk assessment and management of both 'old' and 'novel' toxins; helping the fishery and aquaculture sectors to meet consumers' expectations for safe food.

The increasing demand of food on global and regional scales is keeping the attention of stakeholders on already exploited parts of the sea, as well as new areas, including freshwater. The increasing fishery and aquaculture exploitation has emphasised the many issues linked to human and environmental health, and particularly food safety[1]. The possible contamination of fishery products due to toxins of microalgal origin is one such issue, and has been a major drive for the development and implementation of effective procedures to manage the risks posed by the natural products responsible for human and animal intoxications.

The existence of toxic microalgae has been recognised for centuries, and the contamination of fishery products, particularly bivalve molluscs, represents a natural phenomenon[2]. It's no surprise, therefore, that scientific and technological advancements have marked the efforts to obtain the best possible knowledge and tools for human health protection with regard to fishery products, particularly in the last 20 years. Undoubtedly, the Codex Alimentarius has been playing a key role in the identification of actions framing the management of risks posed by toxic microalgae and the contamination of fishery products[3]. The process has included several steps spanning about 10 years and, importantly, has represented the outcome of concerted actions of stakeholders in the field, in a collaborative international effort of

scientists, industry and political/societal bodies[3]. A set of recommendations drafted in Oslo in 2004 provided a fundamental contribution to this process, and called the attention onto major aspects of risk assessment and management, from a perspective of prevention that consumers might be exposed to hazardous levels of toxins in contaminated seafood[3,4]. The quest for: i) more toxicological data and the characterisation of the mechanism of action of different groups of toxins; ii) fully validated methods for toxin detection in contaminated food; and iii) the implementation of programmes for integrated monitoring of microalgae in waters and toxins in food clearly emerged from those recommendations. Furthermore, the need of operational models for forecasting blooms of toxic microalgae was stressed[4].

Ten years after the consultation in Oslo, I wish to propose a few considerations, to contribute to support further action for the protection of human and environmental health, as well as the support of activities of fishery and aquaculture sectors.

The first consideration regards the body of knowledge available to stakeholders, which supported the risk analysis culminated in the Oslo consultation. The recommendations mentioned above stemmed from significant sets of data supporting risk assessment, leading to proposals for risk management[4]. In keeping with past efforts, the last 10 years have seen the development and validation of powerful instrumental methods for toxin detection in naturally contaminated samples, including the case of multi toxin analysis, as well as the production of standards and reference materials needed to perform analyses[5]. The regulatory acceptance of LC-MS/MS procedures and the replacement of mouse bioassay for the detection of toxin contaminations in shellfish and other seafood[6], in particular, has been a major achievement of the last years[5,7]. On a toxicological ground, more data on environmental and human effects of toxins have been gathered, supporting knowledge-based decisions for risk management[8,9]. Furthermore, advanced bio-molecular tools have been employed to probe mechanisms of action of toxins, providing relevant information for the understanding of the molecular bases of toxicity of both seafood and freshwater toxins, including 'old' and 'novel' natural products[10-12]. The advancements have not been confined to toxins detection and their biological activity, but have included microalgal biology, providing better knowledge regarding the pathways of toxin biosynthesis[13,14], as well as the detection of toxic microalgae by DNA probes[2,15].

Even an outline of the progresses in the field of toxic microalgae and microalgal toxins is obviously beyond the scope of this short contribution. Thus, the quick mentioning of important topics which have received attention, and led to significant advancements, is instrumental to highlighting a couple of points.

The first point can be better approached by inspection of **Figure 1** (page 8), where a simplified scheme of major lines of investigation in the broad field of toxic microalgae and microalgal toxins is proposed, showing links existing as to the understanding of basic scientific issues, their further study aimed at developing tools for risk management, and the primary outcome of these activities. The great complexity of issues at stake emerges from the extreme simplification of this scheme, including the three primary levels of analysis: the algal producers of toxins, the organisms that may become contaminated by feeding on toxic microalgae, and the animals that may become intoxicated as a consequence of exposure to toxins upon ingestion of contaminated

seafood. The three levels of analysis are not isolated, and significant interactions, either actual or operational, exist. Furthermore, multiple topics contribute to the characterisation of processes at each level of analysis, and the few items indicated in the scheme simply pinpoint the many factors to be considered at sub-system levels. Indeed, the knowledge gained through activities at any single level is not 'all-inclusive', and different steps can be proposed in the linear development from basic knowledge to its exploitation as a technological/operational tool for each item in the scheme. Likewise, the elements at the three levels are not isolated from the environment and independent of each other. Thus, every biological entity of the system is affected by external conditions and is subjected to interactions with other individuals of the same species and other species as well.

Considering this schematic representation of complex systems, the importance of integration among players with different expertise and fields of action becomes apparent. Indeed, the extensive interactions of the fishery and shellfish industry and academia have been giving very strong support to basic biological and chemical research, as well as the development of effective analytical tools[4,5].

The concerted actions and interdisciplinary research activities is the proper background of future developments in the area, which represents the second point I wish to consider. The great progress of our knowledge in the field in the last 10 years, in fact, has brought a better recognition of gaps and lacks, posing novel emphasis on the importance of a continuing support to research activities aimed at further advancements in the characterisation of toxic microalgae and microalgal toxins, to support human and environmental health as well as the fishery and aquaculture industry. Based on the scheme of **Figure 1**, a good guess is that many issues remain open and additional questions have been raised by the results obtained so far. This is actually correct, and I will just mention a few lines of major intervention already gathering the attention and action of stakeholders.

In the field of algal biology, the characterisation of the huge dinoflagellate genomes represents a major challenge which will demand tremendous research efforts of the scientific community. The full

clarifications of biosynthetic pathways of toxins in microalgae, and the understanding of major factors regulating the production of these agents, represent other relevant topics, whose developments will need better understanding of the physiology of toxic microalgae in the wild. A more in depth knowledge on toxin metabolism in contaminated species, and the possible effects those stressors may have on host/vector physiology will be additional lines of intervention. Significant advancements in these two areas will have a positive impact on the development of tools for the protection of human and environmental health, as well as fishery and aquaculture activities.

It seems likely that a full clarification of biosynthetic pathways of toxins in microalgae will be mirrored by developments in the chemical characterisation of toxins and better performing instrumental tools for toxin detection in biological matrices. Particular attention in the analytical field is being already given to the development of bio-molecular procedures for toxin detection, primarily antibody- and receptor-based methods. In the future, increasing efforts in this area will be devoted to find the best analytical settings for accurate quantification of toxicologically relevant components in rapid, high-throughput, user-friendly and cost-effective formats.

Research activities aimed at the development of toxicity-oriented tools for toxin detection will also involve cell-based methods[16], in keeping with the clarification of toxicity pathways and an increased use of cellular systems in toxicity testing[17]. Significant advancements in this area will be possible only if appropriate characterisation of mechanisms and modes of action of toxins will be obtained. This kind of progress should eventually support the development of simplified bio-molecular procedures for sample analysis under cell-free conditions, as exemplified by the receptor binding assay for saxitoxin-group toxins[18].

With regard to toxicity studies, at both a molecular and organismal level, the importance of gathering knowledge of responses ensuing from the combined actions of different classes of toxins in mixtures will most likely mark future investigations. Considering seafood safety, it has to be expected that consumers

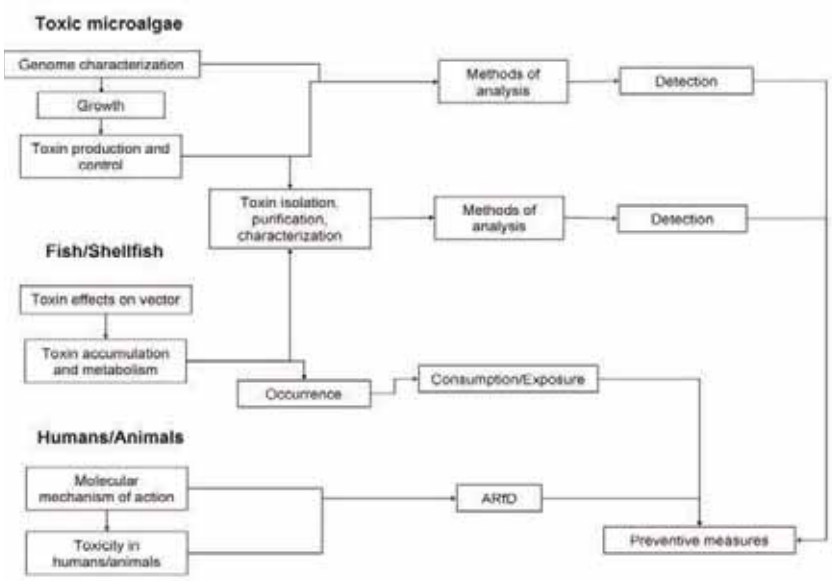

Figure 1: The three basic biological levels involved in safety issues posed by toxic microalgae. Schematic representation of major fields of studies supporting risk assessment, and the development of tools for the management of risks posed by toxic microalgae and biotoxin contamination of seafood. ARfD, acute reference dose.

are being exposed to mixtures of stressors, rather than to a single agent at a time, and microalgal toxins might actually represent only a portion of the burden of toxicants ingested with seafood. Thus, the protection of human health and the marketing of safe seafood will certainly demand a better understanding of the toxicity of mixtures. The extreme complexity inherent into this issue, due to the variety of combinations of agents and their relative concentrations, represents a major challenge for toxicologists.

Obtaining 'operational models for forecasting blooms of toxin-producing microalgae in time and space', as recommended by the Oslo report[4], will perhaps represent one of the most challenging topics in the field, and will keep stakeholders busy for quite some time. The modelling of harmful algal blooms has been already approached in the last years by distinct theoretical and operational approaches[19], and most effective advancements are expected in the next 10-15 years. Progress in this area will be accompanied by development of remote sensing devices, such as the Environmental Sample Processor[20], and it is expected that data collection by robotised equipment will exploit the most advanced knowledge and analytical tools obtained for the detection and quantification of relevant microalgae and toxins in open sea.

The protection of consumers from intoxications due to seafood contaminated with microalgal toxins, as well as the activities in fishery and aquaculture sector have been supported by the advancements of knowledge on toxic microalgae and microalgal toxins in the last 10 years. The awareness of the complexity of issues to be approached in this field has increased in the scientific community and other stakeholders, giving better theoretical and operational tools for further progress in the risk assessment and management of both 'old' and 'novel' toxins. These are good news for future actions for seafood safety.

About the Author

Gian Paolo Rossini is full professor of Biochemistry at the Università di Modena e Reggio Emilia (Italy), where he teaches general and applied biochemistry. He obtained his degree in Biological Sciences at the Università di Bologna (Italy) in 1976. From 1979 to 1981 he was a Research Associate at The University of Chicago, USA. Gian was a Guest Scientist at the Karolinska Institutet, Sweden from January to December 1985, and at the Institut National des Sciences Appliquées de Lyon, France from September 1994 to Auguts 1995. Over the last 20 years, his investigations have been focused onto the molecular mechanisms of action and the toxicity pathways of microalgal biotoxins as well as the development of cell-based, functional methods for the detection of biotoxins in contaminated materials. He has been team leader/coordinator in several local, national and international research projects. He has contributed to the drafting of several reports for international organisms. In 2001 he was an invited expert in the 'Working Group on Toxicology of DSP and AZP' of the EU Commission. In 2004, Gian was invited to participate at the 'Joint FAO/IOC/WHO ad hoc Expert Consultation on Biotoxins in Molluskan Bivalves' held in Oslo, Norway. And from July 2006 to December 2009, he was a member of the working group on marine biotoxins of the European Food Safety Authority (Scientific Panel on Contaminants in the Food Chain).

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Tribology: a new tool for the food rheologist's toolbox

Rheology is a powerful tool that can help link food physicochemical properties, structure, and sensory texture to form a cohesive, fundamental understanding of structural and physicochemical contributions to food texture. Yield stresses, fluid flow profiles, and fracture properties of firm solids are relatively easily determined with standard rheometry. However, there are still aspects of food texture that cannot be measured via standard rheological testing. Food texture in the later stages of mastication, when the food is being prepared for swallowing, shows poor correlation to mechanical measurements[1].

Additionally, certain aspects of mouthfeel, such as grittiness and astringency, are not detectable with rheometry[2-6]. For example, it is possible to design a fluid with the exact viscosity, sweetness, flavour, pH, and colour of pomegranate or cranberry juice. Rheological testing will not differentiate between the actual juice and the mock juice, but a simple sensory difference test will quickly confirm that the two liquids are indeed different. The difference lies in the mouthfeel: cranberry and pomegranate juices are astringent. Astringency, the feeling of mouth-puckering or drying[7], is not detectable through rheometry, nor are many other mouthfeel terms such as grittiness or mouthcoat. A new tool is

needed to provide mechanical measurements of astringency and other friction-based mouthfeel sensations.

Tribology, the study of friction, lubrication, and wear between two sliding surfaces, has been used for decades by the chemical and material engineering industries to determine the friction behaviour of various substances, such as lubricating oils and rubber[8]. Practical applications of this testing are relatively straightforward: lubricants that provide low amounts of friction between sliding steel surfaces are useful to reduce friction from surface-surface contact, such as in mechanical pivots or sliding pistons. Likewise, rubber for car tyres needs to provide sufficient friction for the tyres to grip the road, but not so much friction that rotation of the wheel requires significantly more energy and results in rapid wear of the tyres. More recently, tribology has been used in food research to study food friction behaviour. To date, tribological studies of foods comprise mainly model systems such as oil-in-water emulsions[6,8-12], although various dairy products[3,5,13,14], chocolate[15], and mayonnaise[3,4,16] have also been evaluated.

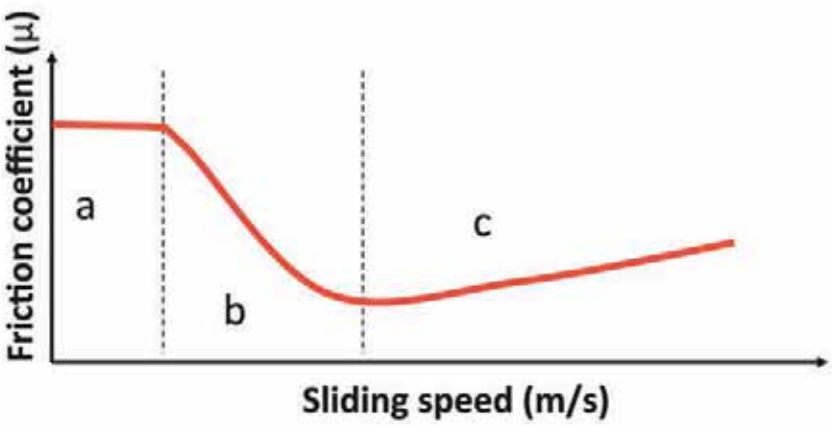

Figure 1: Stribeck curves showing three distinct regimes[2,8]: (a) the boundary regime; (b) the mixed regime; (c) the hydrodynamic regime

The most common tribological apparatus involves an indenter or ball that slides against a flat surface, with or without inclusion of a lubricant between the surfaces. Steel surfaces (hard contact) are generally used for tribological studies of machine wear and friction[8]. Tribological studies of foods, on the other hand, use elastomeric surfaces (soft contact), such as silicone rubber. These softer surfaces are considered to be a more accurate mimic of oral surfaces, and thus provide a better approximation of oral friction behaviour than hard contact surfaces[2,11,17]. Plots of friction coefficient (derived from torque measurements) versus sliding speed are generated from tribological data. These plots are called Stribeck curves and, for Newtonian fluids, have a similar shape to the plot shown in Figure 1. Stribeck curves have three distinct regimes[2,8]. At low sliding speeds, in the *boundary regime* (Figure 1a), friction is high due to contact between surface asperities and the thickness of the lubricating layer is insufficient to separate the surfaces. As sliding speed increases, the amount of fluid between the surfaces also increases, providing greater, but still incomplete, surface separation. This is the *mixed regime* (Figure 1b). At high sliding speeds, the surfaces completely separate, all hydrodynamic load is carried by the fluid, and lubrication behaviour shifts to the *hydrodynamic regime* (Figure 1c). Oral sliding speeds have been estimated to be between 30-100mm/s[18], and food lubrication behaviour in the mouth is generally considered to be in the boundary-to-mixed regime[2,8,19,20].

General assumptions behind the Stribeck curve and the calculation of friction coefficient include the use of hard (i.e. nondeformable) surfaces and Newtonian lubricants. However, tribological testing of foods is generally performed on elastomeric surfaces, as previously stated. Deformation of the surface can result in a change in contact area between the surfaces, which can affect friction measurements. Additionally, most foods are not Newtonian, and phenomena such as yield stresses and shear-thinning can significantly impact friction behaviour. There is also difficulty in measuring food friction behaviour at sliding speeds high enough to generate hydrodynamic behaviour. Thus, friction coefficients that are calculated using standard equations may not take the shape of the traditional Stribeck curve. There are methods to mitigate effects from surface deformation and non-Newtonian effects: contact area at each sliding speed may be calculated based on Hertzian theory[21] and dynamic viscosity at high shear rates (>10^4 s^{-1}) may be used to account for samples with different viscosity, or when viscosity changes with shear[21,22]. In addition, measuring food tribological properties at high sliding speeds may not be necessary to replicate the behaviour seen in the mouth, as the tongue is only capable of moving at sliding speeds up to 200mm/s[23]. Nevertheless, examining the friction behaviour of foods over the entire Stribeck curve may still be beneficial for properly describing oral friction behaviour, as multiple sliding speeds occur simultaneously during mastication.

One of the major objectives of food tribological research is to relate food sensory texture to mechanical friction measurements. A recent review of rheological and tribological contributions to food texture proposed that oral behaviour of food during the initial stage of oral processing is dominated by bulk rheological behaviour but tribological (thin-film) behaviour dominates during the later stages of mastication[2]. Thus, oral evaluation of foods involves sensation of both mechanical and friction behaviour, as well as compositional and physical properties (e.g. pH, particle size, fat content, and moisture content)[2]. This reasoning has led to the hypothesis that measuring food friction behaviour may yield information on food texture that is not provided by traditional mechanical testing[2-6] and improve understanding of food structure-texture relationships.

One of the major objectives of food tribological research is to relate food sensory texture to mechanical friction measurements

Several studies have found relationships between emulsion mouthfeel terms and friction coefficient[8,9,12,20]. Fat content appears to be the major contributor to both emulsion texture and friction behaviour. Fat content has also been shown to have a significant impact on the friction behaviour of other foods such as milk[5], custards[3,13], mayonnaise[4], and yoghurt[24]: fat is an excellent oral lubricant and can significantly reduce friction coefficient. Particle size has also been found to impact friction behaviour[3], although the results were not always compared to sensory texture data. Interestingly, large particles do not necessarily cause an increase in friction behaviour. Instead, the important factor to

consider is particle shape and size in relation to gap height during sliding[3]. Particles that are larger than the gap between sliding surfaces are excluded from the gap, resulting in different friction behaviour than that measured at a larger gap (higher sliding speeds and a different lubrication regime)[20]. These results are especially important in fluid food design: care must be taken when measuring friction behaviour of fluids with relatively large particles and comparing the results to sensory texture. What may be detectable as a feeling of particles on the tongue may not be reflected in an increase in friction during mechanical testing (or vice versa) if the gap heights or sliding speeds used are dissimilar.

There has been less study on the effect of nonfat ingredients (e.g. starch, proteins, hydrocolloids) on food friction behaviour. Additionally, there has been little study on the relationships between friction behaviour and sensory texture. Studies examining the relationships between friction behaviour and sensory texture are mainly limited to foods with varying fat content. Fat content is a major driver behind sensory texture[5,8,9], especially in fluid systems; however, other ingredients can also significantly contribute to sensory texture. Structural features, e.g. a weak gel network or protein conformation, can also impact friction behaviour. Further study is needed to examine the effects of these ingredients on friction behaviour and determine their contribution to friction-based sensory terms.

Another factor in food texture that is beginning to receive more attention in tribological studies is the contribution of saliva to food texture and textural changes. Saliva, a viscoelastic fluid, is more than 99 per cent water[25]. The remaining fraction comprises buffering salts, enzymes, mucins, other glycoproteins, immunoglobulins, and

peptides[25,26]. Mucins in particular are primarily responsible for saliva's characteristic mechanical behaviour[26]. When saliva is mixed with food during mastication, the resulting product usually possesses significantly different friction and mechanical behaviour than the original food, particularly during long mastication times (>10s). Nevertheless, saliva is generally not added to foods prior to or during mechanical testing even though the importance of saliva in food friction behaviour has been noted by several groups[27,28]. Additionally, studies on the friction behaviour of whole saliva, as well as saliva mixed with various foods have proposed that saliva, an excellent

Several studies have found relationships between emulsion mouthfeel terms and friction coefficient

oral lubricant[26], is a primary factor in oral lubrication processes[24,29]. Furthermore, food saliva interactions may impact food friction behaviour[24]. For example, the complexation of astringent compounds with saliva, resulting in an astringent mouthfeel, can be observed in tribological tests via an increase in friction coefficient upon addition of saliva to a solution containing astringent compounds[27]. In addition, food saliva mixtures have been shown to have different friction behaviour than either the food or saliva alone[3,24]. The changes in friction behaviour were shown to be from saliva itself rather than a dilution effect, supporting the idea that saliva significantly impacts friction behaviour.

Although testing foods with saliva may yield valuable information about changes in food frictional behaviour during the mastication process, saliva can be a difficult research material to use. In addition to varying among individuals, saliva composition is dependent on health status, hunger, stress, time of day, and mechanical stimulation in the oral cavity[25,26]. Carefully following saliva collection and storage protocols and pooling saliva from multiple volunteers can reduce the inherent variation in salivary composition. However, some variation in saliva composition, and thus behaviour, may still be present despite these precautions. On the other hand, an artificial saliva able to mimic the mechanical and friction behaviour of human saliva, as well as replicate the physicochemical changes in food caused by human saliva, is not currently available. Therefore, human saliva is used in tribological studies seeking to examine the effects of saliva on food friction behaviour.

Is tribology the Holy Grail of rheological testing? Will it be able to replace descriptive sensory analysis, the current gold standard for determining food texture? The most probable answer to both of these questions is no. The human mouth is a complex environment in which many physicochemical changes occur and multiple textural attributes are evaluated simultaneously. In fact, the capabilities of sensation and evaluation in the oral cavity rival the most modern food research lab facility: no single test or instrument is capable of simultaneously evaluating mechanical and friction behaviour, pH, volatile composition, fat and moisture content, and structure, as well as the changes in these properties over a range of timescales. However, combining the results of multiple tests gives a clearer picture of food texture and the mechanisms behind it. Although tribological testing may not be able to completely describe food texture, using tribology to evaluate food friction behaviour provides additional information on the role of friction in food texture, particularly when saliva is added during tribological testing. While not the Holy Grail of instrumental measurements, tribology is an additional tool for measuring the complex relationships between food physicochemical properties, structure, and texture.

About the Author

Helen Joyner has a B.S. in chemical engineering and an M.S. and Ph.D. in food science. She is currently an Assistant Professor at the University of Idaho in the School of Food Science. Her research focuses on rheological and tribological behaviour of food products, with the goal of determining relationships between structure, mechanical/friction behaviour, and sensory texture. The results of this research may be applied to many different food products, allowing more precise control of texture through structural manipulation.

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From targeted to untargeted detection of contaminants and foreign bodies in food and feed using NIR spectroscopy

For decades, Near InfraRed Spectroscopy (NIRS) has been widely used in the food and feed industry in order to implement rapid, relatively inexpensive and efficient control tools to assure the quality of products. NIRS is a branch of the molecular vibrational spectroscopy that refers to the measurement of radiation intensity (i.e. absorbance) as a function of frequency ranging in the electromagnetic spectrum (usually expressed as a function of wavelength [nm], but with the introduction of Fourier transform based instrument also as a function of wavenumber [cm^{-1}]) in the 780-2500 nm (12820 – 4000 cm^{-1}).

The radiation intensity resulting from the interaction between the infrared radiation and the matter is acquired using a spectrometer (also called spectrophotometer) designed to generate, at different frequencies, the NIR absorbance spectrum of the analysed product, expressed as Log 1/R or Log 1/T for reflection and transmission analysis modes, respectively. As a result, the NIR spectra of food and feed products correspond to absorption bands characteristic of the vibration of O-H, C-H, N-H, S-H and C-C groups. NIR spectra can be affected by

several factors such as the physical state of the product (e.g. solid, liquid or gaseous), the temperature of the sample, the particle size (e.g. powder products), the heterogeneity of the material and the sampling errors, the presence of damaged products and impurities, as well as the environment analytical conditions (e.g. temperature and humidity of the laboratory versus temperature of the line of production).

Figure 1: NIR instrument equipped with a wheel of 30 sample holders (Bruker MPA)

The great success of NIRS is mainly due to its relatively easy implementation procedure; its capacity to simultaneously determine several parameters and the fact that this technique allows implementing rapid analytical solutions. For the food and feed industries, this means mainly a method that can provide an analytical answer in short and real-time; but also a method that provides several analytical answers from a unique analysis. The rapid determination of valuable constituents and parameters is essential for the analysis of raw materials, during the process and of the end-products. The aim is usually to maximise profit by raw product check and effective process control, as well as to avoid financial loss by not delivering products with unwanted characteristics. The existence of international standards and guidelines (e.g. norms ISO 12099:2010 and EN 15948) has reinforced the position of NIRS methods in the global analytical scene[1].

NIRS trends

NIRS instrumentation falls usually into two types: (i) dispersive instruments that consist of a monochromatic system that allows successive portions of a polychromatic light to be sent to the sample, a sample compartment and a detector sensitive in the NIR region; and (ii) Fourier transform (FT) instruments that are similar to the dispersive instruments except that the monochromator is replaced by an interferometer that uses a beam splitter to decompose the light into two beams that are recombined before being sent to the sample compartment. An important aspect of the NIR instrumentation is the sample compartment and even more important the sample accessories allowing the maximum benefit to be achieved from the rapid feature of spectroscopy. The sample compartment of an NIR spectrometer varies from a few centimeters to infinite, depending on how the measurement is designed. Sample presentation techniques are based on different accessories used to present the sample to the instrument and vary depending on the way the spectral information is collected (i.e. transmission, reflection or transflection mode). Over the last decade, companies providing NIR spectrometers have invested a lot of energy in the design of high throughput sampling accessories (based on a fibre optic or an auto-sampler) allowing the analysis of high numbers of samples by unit of time.

Another high active area is the *in situ* analysis using NIR microscope or NIR hyperspectral imaging instruments. The use of hyphenated techniques that combine molecular vibrational spectroscopy devices with specific tools as microscopy or imaging allows adding the spatial dimension to the analysis, which is an interesting solution to address food and feed problems[2-5]. Using such instruments, up to several thousand of spectra per sample can be simultaneously collected, which are gathered in order to generate a hypercube that includes the wavelengths, the absorbance values and the spatial information. NIR microscopes, also known as point scan or staring instruments, allow acquiring spectra at successive single spatial locations using a mapping mode system. The hypercube of a sample can be obtained more quickly using whiskbroom or pushbroom hyperspectral imaging systems. Whiskbroom hyperspectral imaging system (also known as plane-scan

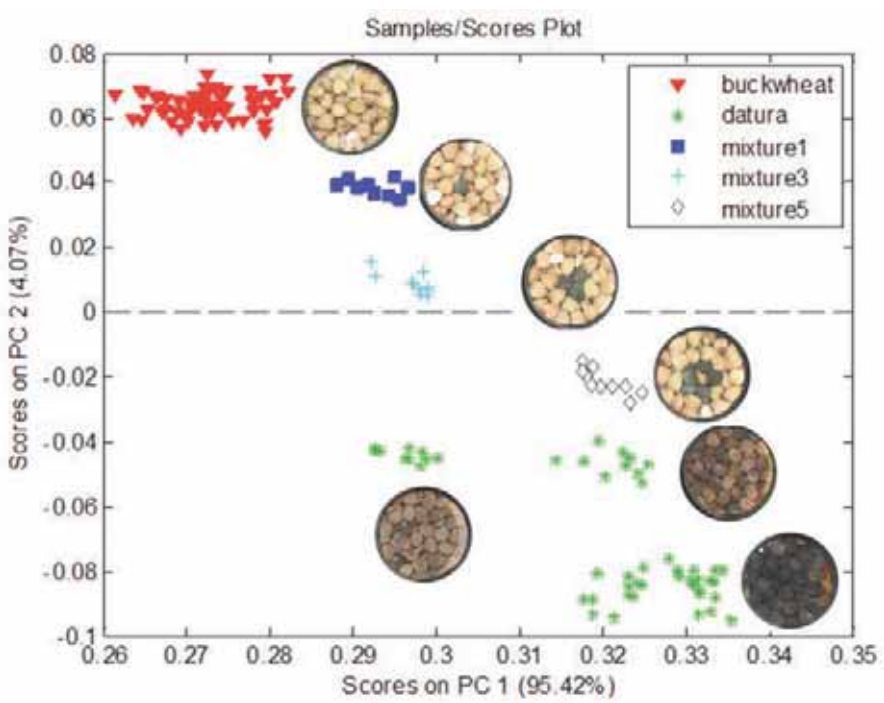

Figure 2: Discrimination of Datura vs. Buckwheat based on PCA analysis

hyperspectral imaging) allows the collection of the absorbance intensities for successive wavelengths. With pushbroom hyperspectral imaging systems (also known as line-scan hyperspectral imaging), the full spectra of the pixels in a line is simultaneously collected. This kind of instrument requires a moving sample stage that can present the successive lines of the scene to be analysed.

The high throughput sampling accessories and the hyperspectral imaging systems are adequate tools to bring the NIRS technology in the field of the detection and quantification of contaminants. Indeed, they allow scanning a sample portion as small as possible in order to lower the limit of the detection of NIRS techniques to the control requirement. This rule is, of course, only valid for contaminants present in the form of particles/bodies. In this paper, three case studies have been selected from projects using NIRS-based techniques to detect and quantify contaminants in agro-food productions:

Case study 1

Detection of plant contaminants in whole grains by NIRS (right sampling procedure allows detecting contaminants at required level)

Recently, we saw a reemergence of the presence of poisonous plants. The intoxication which occurred in France in 2012 was linked to the consumption of bakery products made using buckwheat flour contaminated with *Datura stramonium*, a wild-growing plant found in several crops and well known for its high content in toxic alkaloids. Until now, all the published studies dealing with the detection of Datura during harvest have used visual inspection, optical microscopic or chromatographic methods, which have the drawback of being slow, usually destructive and requiring skilled analysts.

At CRA-W, NIR spectroscopy has been assessed for the detection of the presence of Datura seeds in cereals using an NIR instrument (Bruker MPA) equipped with a wheel for the automatic analysis of 30 vials (**Figure 1**, page 18). With this device a total of 140 spectra were acquired: 60 pure buckwheat spectra, 50 pure Datura spectra and 10 spectra from three different mixtures of buckwheat kernels contaminated with one, three and five Datura seeds respectively placed at the bottom of the vials. **Figure 2** (page 18) shows the score plot of the two first principal components (95.4 per cent and 4.1 per cent of the total variance of the data set respectively) obtained from the PCA analysis of the 140 spectra. According to the score plot, different groups can be observed. Samples of pure buckwheat kernels (red triangle symbol) are located on the top left part of the graph. They are clearly separated from the pure Datura seeds (green stars symbol) that are located to the bottom right part of the graph. The adulterated buckwheat samples with one, three or five Datura seeds (blue square, blue cross and white lozenge symbol respectively) appeared to be situated in the gap between both pure classes and are discriminated according to the second PC with a certain correlation to the degree of contamination. This discrimination is based on the differences in the chemical composition of the seeds analysed. This study demonstrated that the combination of NIR spectroscopy with simple chemometric tools can be used as a fast alternative for the detection of undesirable substances in whole grains.

Case study 2

Detection of plant contaminants in whole grains by NIR hyperspectral imaging (use of one analysis for the simultaneous detection of several contaminants)

In recent years, NIR hyperspectral imaging (**Figure 3**, page 20) has proved

Figure 3: NIR hyperspectral imaging instrument equipped with a grain feed system on a conveyor belt (BurgerMetrics)

its suitability for quality and safety control in the cereal sector by allowing spectroscopic images to be collected at single kernel level[6,7]. Contaminants in cereals include, *inter alia*, impurities such as straw, grains from other crops, insects and other undesirable substances such as ergot (sclerotium of *Claviceps purpurea*). For the cereal sector, the presence of ergot creates an important toxicity risk for animals and humans because of its high level of alkaloid content. The current work, performed in the framework of the EU CON*ffi*DENCE project[12], aims to detect and quantify the presence of ergot bodies in cereals using NIR hyperspectral imaging. In this project, several instrumentation approaches (plane and line scan systems), and chemometrics tools have been tested at laboratory level for the development of a complete procedure for detecting ergot bodies in cereals[8].

A study was sought to transfer and validate the developed procedure using NIR hyperspectral imaging from laboratory to industrial level. All the analyses performed have shown stable and repeatable results with a correlation higher than 0.94 between the predicted values obtained by NIR hyperspectral imaging and those supplied by the stereo microscopic technique, which is the official reference method. The validation of the protocol on blind samples showed that the method could identify and quantify ergot contamination. The transferability of the method has been also demonstrated. This study has been performed on samples with an ergot

concentration of 0.02 per cent, which is lower than the EC limit for cereals destined for humans (0.05 per cent)[9].

The proposed protocol has been extended to the development of procedures for a larger set of contaminants. Figure 4 shows a set of impurities susceptible to be found in a wheat sample (e.g. oilseed rape, Datura seed and ergot body) and its corresponding predicted image obtained after the application of the developed procedure (applying several Partial Least Squares Discriminant Analysis (PLSDA) models). The figure clearly allows discrimination between the different impurities. From laboratory experiments and industrial tests, one can conclude that NIR hyperspectral imaging and chemometrics tools can be used as a control method to develop a protocol to assess the presence and the quantity of impurities and undesirable substances in cereals.

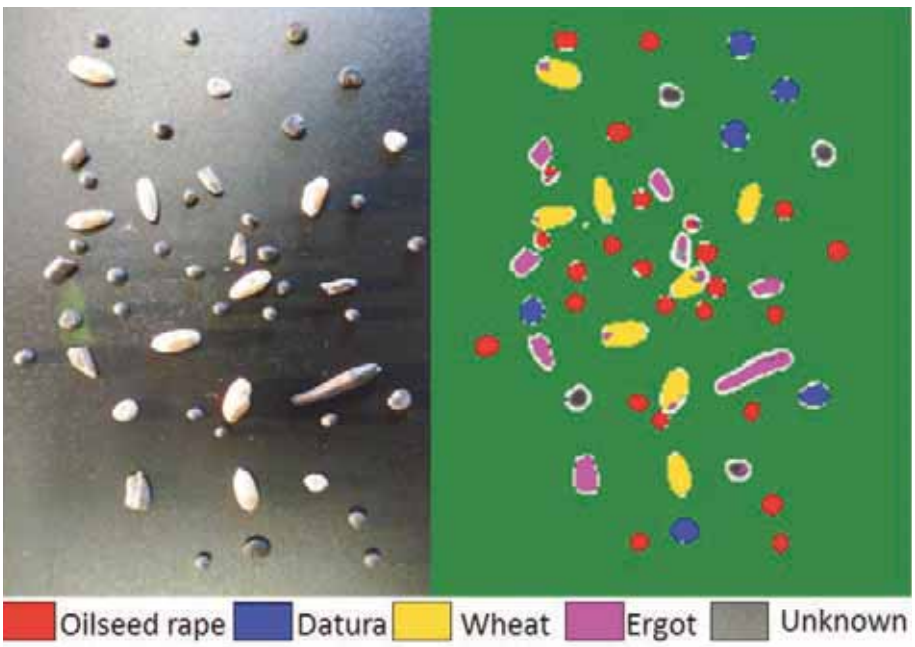

Figure 4: Identification of several known and unknown impurities in wheat based on chemometrics tools

Case-study 3

Untargeted on-line detection of contaminants in feed (look to the unexpected)

In recent years, feed safety has become an increased concern for consumers due to several important crises related directly or indirectly to human health. In 2007, a pet food recall was initiated in North America after a number of cats and dogs became sick and died after eating contaminated pet food with melamine. Again, in 2008, more than 300 tons of soymeal destined for organic chicken in France were withdrawn from the market as authorities discovered melamine levels 50 times higher than the permitted standard. These crises, linked to animal deaths and indirectly human health, put into evidence the

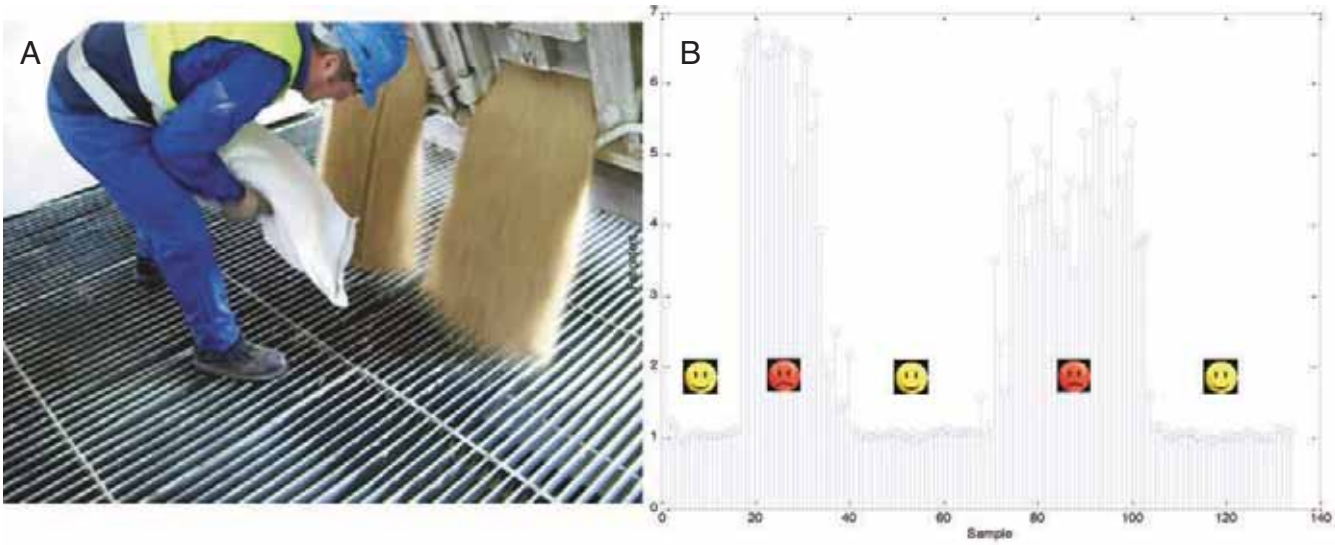

Figure 5: (a) Soybean meal contamination when loading the truck at the entrance of the feed factory; (b) Spectral similarity criterion used to characterise soybean meal and detect the presence of possible contaminants

need of a sensitive, reliable and rapid procedure for the determination of melamine and other contaminants in feed[10,11].

In the framework of the EU project QSAFFE[13] CRA-W proposed a procedure based on NIR spectroscopy and chemometrics in order to characterise soybean meal and to detect the presence of any possible known or unknown contaminant before reaching the feed chain. Using statistical tools to interpret multivariate data obtained from the NIR analysis of soybean meal samples, has led to the creation of some decision rules. They allow checking compliance against specifications in order to decide whether to reject or accept compliance. The procedure was validated at laboratory level and adapted to be applied at the feed mills in order to detect anomalies due to an eventual addition of contaminant or not authorised additives. At the feed mill, a complete experimental plan was designed where trucks containing soybean meal

were contaminated during the unloading with certain types of contaminants (**Figure 5a**, page 22). The application of the decision rules allowed to detect the presence of the contaminants during the unloading of the truck are indicated in **Figure 5b** (page 22) where two clear contaminations can be detected using a simple criterion of NIR spectral similarity (Mahalanobis distance).

Conclusion

The results obtained in these different case studies showed that NIR, combined with some simple chemometrics tools, fits the purpose of authenticating products and detecting targeted and untargeted contaminants at the laboratory and factory levels.

About the Authors

Dr. Ir. Vincent Baeten is head of the Food and Feed Quality Unit of the Valorisation of Agricultural Products Department of the Walloon Agricultural Research Centre (CRA-W, Gembloux – Belgium). The Food and Feed Quality Unit is involved in the development of methods based on spectroscopy (NIR, NIR imaging, MIR, Raman, fluorescence), optical microscopy and chemometrics. The Food and Feed Quality Unit is accredited (ISO 1705) for 3 analytical methods. Dr. Ir. Vincent Baeten has about 20 years of experience on European projects dealing with the development of spectroscopic methods. In the last 10 years he has participated to several projects dealing with quality and safety of food and feed including aspects of traceability and authentication (STRATFEED, TYPIC, MEDEO, CO-EXTRA, TRACE, SAFEED-PAP, CONFFIDENCE, QSAFFE). Recently, he has been awarded of the 2011-Q-Interline Sampling Awards for the outstanding contribution in sampling applied to spectroscopy methods.

Pierre Dardenne is an Agronomy Engineer from Gembloux Agricultural University. In 1980, he was employed by the Walloon Agricultural Research Centre (CRA-W) to lead researches in NIRS, a role he still holds today. In 1991, he got his PhD in Agronomical Sciences at Gembloux Agricultural University in the field of spectroscopy and chemometrics. He has 30 years of expertise in the development of agronomical and agro-industrial applications in NIRS and is involved in several European programmes. Since 2000, Pierre has led the Valorisation of Agricultural Products department at CRA-W. He is leading other groups of scientists working on biomass, feed and food chemical composition, contaminants (heavy metals, antibiotics), milk microbiology and GMO detection. Authenticity, anti-fraud and food safety are keywords in many research programs of his department. The department has 90 employees with 35 scientists.

Ir. Philippe Vermeulen is a Research Engineer at the Valorisation of Agricultural Products Department of CRA-W. He received his Engineering degree in Agricultural Sciences from the Catholic University of Louvain in 1988. Philippe worked for 10 years in the area of breeding on a European Research Program in hybrid wheat for a private company called Hybritech-Monsanto. Since 2001, he has worked at CRA-W inside the Food and Feed Quality Unit, and is involved in the development of NIR methods for the control on-line of agro-food products and feed.

Juan Antonio Fernandez Pierna received his degree in Physical Chemistry at the University of Zaragoza, Spain in 1997. In 2003 he received his PhD in Pharmaceutical Sciences (Chemometrics) at the Analytical Chemistry department of the Vrije Universiteit Brussels under Professor D. L. Massart, with a thesis entitled 'Improvements in the multivariate calibration processes'. Since 2003 he has worked as a research assistant at the CRA-W in Belworks as research assistant at the CRA-W where he has been working for the statistical treatment of the data, the application of chemometrics and the validation of methods. From end 2009, he is also responsible of the Hyperspectral Imaging laboratory installed at the Food and Feed Quality Unit. He is the author or co-author of numerous chapters and around 65 scientific papers mainly related to the statistical treatment of spectroscopic data (including homogeneity detection and uncertainty estimation), food and feed authentication and imaging techniques. He is a member of the Belgian Chemometric Society and is still involved in various EU projects.

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12. http://www.conffidence.eu
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Quantitative determination of taurine in energy drinks by ^{1}H NMR spectroscopy

A look on the supermarket shelf shows a widespread variety of different energy drinks. Besides a multitude of different brands, energy drinks with sugars replaced by sweeteners, different sorts of fruit concentrates, whey or tea as an additional ingredient and concentrated energy drinks in terms of so called 'energy shots' are available. The consumption of energy drinks is generally increasing and, referring to a survey about energy drink consumption in Europe in 2012, especially adolescents (aged between 10 and 18 years) consume such energy drinks[1].

By law, the designation 'energy drink' describes caffeinated beverages that contain one or more of the substances taurine, glucuronolactone and inositol[2]. The term 'energy' promises a positive, vitalising effect, associated with the intake of energy drinks. Among others, this claim is related to high concentrations of taurine. Taurine is an amino sulfonic acid that naturally occurs in animal products, as the most abundant free amino acid in animal tissues[3]. In Germany, taurine concentrations up to 4000 mg/l are legally granted for energy drinks[2]. Thus, the consumption of energy drinks highly increases the daily intake of taurine from omnivore diets, which is varying between 40 mg/day and 400 mg/day[4]. Actually, a multitude of physiological effects are described for taurine, whereby both negative and positive effects are mentioned. However, health effects according to energy drink consumption are not conclusively explained yet and due to insufficient data about possible chronic toxicity or carcinogenicity, no upper safety level for the daily intake of taurine exists so far[1].

In consideration of the limited state of knowledge about the long-term effects caused by energy drink consumption and coexistent high taurine concentrations in energy drinks, it is important to control the legal limits for taurine. Quantification of taurine is commonly performed by chromatographic techniques[5], based on classical amino acid analytics using UV-detection after derivatisation with ninhydrin. As derivatisation is always accompanied with the risk of incomplete reactions, we recently described the approach of straightforward quantification of taurine by means of ^{1}H NMR spectroscopy as an applicable alternative tool6.

Basics of ^{1}H NMR spectroscopy

The principle of NMR spectroscopy is a measurement of the behaviour of spin-possessing nuclei (as in this case ^{1}H), when these nuclei are placed into a strong static magnetic field and subsequently excited with a radio frequency pulse. NMR spectroscopy is mainly known as an analytical tool

for structural clarification, although it is highly suitable in the context of quantification purposes, since the signal intensity is directly proportional to the number of atoms evoking it[7]. ^{1}H NMR spectroscopy actually presents a multitude of advantages[8], compared to other quantification techniques. For instance, it is known to offer high reproducibility and a huge dynamic range for linear quantification conditions at the same time. Furthermore a direct measurement without extensive sample preparation is possible, which makes it both time-saving and less prone to errors caused by inevitable variations in sample preparation steps.

Just as other quantification tools, NMR requires the presence of distinct signals for integration and quantification. In case of overlapping signals in ^{1}H NMR spectra, signal separation can be obtained by a variety of different methods. For instance, the effect of solvents, pH values, ion concentrations or different temperatures can be utilised for signal separation[7]. As previously described, we achieved a distinct taurine signal in energy drink spectra by means of an appropriate pH adjustment[6].

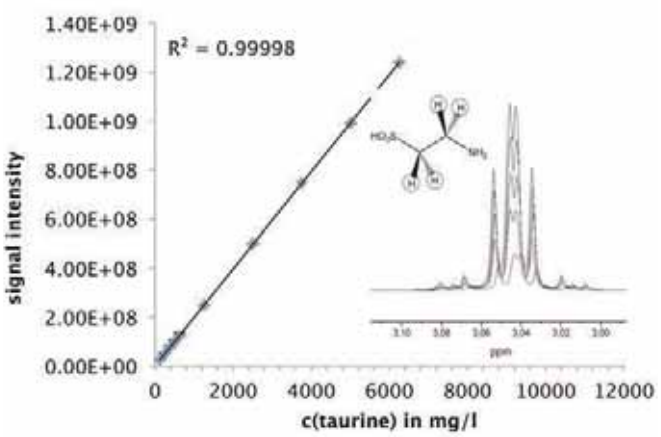

Figure 1: Calibration graph of aqueous taurine solutions ranging between 125.1 (1mM) and 625.7 mg/l (5mM) and between 1251.4 mg/l (10mM) and 6257.0 mg/l (50mM); right-sided: according 1H NMR spectra of aqueous solutions with taurine concentrations ranging between 1251.4 mg/l (10mM) and 6257.0 mg/l (50mM)

Quantification of taurine using ^{1}H NMR spectroscopy

We analysed 20 different energy drinks, varying in brand and ingredient composition with a Bruker Avance 400 spectrometer, an inverse probe head with Z-gradient coils, a BCU 05 temperature unit, an automatic sample changer (B-ACS-60) and TopSpin 3.0 software, all purchased from Bruker Biospin GmbH (Rheinstetten, Germany). Solvent suppression of water in the samples was achieved using a Bruker 'noesygppr1d' experiment with a NOESY-presaturation pulse sequence.

Sample preparation was carried out very simply by adjusting the pH values of a mixture of degassed energy drink, D_2O (as locking substance) and TSPd4 (3-(Trimethylsilyl) propionic acid-D4 sodium salt, as referencing standard). Subsequent measurement of ^{1}H NMR spectra took no longer than 31 minutes. Finally, quantification was performed by external calibration of aqueous taurine solutions, with concentrations ranging between 125.1 (1 mM) and 625.7 mg/l (5 mM) and between 1251.4 mg/l (10 mM) and 6257.0 mg/l (50 mM). The calibration results are displayed in Figure 1.

The high regression coefficient of the calibration graph (R^2 = 0.99998, linearity verified by Mandel fitting test with a 95 per cent confidence level) shows optimal quantification conditions. As previously mentioned, concentrations up to 4000 mg/l are legally granted for taurine in energy drinks in Germany[2]. Moreover concentrations up to 300 mg/l taurine are

legally granted for flavouring[9]. Since producers generally make full use of the legal limits, the measured samples showed either values around 4000 mg/l or 300 mg/l taurine.

In order to validate the results for taurine concentrations given by ^{1}H NMR spectroscopy, the next step was to compare these results with the results of classical LC-UV/vis measurement using derivatisation with ninhydrin. Hence, all energy drinks were also analysed using LC-UV/vis method. For this, an amino acid analyser (Sykam, Eresing, Germany) with a cooling part for reagents (S 7130), an autosampler (S5200), a quaternary pump-system, a module for amino-acid derivatisation (S4300) and Chromeleon 6.6 software (ThermoFisher Scientific, Waltham, Massachusetts, USA) were used. Chromatographic separation was carried out with an ammonium-filter-column prior to a cation-column, elution with buffers showing pH values increasing from 2.9 at the beginning up to 10.5 at the end and detection was achieved at λ = 570 nm.

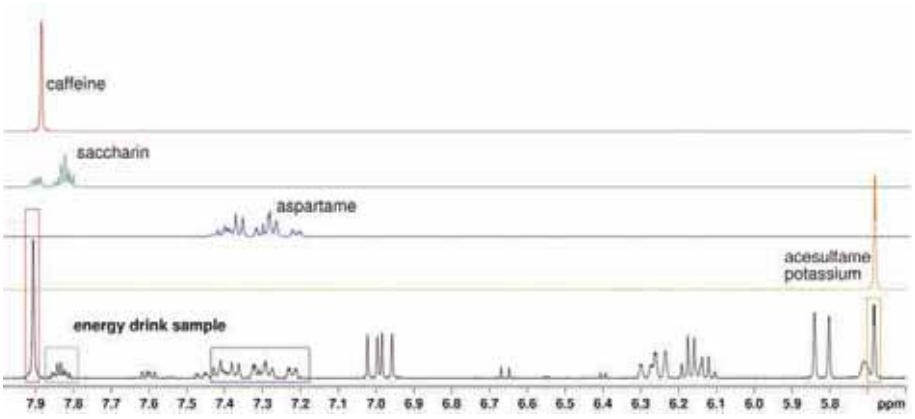

Figure 2: 1H NMR spectra of an energy drink sample and reference substances caffeine, saccharin, aspartame and acesulfame potassium

The deviations between ^{1}H NMR and LC-UV/vis results were always less than the expanded measurement uncertainty of the LC-UV/vis method itself (12.2 per cent using a 95 per cent confidence interval). Thus, applicability of quantitative 1H NMR analysis for taurine determination in energy drinks can be assumed.

Validation of quantitative NMR results, based solely on a comparison to LC-UV/vis data, is difficult due to the fact that LC-UV/vis data itself underlie measuring errors and hence, cannot serve as absolute values. Minor deviations between the two methods may be attributed to a systematic error for both techniques and do not guarantee an according high correctness of results. Furthermore huge deviations of NMR data from LC-UV/vis data cannot surely be attributed to bad results of the NMR method, as this may also be referred to measurement errors of LC-UV/vis data. For a validation of quantitative NMR results independently from LC-UV/vis results, spiked recoveries were analysed. The values of recovery rate were ranging between 97.1 per cent and 108.2 per cent and consequently confirmed suitability for the NMR method.

Suitability of ^{1}H NMR spectroscopy for simultaneous analysis of several ingredients

Besides the already mentioned advantages of ^{1}H NMR spectroscopy, further applications are described in the following part. An important benefit is presented by the high information content of ^{1}H NMR spectra, as all hydrogen atoms are detected (considering adequate concentrations higher than the limit of detection). Hence, ^{1}H NMR spectra can easily be used as a tool for simultaneous qualification and quantification of several substances. Previous literature regarding NMR analysis of juice[10] and wine[11] illustrates the capabilities of NMR for multi-quantitative determinations.

Thus, ^{1}H NMR spectra of energy drinks reveal not only information about taurine concentrations but also additional information for further analytes. For instance, spectra that have been measured in the context of taurine quantification can subsequently be analysed in view of the content of caffeine or sweeteners. Figure 2 shows ^{1}H NMR spectra of an energy drink sample as well as aqueous solutions of caffeine, saccharin, aspartame and acesulfame potassium. It clearly shows characteristic signals for each reference substance in the ^{1}H NMR spectrum of an energy drink sample, that can serve for qualitative and quantitative analysis.

For sugar-free energy drinks, the signals obtained by ^{1}H atoms of the N-methyl groups in caffeine (apparent at the spectral region from 3 to 4 ppm) can also serve as reference signals, whereas an overlap with intensive sugar signals hinders an interpretation for sugar-containing samples. Qualitative analysis of ^{1}H NMR spectra can be very useful in order to verify if the sample's ingredients are labelled correctly. Once that calibration is performed, quantification can be done in view of the respective legal limits.

Thus, ^{1}H NMR spectra can be used as a screening tool for various

substances. In case of suspicious signals or unusual signal intensities in ^{1}H NMR spectra, further verification by classical analysis can be achieved. This enables a highly risk-orientated analysis and optimised workflow for food surveillance.

Summary

Taken together, quantitative NMR spectroscopy presents a very suitable approach for the quantification of taurine in energy drinks and hence, an alternative to LC-UV/vis analysis. Moreover the potential for simultaneous qualitative or quantitative analysis of further ingredients has to be emphasised, which is a key advantage of NMR. High costs for spectrometers are frequently complained as a major disadvantage of NMR. However, if a spectrometer is available, NMR applications offer cheap analysis because of simple and non-elaborative sample preparation, very small amounts of chemical wastage and short measuring times. Hence, in case of a good utilisation and given the long lifetime of spectrometers, the argument of high costs is actually weakened. The multitude of recently developed effective applications of ^{1}H NMR spectroscopy shows the potential of the technique and presumably the implementation of ^{1}H NMR spectroscopy in the context of food surveillance will become more important in the future.

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About the Author

Monika Hohmann studied Food Chemistry at the University of Würzburg, Germany, from 2006 to 2011. In 2012, she took the exam as a state-certified food chemist at the Bavarian Health and Food Safety Authority. Since then, she has been working as a doctoral candidate at the Bavarian Health and Food Safety Authority, under the doctoral supervision of Prof. Dr. Ulrike Holzgrabe (Institute of Pharmacy and Food Chemistry, University of Würzburg, Germany). She carries out research in method development and application of 1H NMR spectroscopy in the field of food surveillance, which aims at replacing extensive classical methods and in addition at gaining further information about food authenticity by means of multivariate data analysis.

ROUNDTABLE

Chris Piotrowski
Director, Aunir

Gustavo Caneda
Strategic Marketing Manager,
Ocean Optics

Lars Nørgaard
Senior Manager, PhD,
Affiliate Professor, FOSS

Moderator: **Dr Ir Vincent Baeten**, Head of the Food and Feed Quality Unit, Walloon Agricultural Research Center

What are the perspectives of vibrational spectroscopy techniques in the untargeted detection of mislabelling and contaminants in food and feed chains? Are we able to detect the next melamine crisis?

Piotrowski: NIRS is a valuable tool in the detection of organic substances. If a suitable calibration is available for the substance, NIRS is capable of detecting it. The main challenge is the speed at which a new calibration can be set up. Creating a suitable database can take time. Close links between the food/feed supply chains and quality control partners should be maintained to identify and highlight critical control points in processing so that monitoring steps can be put in place to avoid such crises.

Caneda: Vibrational spectroscopies have a host of advantages for the control of mislabelling and contaminants in food. They are fast, require little/no sample preparation and are hygienic. The biggest challenge is that with contaminants the detection levels are very low. For example the FDA has established a 1ppm limit for melamine in milk formula. NIR is best where speed is important, although very robust calibration models are required to detect contaminants at trace levels. Raman can be used for detection down to ppb and ppt levels using SERS but requires sample preparation. In conclusion, these techniques are ideal to deal with high volume of samples accurately and quickly. They do however require a serious and rigorous calibration procedure with strong field validation.

Nørgaard: In the complex food supply chain there are three main areas to be concerned with: adulteration of incoming raw material, process deviations and mishaps, and deviations from end-product quality. Vibrational spectroscopy – with mid-IR and NIR targeting different sample matrices – matches the requirements to an analytical platform which is capable of detecting the yet unknown adulterants or contaminants in a high sample-throughput industrial setting. Mid-IR-based global untargeted models for raw milk analysis are examples of already commercially available global applications. The untargeted mid-IR or NIR approach should always be considered a screening tool which alerts to the need for further investigations to determine the nature of the abnormality. So yes, we have the methods to screen for the next melamine crisis!

Do you think that vibrational spectroscopy is the right method for official controls? What is the place of such technique in official lab?

Caneda: The demands placed on testing labs are only going to increase as more food with stricter controls require ever more testing. This means that the throughput of samples in official labs will go on increasing. With traditional techniques, the sample preparation is often very time consuming and requires costly reagents and consumables, this is a bottleneck. It is also an environmental issue to use ever more chemicals. Vibrational techniques like NIR and Raman can take raw samples and test them in an unadulterated state. Currently, specific chemical lab tests are the gold standard for this kind of testing but we strongly believe that the advantages of sampling speed and cost enabled by modern techniques like NIR and Raman, will bring a sea change for how this testing is approached in the years to come. Chemical will become the way to validate and calibrate higher throughput instruments using vibrational spectroscopy.

Nørgaard: Vibrational spectroscopy can be used for screening of adulterants or contaminants in feed and food products – also in the official control laboratory. Due to the non-invasive property and very short analysis time it is a powerful screening technique with an excellent cost-benefit level compared to other techniques. However, it is important to recognise that a positive result provided by any screening method always has to be verified by primary reference methods – and such methods are often readily available in the official control laboratory. The Food and Drug Administration has introduced a regulatory framework for Process Analytical Technology implementation boosting vibrational spectroscopy for control in the pharmaceutical industry, and the same routines are now seen in the food segment.

Piotrowski: There are numerous advantages to using NIRS. As long

as the material being measured is organic and the levels present in the sample are high enough to overcome the noise of the instrument, NIRS can be used in an official capacity. Wet chemistry will always be necessary but NIRS allows more frequent analysis which can help to identify changes to expected results quicker and more cost-effectively than with wet chemistry alone.

What is your strategy to set up the right sampling for heterogeneous samples?

Nørgaard: For liquid samples, where mid-IR spectroscopy based methods are a natural choice, the analytical sampling is of course much easier to perform than for powder or solid samples. In a new solution for wheat and barley whole grain grading we utilise the power of vision based analysis, both in the visual and NIR spectral range, to overcome the sampling problem by analysing every single seed in the sample. In this solution it is possible to classify 10,000 single seeds for 10-15 defects in around three minutes – a truly impressive system for analytical sampling of heterogeneous samples.

Piotrowski: There are three factors to consider with sampling; the frequency, the technique used and the ability to replicate the sample. It is very important to be as consistent as possible when sampling. At Aunir, we have standard sampling procedures we recommend for our clients to ensure that sampling is carried out as frequently as is efficient, and that any changes in personnel do not effect results.

Caneda: Food and feed samples are almost invariably heterogeneous so the sampling is always a priority. In the real world, users need an accurate and representative result of the sample and inhomogeneous samples are the big challenge. Our strategy is to use dynamic techniques that allow the spectrometer to collect many spectra over a large sample (the speed of NIR being key here). Then using averaging you can produce a result that is accurate, representative and much closer to the real product or process you are trying to control. This is also a critical part of producing high quality calibrations for NIR. Different standard accessories can be used for the sample whether it is liquid, solid, or slurry. Alternatively we can develop custom solutions.

What are the key criteria of good spectroscopy software?

Piotrowski: Aunir is a leader in the development and supply of NIRS solutions. We pride ourselves on having a large dataset with multiple

mathematical transformations, plus good varietal, seasonal and geographical spread of the samples in it. Aunir is dedicated to providing calibrations so we are constantly updating our database and adjusting the software to ensure the most accurate calibrations are on offer to customers.

Caneda: Software is the hardware integrator. Without a user friendly design, all the advantages of the sensing and sampling hardware are lost. The first level is to bring it all together and display clean and clear spectra that can control the measurement set up and allow users to quickly verify the result. Then you need functionality to help automate the sampling and repeatability of the process. The next step is to integrate the modelling and calibration routines. Finally you need fast and efficient saving and storing of current and historical data. Ideally we'd like to see the industry adopt common formats that can be used more interchangeably, helping to build up more data to create better and more accurate calibration models.

Nørgaard: Good spectroscopy software is essential for realising the solution's full potential to create value to the users. The software must be dedicated with respect to application development and offer seamless integration with databases, and instrument and networking software. The software should provide options to identify, to qualify and to quantify – IQ^2 – the sample in question in a logical way with efficient cutting-edge chemometric algorithms. As an example, an identification model can determine if we have skimmed milk powder or whole milk powder in the process pipe or if the sample is deviating from these groups. The qualification model zooms into the actual product – often through a tighter model – and finally quantification of composition can be performed confidently.


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What will be the next revolution in NIR spectroscopy?

Nørgaard: FOSS believes that the next revolution in NIR spectroscopy, among other things, will address the difficulties in analysing heterogonous samples. With, for example, hyperspectral NIR cameras it will be possible to predict the chemical composition for a large number of small subsamples without sacrificing speed and in addition decrease the limit of detection for adulterants and contaminants. Also the next generation of process analysers will make it possible to provide much more confidence into feed and food manufacturing.

Caneda: In terms of the technology, there is definitely a drive for smaller spectrometers that can be integrated into more handheld, consumer and medical devices. For the food industry this is a tremendous opportunity for the identification and authentication of ingredients and quality control from the finished goods warehouse to the supermarket aisle. The idea that anyone could have a handheld NIR spectrometer to check the quality of their food in restaurants or at home is getting closer to reality than many might think.

Piotrowski: There are several revolutions on the NIRS horizon that we can see. Everyone is constantly striving for improved accuracy – this will continue to improve in the coming months and years. The use of NIRS will increase as industries realise the value that it can deliver. Hand-held NIR devices are coming onto the market which enables NIRS to be used in more and more portable situations. In conjunction with this, automatic scanning on production lines will become standard for a wider range of measurable factors. Acoustic Optical Turnable Spectra (AOTS) technology is being developed that allows 5,000 scans per second. Use of this technology is predominantly in the beverage and oil industry at present but this will be used increasingly for solids on fast moving production lines in the near future.

About the participants

Chris Piotrowski has worked in the NIR industry since 1978. His early years were spent gaining knowledge and skills as an analytical chemist. By the late 1980s he was tasked with introducing an NIR network to the 10 feed mills of J Bibby & Sons. Following this successful implementation he returned to running both the analytical and NIR divisions of Central Laboratories. In 1995 Central Laboratories was set up as a commercial laboratory serving the food and feed industries. The laboratory grew rapidly covering a diverse range of techniques, including analytical chemistry, microbiology and NIR spectroscopy. In 2008 the decision was made to sell the analytical division of Central Laboratories to AB Agri allowing Chris and the team to focus on the core activity of NIR under the new name of Aunir.

Argentinian **Gustavo Caneda**, a chemical engineer, spent his early career working in industrial processing plants. In 1991 he discovered the exciting possibilities afforded by the power of NIR spectroscopy. This led him to invest his time developing strong experience in chemometric modelling and NIR quality and process control, including as a trainer and consultant to companies worldwide. In 2001 he founded TecnoCientifica, creating his own line of bench top and On-Line NIR instruments using Ocean Optics technology. In 2014 Gustavo joined Ocean Optics to add his experience and help the company to further develop dedicated Industrial Solutions using NIR, UV-Vis and Raman spectroscopies.

Lars Nørgaard is Senior Manager, PhD, Affiliate Professor at FOSS and Head of Team Chemometric Development at FOSS Product Innovation. He holds a Master's of Science in Chemical Engineering and a PhD in chemometrics and analytical chemistry. He has more than 20 years of research and teaching experience within chemometrics and spectroscopy for food-agri and pharmaceutical applications. Before joining FOSS in 2010 Lars was head of Department of Food Science at University of Copenhagen, and he was appointed affiliated professor at University of Copenhagen in 2011.

Advancing food safety

IAFP, the Annual Meeting of the International Association for Food Protection, has earned worldwide recognition as the leading food safety conference and is expected to draw more than 3,000 of the top industry, academic and governmental food safety professionals from six continents. This renowned event owes its reputation and success to the quantity, quality and diversity of each year's program; the quality and relevance of exhibits sharing the latest in available technologies; leading experts speaking on a variety of timely topics; and special recognition of outstanding professionals and students for their contributions to food safety.

The meeting will take place in Indianapolis, Indiana on 3 – 6 August 2014. "IAFP 2014 in Indianapolis is set to be another great one," said Don Schaffner, IAFP President. "As always, our Program Committee has been hard at work putting together a stellar program. This year's competition for symposia and roundtable spots was as strong as ever. The Committee had a tough job awarding one spot for every two ideas submitted. Similarly, they worked hard to ensure that the technical presentations and posters met our high standards.

"But while attendees may come for the quality speakers and valuable information, I can tell you from personal experience that the real value of attending our Annual Meeting happens in the hallways, at the social events and after hours. The calibre of attendees and the quality and substance of the discussions that happen in and around the structured program is unparalleled. I truly hope you'll be able to join us in Indianapolis this summer. And please, if you see me at the meeting, don't hesitate to come up and say 'hello.' It's great to see old friends, but all of us at IAFP love to make new friends as well."

For those wishing to take advantage of an extra day of food safety-related education, three Pre-Meeting Workshops will take place on Saturday 2 August. They include: Addressing the Challenges of Adopting Molecular Methods in Food Safety Laboratories; Advanced Cleaning Technology for Food Processing Equipment; and Validating Pasteurisation Processes for Low-Water Activity Products. That same evening, IAFP President Schaffner will welcome Annual Meeting participants who arrive early to

Sunday 3 August will be filled more than two dozen Committee and Professional Develop Group meetings

the Welcome Reception, which provides the perfect venue for socialising, introductions, reconnecting and networking among Association Members, new Members, colleagues, students, and first-time attendees.

Sunday will be filled with more than two dozen Committee and Professional Development Group (PDG) meetings, all aimed at offering attendees the opportunity to share a wealth of knowledge and expertise while guiding the efforts of IAFP.

Panel discussions are a key feature of IAFP

IAFP 2014 officially begins on Sunday 3 August 2014, with the Opening Session. Recipients of the Fellow Award, the Travel Award and the Student Travel Scholarship will recognised, followed by the Ivan Parkin Lecture. This year's honored lecturer is William (Bill) Marler, Managing Partner with Marler Clark, LLP PS, speaking on '20 Years Later, Where Were We, Where are We and Where are We Going?' An accomplished personal injury trial lawyer and national expert in foodborne illness litigation, Mr Marler has been a major force in food safety policy in the U.S. and abroad. Following the lecture, a Cheese and Wine Reception will be held in the Exhibit Hall, where attendees can view more than 150 companies demonstrating the latest products and technologies in food safety.

Monday 4 August launches three days of sessions with more than 900 presentations, including 52 symposia, 15 roundtable sessions, 132 technical presentations, and more than 500 poster presentations. A sample of symposium topics includes: Cyclospora – Recent Foodborne Outbreaks and Challenges; Food Traceability: Important for Food Safety and Indispensable for Food Defense; and Celebrating 100 Years of Food Safety.

Student Members play a key part in the Association and at our Annual Meeting; they are the future of IAFP. This meeting gives students the perfect opportunity to network with seasoned food safety professionals, become involved and foster long-lasting relationships with their student peer group, and share their research and results during their presentations. Students are provided with dedicated events such as the Student Luncheon and the Student Mixer, where they can be among other young leaders to effectively exchange information and experiences.

Other scheduled events taking place throughout the conference include the Silent Auction, which generates valuable revenue each year for the IAFP Foundation, and the Annual Business Meeting.

Wednesday afternoon's Closing Session will officially end the educational part of the conference with the John H. Silliker Lecture, 'Bringing Science-based Risk Analysis to Practice to Further Improve Food Safety' featuring Leon Gorris. Dr Gorris is Director for Regulatory Affairs in North Asia for Unilever R&D Shanghai, China and has served in various roles with Unilever since 1998, including as Food Microbiology Expert and as Consumer Products Safety Risk Assessor.

Wednesday evening's Awards Banquet will appropriately close out IAFP 2014 by honoring outstanding food safety professionals for their contributions during the past year and throughout their careers. Special recognition will also be given to six students who will receive a Developing Scientist Award for research presented during the conference.

The Indiana Convention Center will host IAFP 2014

IAFP 2014 promises to be another exceptional and record-breaking event in Advancing Food Safety Worldwide®. We look forward to seeing you there!

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International Association for Food Protection

For more information or to register for the event, please visit :
www.foodprotection.org/annualmeeting

Connect4Action: Improving communication between key players in the food innovation process

Novel food products and technologies sometimes fail in the marketplace due to a lack of communication between food product/technology experts and consumer insight experts in the innovation process. The objective of Connect4Action is to improve communication between consumers, consumer scientists, food technology developers, and other key players in the new product/technology development and commercialisation process. This will contribute to an improvement of the multidisciplinary dialogue and is expected to result in new food products that are superior in targeting the true needs and desires of consumers, thereby lowering the failure rate of new food technologies in Europe.

Consumer-to-business and business-to-consumer; what is missing?

Improved communication between food technology developers and consumers finds its basis in making the voice of the consumer heard, as a starting point for new product ideas and consumer-desired product design. Two literature reviews regarding consumer-led new product development (NPD) and the food innovation process were conducted within the Connect4Action project. The first focused on communication between consumers and those responsible for consumer science within companies (external communication). The second review focused on communication between functional disciplines (mainly food technology developers and consumer scientists) within the company (internal communication).

Communication between food companies and end consumers takes place in three different processes of information exchange:

- Understanding of consumer needs and wants (consumer-to-business communication)
- Identification of consumer reactions to ideas developed by the company (business-to-consumer communication)
- Interactive approaches, where end consumers become co-designers of the new product (two way business-and-consumer communication).

The literature review shows that current insight is dominated by business-to-consumer and consumer-to-business communication. However, both of these fields have developed in poorly integrated ways with a lack of truly coherent approaches. What is missing is a shared thinking, as well as agreed models and approaches on how consumers make decisions in terms of new product acceptance and adoption. Although a large variety of drivers of new product acceptance have been identified, the lack of shared models and thinking prevents a critical insight into the relative importance of these drivers in different contexts and situations. Interactive means of communication, in which consumers are actually co-designers in the NPD process (rather than kept at arm's length), have emerged in several industries. However, the literature review shows that such approaches are virtually absent in the food industry.

Internal communication on the other hand involves the communication between functional disciplines within the company. The literature review identified a large number of potential barriers and facilitators for an effective and efficient internal communication. These reside primarily at the level of organisational structure, team composition, management support, and knowledge management. In addition, the optimal intensity of communication between different functional disciplines was found to depend on the level of external (market turbulence) and internal (technological) uncertainty, and the phase of the NPD process, with a higher need for communication at an earlier point (e.g. opportunity identification), compared to later stages.

An important conclusion is that there is a shortage of studies aimed at the food industry. Optimal communication models designed for the food industry are required, encompassing validated and interactive means of communication. Further research should focus on specifics of the food industry (e.g. dominance of SMEs in this sector) with a focus on understanding the internal communication process itself (rather than focusing on its outcomes), leaving room for the identification of cultural differences and the role of those in business culture.

Involving key players in the common dialogue

In addition to science and product experts working with technology and processing issues, new technology development (NTD) and NPD need input from different actors in the food domain. Consumers, regulators, and different interest groups should be considered as possible stakeholders affecting the final acceptance of new technologies and products. These possible interest groups have to be identified on a case-by-case basis and assess whether involving them in the process would be beneficial to the NTD and NPD processes. An open dialogue with different interest groups may enable an early detection of possible barriers and facilitators for the acceptance of new products and technologies.

In the innovation process, food science and technology background provides expertise to develop tangible product attributes, while consumer and market experts try to understand what the consumer needs and desires are and how to communicate the product benefits to the consumer. Combining these different types of expertise requires successful communication within the innovation process. In Connect4Action, we have extracted recommendations on facilitators and possible barriers of communication from the literature and a survey with stakeholders.

What thwarts and what enhances effective communication?

The main barrier for successful communication is the different language that people coming from different disciplinary backgrounds use. To overcome this barrier there needs to be sufficient time and possibility to develop a shared language and understanding of the goals in innovation processes. Organising the innovation activities in cross-functional teams promotes the communication between the disciplines through formal meetings where experts with different backgrounds participate. Furthermore, knowledge management systems, which allow efficient data sharing so that different experts have an access to information gathered both by consumer and technology experts, can promote communication.

There is, however, a balance between formalising and centralising the NPD and NTD activities. Decentralised teams are empowered to make decisions about how to proceed (or not) in NTD/NPD processes, but at the same time the decisions made in single teams may not be accessible to the whole organisation. Formalisation of activities will improve communication between marketing and R&D because the team members are forced to share information at scheduled face-to-face meetings or via knowledge management systems. A high level of formalisation leads to better integration between marketing and R&D due to less role ambiguity and conflict between functions, but should also allow the more informal encounters. Finding the right balance of empowering the teams to pursue their set goals and keeping up sufficient information flow from development teams to the other parts of the organisation requires case-by-case assessment.

Building successful communication between food and consumer experts in NTD and NPD processes requires support from management by organising activities in cross-functional teams, providing necessary resources for adequate knowledge management systems and time for building the shared language. Rewarding the innovation teams on their shared performance achievement of commonly set goals is important rather than rewarding each discipline based on their individual tasks.

A common language between consumer and food experts enables developing shared goals at the early stages of idea generation and screening. If different experts working in the innovation process have a collective understanding of what the goals of the process are, they are able to work more independently in the latter stages of development without losing common direction. Furthermore, better understanding of other professionals' input creates trust and makes the information coming from other experts more actionable.

The more radically innovative the new technology or product is, the more important it is to involve both consumer and food experts in the development process at early stages. The same applies to market uncertainty. Radical innovations or high market uncertainty indicate higher risk for consumer acceptance, and therefore understanding the market and being able to anticipate the market responses is crucial for the success of new technologies and products.

Connect4Action in the spotlight

At the moment, we are finalising the toolbox and accompanying training modules that have been developed with the aim to assist communication during the food innovation process. More information can be found on our project website: www.connect4action.eu/toolbox. These, and other updates, are managed by the European Food Information Council (EUFIC); dissemination leader in this project.

The project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration (Grant Agreement No 289023).

To learn more about the Connect4Action project, visit the project website: www.connect4action.eu or contact project coordinator, Karin Zimmermann at: karin.zimmermann@wur.nl

Upcoming events

Connect4Action is organising two final workshops on this toolbox. The trainings are targeted at industry (25 November 2014, Uppsala) and young academics (30-31 October 2014, Brussels) and will present a final toolbox as well as training modules. Participants will learn how to apply these at their own company or university.

If you are interested in participating in one of these workshops, please contact Katerina Palascha at: katerina.palascha@eufic.org. A few travel scholarships are available. Please also check www.connect4action.eu for updates on the final conference which will take place in Brussels on 29 October 2014, in conjunction with our partner project RECAPT.

Further reading

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Patricia Aron Senior Hops Chemist, MillerCoors

A perspective on beer flavour stability

The educated beer consumer's heightened expectations have changed the game in terms of beer quality. Today's beer drinkers are more sophisticated, fickle and less forgiving when it comes to beer flavour. Consequently, flavour instability is now one of the most critical quality problems faced by the brewing industry. Achieving beer quality in terms of flavour and flavour stability can be complex, especially considering the variety of acceptable beer flavours and styles appreciated by consumers. Therefore, there is a need for brewers to understand the underlying mechanisms known to cause flavour changes during beer aging, and which quality control mechanisms and quality assurance techniques can help them in their journey toward greater beer quality and consistency.

Beer flavour stability

In a perfect world a beer would taste the same at package release as it does at the end of its shelf life. In reality, beer begins to undergo flavour modifications as soon as it leaves the brewery with more brewers now competing in expansive domestic and global markets. Shipment of beer to distant markets takes time and thus requires best-buy/pull dates of six months to a year. When shipped and stored under refrigeration, beer is likely to stay fresh up to anticipated shelf life. That said, the shipment and storage of beer under refrigeration adds cost and is not always feasible.

Most beers have expected shelf lives of 17-26 weeks. Imports have longer shelf lives and draught shorter at 9-13 weeks. A typical domestic lager stored at room temperature (75°F) is expected to stay fresh for up to 17 weeks. Yet, for each day stored 10 degrees above 75°F, a domestic lager will lose up to two days of shelf life (**Figure 1**, page 37). Prediction of a beer's actual shelf life can be achieved by force aging under accelerated conditions (augmented temperature). Evaluation of beer flavour changes at several time points provides useful information for shelf life prediction and insight into potential process modifications a brewer may need to make to increase product flavour stability. All that is required to

gather this type of information is a discerning palate, or a collective group of discerning palates in the form of a sensory panel. For those brewers especially concerned about flavour consistency and stability, the next step is to look at the installation of instrumentation for quality control and quality assurance monitoring.

Impact on flavour

Despite numerous focused studies and application of highly sophisticated analytical instrumentation, forming a comprehensive picture of beer flavour instability remains a challenge. In general, beer aging is characterised by decreased bitter taste, increased sweet/caramel taste, ribes (black currant), toffee and sherry-like aromas. However, each brew is truly unique and no single compound or measurement exists to adequately evaluate the many facets of beer aging for all styles. Pale and dry-hopped beers are generally of higher susceptibility than dark or heavily kettle-hopped beers.

Hundreds of compounds from various chemical groups have been associated with beer flavour modification during aging[1]. These components may take part in one or more chemical reactions including, but not limited to: Maillard reactions, formation of linear aldehydes and esters, ester degradation, acetal formation, etherification, degradation of hop bittering acids and polyphenol formation/interaction. Occurrence of each reaction largely depends on the beer type, raw materials used and exposure to the major beer enemies: oxygen, light, temperature and time[2,3]. Although many of the aging mechanisms are associated with oxidation, non-oxidative mechanisms may also occur[4].

Because yeast is the engine of beer making, yeast health is vital

Non-oxidative flavour modification reactions include esterifications, etherifications, Maillard reactions and glycoside and ester hydrolysis[3]. These types of reactions can be impactful for beers that are bottle conditioned and for dry-hopped beers that are high in glycosidically-bound aroma precursors. During bottle conditioning, hungry yeast will attack bound sugars of glycosides to release volatile floral aromatics such as linalool and geraniol. Several studies show that refermentation or bottle conditioning can markedly improve the overall profile of the beer. Other reactions, such as esterifications between carboxylic acids and alcohols may positively change the aroma profiles from pungent to fruity. Maillard reactions tend to lead to flavour formation of caramel or cooked notes, but are of potentially low impact given their high flavour thresholds.

Oxygen in beer

Limiting dissolved oxygen levels in packaged beer to below 50ug/L should prevent most undesirable effects on flavour stability. However, this is not necessarily achievable in all packaging facilities, and thus quality control criteria for packaged oxygen can be set at 0.2 mg/L or less, with modern filling equipment capable of achieving 0.1 mg/L total package oxygen[1,5]. Reactive oxygen species (ROS) are thought to be largely responsible for aged beer flavour formation[6,7]. Such reactive oxygen species can be of the radical form (nitrogen and oxygen) or non-radical form in that they have the potential to convert to oxidising radicals. The measurement of total package oxygen in finished product is a good quality assurance practice for the modern brewer. However, in practice molecular oxygen levels do not directly equate to flavour deterioration. Oxygen related reactions are thus thought to proceed via free radical formation through transition metal catalysis.

Metals as protagonistic catalysts

Metals play a defining role in beer style. When concentrations reach beyond what is needed for pH adjustment and yeast health (e.g., zinc), metals become protagonists of beer flavour and flavour stability. Excess iron in beer can lead to metallic off-taste and high manganese has also been attributed to sherry-like, off-flavour formation during beer aging iron can also behave as a catalyst that facilitate oxidation mechanisms through radical generation via the Fenton and Haber-Weiss reactions. Copper and manganese are also capable of catalysing reactions to produce ROS and is suspected to act synergistically with iron to catalyse oxidative staling reactions[8]. Because even trace amounts of transition metals may cause detriment to beer flavour stability, the brewer should make every effort to keep levels under 50 ppb at all stages of the brewing process. That said, raw materials (water, malt and hops) can have higher than acceptable levels of transition metals. Typically, transition metal concentrations will decrease during fermentation, as yeast absorb and intracellularly distribute transition metals. Over several repitches, this can affect yeast health and subsequently affect the flavour stability of the finished product. Brewers also need to pay attention to interaction with filtration media such as Diatomaceous earth, which has the potential to contribute to beer soluble iron. In a well-equipped quality assurance lab metal content can be monitored by inductively coupled plasma – atomic emission spectroscopy (ICP-AES).

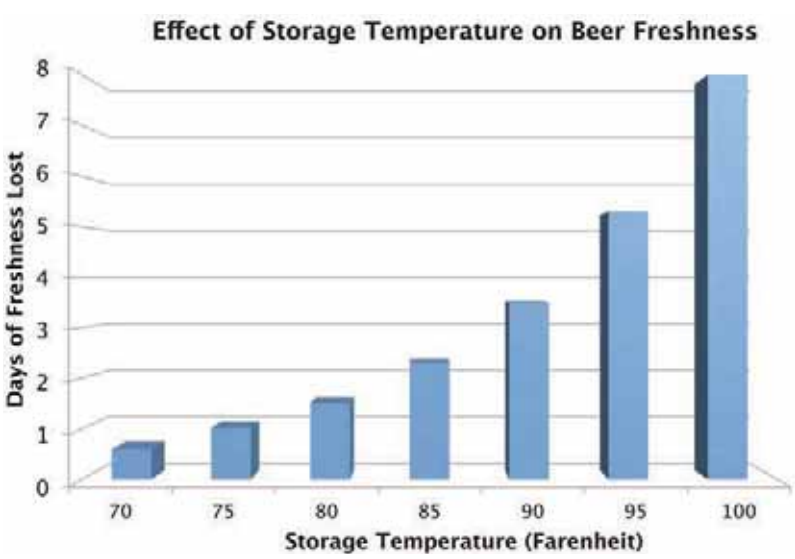

Figure 1: The accelerated aging that takes place when beer is stored at varying temperatures above 75°F (most shelf life is based of storage at 75°F). An increase of 10°F can result in more than two days of lost shelf life per day. Beer stored at 100°F will lose eight days of freshness for each day it is stored at 100°F, effectively shortening the shelf life from 17 to two weeks.

Aldehydes as staling indicators

Aldehydes are monitored as staling indicators in beer because of their tendency to increase during aging and

their relatively low flavour and aroma thresholds (sub-ppb). During beer aging, amino acids such as leucine and phenylalanine can undergo Strecker degradation to form aldehydes of high aroma impact: Isovaleraldehyde (threshold 46 ppb as malty, cherry, apple, almond) and phenylacetaldehyde [threshold <100 ppb as floral, roses[9]]. Strecker degradation begins during the brewing process and will progress during beer storage. Aldehydes pertinent to beer aging can result from oxidation reactions, Maillard reactions and from the degradation of proteins. The formation of trans-2-nonenal (cardboard/papery flavour) in beer is thought to align well with oxidative aging. Trans-2-nonenal levels of less than 1 ppb can significantly impact the flavour of a pale lager beer. Hence, brewers with access to sophisticated instrumentation can monitor levels of trans-2-nonenal and its precursors throughout the brewing process. Despite its relatively high flavour threshold, the Maillard product furaldehyde forms due to heat stress and is thus used as a staling indicator relevant to temperature exposure. Other equally flavour-inactive aldehydes are monitored and used as aged beer flavour indicators because their concentrations increase along with increases in known oxidative flavours[9]. Although a majority of aldehydes found in beer are thought to derive from malt precursors, hop acid side chain degradation (organic acids) and yeast metabolism (short chain fatty acids) can also lead to aldehyde formation. Aldehydes can be quantified via use of Gas Chromatography with Flame Ionization Detection (GC-FID) or mass spectrometer detection (GC-MS). This can be done in conjunction with a derivatisation agent and solid phase micro extraction fibres (SPME) or stir bar sorptive extraction (also known as twister) (SBME). GC-Olfactometry (GC-O) can also be incredibly useful to monitor flavour formation.

Yeast and flavour formation

Healthy, happy yeast will cleave sugars to produce ethanol, carbon dioxide, and heat as well as various secondary metabolites: esters, carboxylic/fatty acids, phenolics, sulfur components, etc.[1]. Yeast ability to produce specific secondary metabolites, both desirable and undesirable, varies by strain, and thus strain can be highly impactful on overall beer flavour. In general Ale yeasts (*saccharomyces cerevisae*) produce more esters than lager yeasts (*saccharomyces pastorianus*). Both ale and lager yeasts produce Ethyl acetate (solvent, nail polish). Ethyl acetate is ethanol ester, and is most predominant in beer because ethanol is the predominant alcohol produced by beer yeast. Ethyl acetate has a relatively high aroma threshold in beer which is why even higher concentrations do not impact the overall aroma profile of the beer. Other esters such as isoamyl acetate (banana) are detectable by nose in even the lightest of lager beers because they have a relatively low flavour threshold and can reach significant levels during fermentation. As beer ages, levels of fruity and floral esters tend to decrease. However, some esters may actually form during aging due to ethanolic esterification of carboxylic acids during beer storage. Acetate esters of higher alcohols and ethyl esters of long chain fatty acids (butyric, caprylic and proprionic) can result in pungent off flavours described as 'cheesy', 'goaty' and 'milky'. Higher alcohols or fusel alcohols can be important flavour contributors, imparting solvent and floral notes to beer. During aging, ethanol can also oxidise into acetaldehyde (green apple note). Increases in acetaldehyde can be monitored as a staling indicator in beer. Yeasts also produce the notoriousvicinal diketones (VDKs) such as diacetyl, which has a flavour note of butter. Typically VDKs and precursors are monitored at the end of fermentation to ensure that levels are low enough for aging and package release. Consequently, any VDK precursors remaining in beer can lead to off-flavour formation post-package. VDK analysis can be done using spectrophotometry; however, it is relatively less sensitive than GC-ECD. Not all, but some ale yeast used for Saisons, Heffeweisens and Belgian style ales possess the enzyme necessary to convert ferulic acid to the phenolic 4-vinylguiaicol (clove-like), which carries through to the finished beer. However, this phenolic note tends to decrease during beer aging.

Other potent flavour compounds that derive from yeast belong to the sulphur family. Yeasts metabolise sulphur containing amino acids such as cysteine and methionine to produce some rather pungent molecules ranging from rotten eggs, onion, burnt match to cooked cabbage and sweet corn. Some sulphur components such as dimethyl sulfide (DMS, sweet corn note) originate from malt constituents. DMS can be removed by vigorous wort boiling in the kettle. However, if there is any S-methylmethionine precursor left in the wort, it may convert to DMS in the packaged beer.

Because yeast is the engine of beer making, yeast health is vital. The presence of spoilage bacteria can significantly alter pH and flavour attributes of beer. If a consumer detects flavours such as musty, mouldy,

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medicinal, sour, bready, horsey or farm-like in their beer, spoilage organisms have surely been at work.

Hop bittering acid degradation

Most commercial beers contain hops or hop products. Hops help the brewer in many ways: they provide flavour, aroma and bitterness, aid in wort clarification, act as antioxidants and antimicrobials, provide foam capacity, texture and can improve the overall flavour stability of beer. The main bittering components of beer, the isomerised-alpha acids, are also susceptible to aging.

Oxidation, heat stress and light are all enemies of the hop acids. Over time, *iso*-alpha acids in beer begin to degrade which results in decreased beer bitterness. Because the trans-stereoisomers of the three main iso-alpha acid analogues are less energy favourable and degrade more quickly during storage, the ratio of trans/cis-iso-alpha acids can be used as a staling indicator. Hop bittering acid content of beers can be monitored using High pressure liquid chromatography (HPLC) in conjunction with solid phase extraction (SPE). Thermal degradation or heat stress of the hop acids (both in the hops and in the beer) can yield pungent organic acids such as butyric (vomit), valeric (cheesy) and isovaleric acids (cheesy/sweaty). In the presence of ethanol, these organic acids may undergo esterification into somewhat less offensive, fruity esters. Hop acids in leaf (whole cone), pellet and extract form are all susceptible to the same degradation reactions and thus, if not stored properly, may develop similar cheesy aromas. Light is an enemy that can be battled, if a brewer so chooses. Exposure to light energy instigates a reaction between iso-alpha acids degradation products and sulphur in the beer matrix to produce lightstruck or skunky character. Brown glass, cans and kegs all block the wavelengths that cause lightstruck character. Conversely, beer packaged in blue, green or flint-glass is susceptible. The culprit, 3-methyl-2 butene-1 thiol (MBT), begins to accumulate within minutes in beer exposed to sunlight. The reaction occurs less readily under UV lights from a lit display case, but it will eventually occur. Modified hop extracts that are not susceptible to photo-degradation are commercially available from several vendors. However, use of modified extracts for beer bittering may result in decreased antioxidant potential and thus sacrificed flavour stability. Hop derived off flavours are best monitored by GC-FID or GC-MS by sampling the headspace. Detection of lightstruck character is easily done by sensory panel due to thiol low flavour thresholds. Analytical detection of thiols such as MBT requires highly sophisticated instrumentation with sulphur detection capabilities.

Endogenous antioxidants in hops and malt

Polyphenol capacity to improve food oxidative stability has been well documented. Therefore, it seems realistic for brewers to look toward polyphenols as potential antioxidants with a capacity to improve beer flavour stability[10]. Hops and malt contain polyphenols of the flavonoid class (proanthocyanidins, flavonols, and flavan-3-ol monomers, such as (+)-catechin and (-)-epicatechin). Although the same polyphenols are known to influence oxidative mechanisms responsible for aged food flavours, the brewing industry is still wavering on their practical effectiveness. To be certain, too little is understood regarding their impact, or their exclusion (as in the use of hop extracts) on aged beer flavour formation.

Addition of hops and hop vegetative matter to the kettle undoubtedly can impart overall flavour, as well as improve overall beer flavour and shelf life. Brewing trials from various geographic origins indicate that the most potent punch seems to come from kettle hopping with pellets and that beneficial flavour attributes do derive from the hop vegetative matter that is considered 'spent' by the hop extract industry (CO_2 extraction removes most of the bittering components and oils)[11]. To date, at least five patents have been filed in reference to the advantages of brewing with hop polyphenols. The main challenge with research on polyphenols lies in the difficulty of extracting and analysing them. Analysis of polyphenols can be done via spectrophotometry, however the methods are largely unspecific. HPLC-MS is much more specific, yet is costly, complicated and time-consuming. Rather than characterising and quantifying specific polyphenols, analysing the total antioxidant or anti-radical capacity of the beer or hop products can be useful. Antioxidant capacity or anti-radical capacity of beer and raw materials may be assessed via spectrophotometric methods (DPPH and FRAP) or via ESR. However, comparison of results from ESR to other antioxidant capacity methods reveals a sort of polyphenol/flavour stability paradox in that the different analytical methods do not always align.

In summary, flavour instability is of growing concern for many brewers. Achievement of beer quality in terms of flavour can be extremely challenging, especially considering the multifaceted mechanisms at play. Having an understanding of beer flavour origins and modifications that occur post-package will help brewers work towards their flavour stability goals, no matter the distance between brewery and thirsty consumer.

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About the Author

Patricia Aron obtained a B.S. in Biochemistry from Elmira College, Elmira, NY. She obtained both her MS and PhD degrees at Oregon State University, Corvallis, OR. During her MS, Patricia studied Enology (Wine Chemistry) and focused on investigating polyphenol extraction during red wine maceration. Patricia's PhD work focused on lager beer flavour and flavour stability as it pertains to hopping technology. Patricia began working at MillerCoors in Milwaukee, WI in 2010. She currently functions as the Senior Hop Chemist in the Brewing Materials Group for MillerCoors Brewing Research, Innovation and Quality department.

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Gaskets and seals for food equipment

You rarely find people talking enthusiastically about seals and gaskets – usually they are only the subject of interest if there is an obvious failure in an application, such as slippery oil puddles on a floor or hot steam spray from a leaking heat exchanger. However, it is the silent seal failures, where, for example, a product can leak into a closed cavity behind a seal and becomes spoiled, that are often of greater concern to the food industry. This article aims to provide an overview about the special requirements of hygienic design seals in food equipment and also the current solution principles of static and dynamic seals. The upcoming new EHEDG guideline will deal with the subject of seals and offer a great amount of help to the designer and also the user who wants to select a good hygienic sealing solution.

How does a seal work?

If you exclude the subject of dynamic leakage or permeation of gases through seal materials, the answer is simple. A seal works – that is, it seals – when the contact pressure between the seal and the mating surfaces of the parts connected is higher than the system pressure. A seal manufacturer will take this simple fact into consideration by designing a proper interference between the seal and the recommended installation groove. The groove is defined by shape as well as dimensions and allowable tolerances. **Figure 1** (page 42) shows the basic principle when a seal is installed by compression and the resulting compressive forces of the seal material create a normal force on the contact surface of the housing. Any added system pressure increases the contact force, because rubber materials act like high-viscous fluids.

Seal materials in food contact

A few sentences about seal materials are required, although this article is focusing on the hygienic design. For seals in product contact, two groups of materials cover the large majority of applications. They are able to create and maintain a proper contact pressure by deformation. The main material group consists of rubber materials, also called elastomers. Rubber materials are highly-viscous polymeric materials which are cross-linked to create the elastomeric properties. The most frequently used materials according to ISO 1629 abbreviation are the synthetic rubbers EPDM, FKM, VMQ, HNBR, NBR and FFKM.

The second material group is the thermoplastic materials which are non cross-linked polymers that often need an elastomeric energiser or metal springs which ensure a long-lasting contact pressure (**Figure 2**, page 42). This is due to the fact that thermoplastic materials suffer from cold-flow and the initial contact forces often diminish too quickly to achieve a good service life of the seal.

For industrial or special applications, other seal or gasket materials like metal or graphite-metal, elastomer-bonded fibre materials or

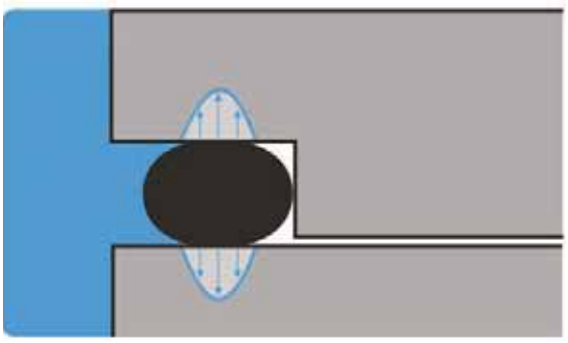

Figure 1: O-ring sealing principle

graphite packings are used. For scrapers, TPE materials, ThermoPlastic Elastomers offer good elastic properties combined with often superior wear resistance. Particularly Polyurethane compounds – which are also available as food grade materials – are utilised.

All materials have to meet the regulatory requirements which are often a combination of formulation and testing requirements. In Europe, the framework regulation (EU) No. 1935/2004 is the main regulation that requires all types of food contact materials to be not harmful to consumer health and to not change the organoleptic properties of the food. Also, it requires Good Manufacturing Practice (GMP) to be applied in the production of the materials, the establishing of a tracking system, proper documentation and a proper labelling of the articles made of those materials. For plastic materials, Regulation (EU) No. 10/2011 must be met. For exports into the United States of America, mainly the 21CFR177 applies. Alternatively, materials or substances may have a registered FCS number allocated to the manufacturer by the FDA.

Polymer seals usually die slowly and quietly

It is important for any user to know that polymeric seal materials will lose their initial resilience and consequently, normal contact forces will slowly diminish until finally, leakage occurs. This stress relaxation of the material is expressed as Compression Set (CS) which is calculated by the following equation defined in ISO 815 Standard: CS%= (h0-h2)/(h0-h1)*100% (**Figure 3**). The standard test piece is a cylindrical button with a diameter of 13mm and 6mm height.

Typical values of food grade rubber materials are compression sets of 20 to 25 per cent after compression by 25 per cent of the original thickness for 22 hours at a temperature close to the limit temperature in air. The lower the CS value, the higher the potential service life. Of course, this process slows down, but never really stops.

In a figurative sense, polymeric seals are shape shifters, meaning that the original cross sectional shape of a new seal is slowly adapting to the housing to which it is installed. It's only a matter of time, temperature and chemical activity, until this change in shape will lead first to microbial permeability and then to fluid leakage. In aseptic processes, this fact may lead to product contamination that may only be detected later on, because there is no visible fluid leakage but there are already movements of the seal in its groove that could transport matter in either direction.

That's why it's of utmost importance for end users to establish preventive maintenance intervals depending on the seal material and the operating conditions in a certain application. Alternatively, the supplier of equipment may establish such intervals for given applications.

Hygienic aspects

First of all it is important to recognise the fact that a seal alone cannot achieve a hygienic joint. The seal and the housing design must match and be designed to achieve a hygienic and easy-to-clean seal when installed. Also, the groove must be cleanable when replacing a worn seal. Cleanability is achieved by good surface quality, sufficient groove radii and groove dimensions that make all areas accessible for cleaning and inspection.

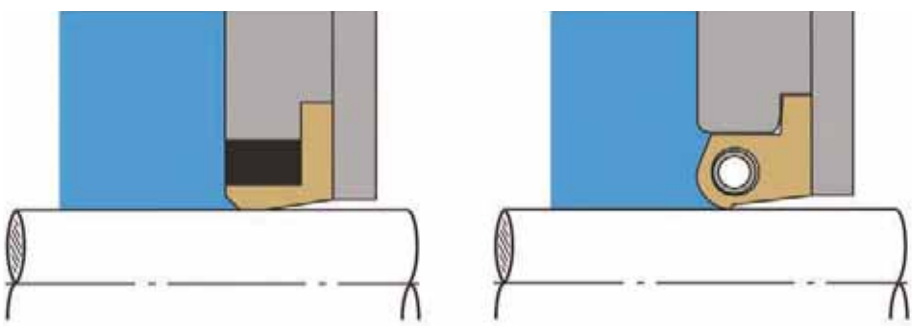

Figure 2: Energised thermoplastic seals

Important hygienic aspects for static seals are:

1) **Selection of a suitable seal material**, providing sufficient chemical resistance in the environment of intended use, meeting regulatory requirements and being non-toxic, non-mutagenic and non-carcinogenic, non-absorbent to microorganisms or spores and non-porous. Pores in rubber could e.g. develop unintentionally during production, when adding too much activator to the formulation in order to accelerate vulcanisation and thus reducing production costs. Also, the material shall not absorb water or cleaning fluids at a level that may compromise hygienic integrity or even destroy the seal. A volume swell of less than five per cent of

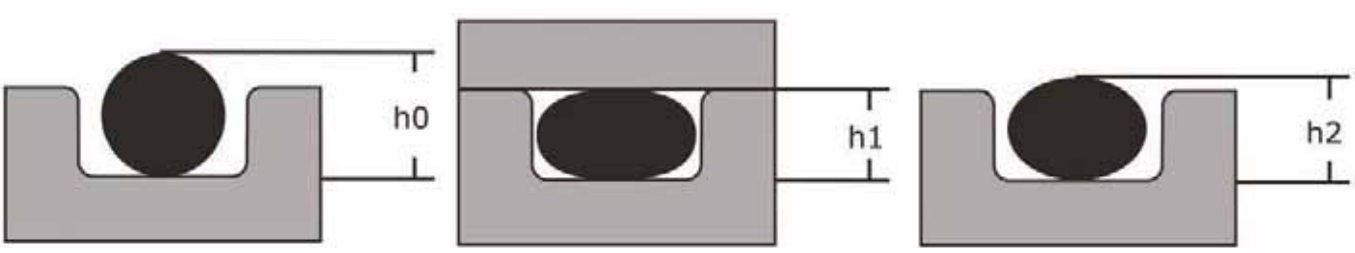

Figure 3: Compression set

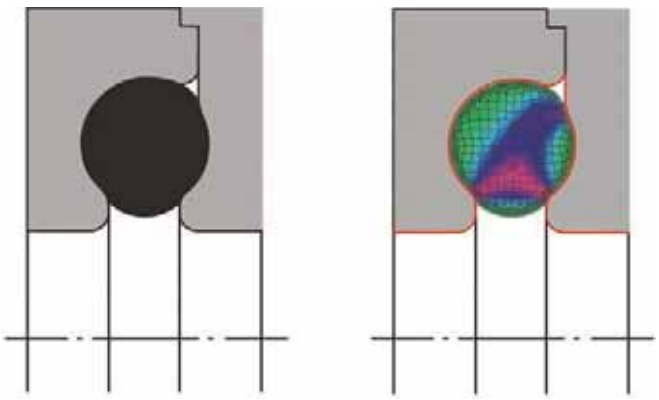

Figure 4: DIN 11864 hygienic coupling

the original seal volume is usually acceptable, e.g. in a groove design according to DIN 11864 which allows for a certain amount of volume swell and thermal expansion.

2) **Front-flush seal design** shall prevent dead areas where a product can stagnate and spoil during production or cannot be effectively removed during CIP cleaning (of course, for manually cleaned equipment, the situation is different). Front-flush design shall ensure a good accessibility of the entire surface to cleaning fluids. A standard 'hydraulic' rectangular O-ring groove design with a short area of metal-to-metal contact on the product side is a hygienic 'no-go'.
3) **Proper alignment** of all parts is important to ensure drain ability and avoid unsupported gasket areas. If a gasket is not completely supported (compressed) from both sides, a non-cleanable crevice condition will emerge.
4) **Controlled compression**, usually achieved by a metal (or plastic) stop on the non-product side of a polymer seal avoids over-compression or under-compression. Both situations can pose a hygienic hazard. Under-compression can lead to bacterial permeability or even fluid leakage, over-compression can damage or even destroy the seal. Standard ISO 14159 recommends 15 per cent compression of a 70 Sh A hardness elastomer O-ring to achieve bacteria tightness. Required compression depends, for example, on the shape of the seal and hardness of the rubber material.

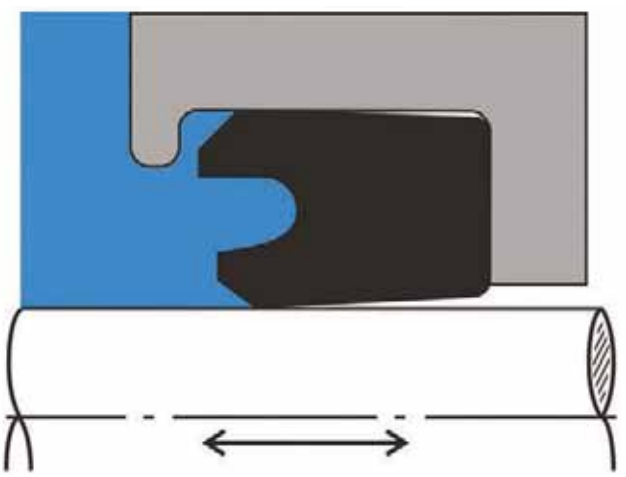

Figure 5: Snap-in rod seal

5) **Groove-fill** and expansion room for polymeric seals. Calculation of a suitable groove for a certain seal would be easy if there wasn't the effect of thermal expansion and volume swell. Rubber materials can

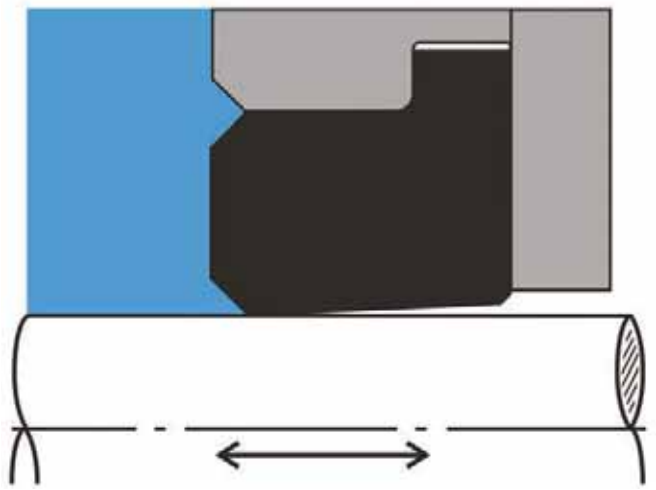

Figure 6: Flanged rod seal

have thermal expansion coefficients of up to 350 x 10^{-6}/K, which is more than 20 times higher than the coefficient of stainless steel. Volume swell is also often an issue, since e.g. rubber materials are usually of either polar or non-polar structure and they are comparable with a high-viscosity fluid, they are supposed to swell when immersed in fluids of the same character. Swelling is a physical and reversible action. For example, a polar Fluorocarbon material (FKM) takes in a certain amount of water or water-based cleaning fluids. If the amount of thermal expansion and the to be expected volume swell are not considered by the groove design, damage of the seal up to the extrusion of seal material out of the groove can be observed.

6) Surface roughness is also a key to a hygienic sealing joint. If the surface of the seal groove is too rough, the valleys of the surface roughness can harbour microorganisms and spores or can even lead to gas or fluid leakage. Also the seal itself should have a good surface quality. A value of Ra 0,8µm for both seal and groove surface usually allows for a good hygienic sealing. For rubber gaskets, it's also important to have no offset between the mould-halves and all flash removed on the finished part. The best hygienic surface is offered by an unharmed, closed 'mould skin'.

Hygienic solutions for static seals

The term 'static seal' is used when the two parts joined do not move in relation to each other, e.g. on pipe couplings or valve halves joined together. For such applications, the O-ring with a round cross section (ISO 3601-1 and American standard AS 568 define dimensions and tolerances) is the most widely used sealing element. One standardised solution is the DIN 11864 coupling (**Figure 4**, page 43) that was developed by the use of Finite Element Analysis (FEA), a numerical method using a lattice structure of the sealing element that uses mechanical data like the coefficient of thermal expansion or stress-strain curves established on test sheets of the material. By FEA it is possible to simulate the behaviour of a seal during installation, under pressure and swell or thermal cycling. This DIN 11864 design considers the material properties of typically used rubber materials like e.g. FKM, EPDM or VMQ and allows for thermal expansion without destruction of the O-ring. Also, the seal element is safely kept in place to avoid a pumping effect that could transport microorganisms from the environment to the product area. The highest contact pressure is directly at the break-off point on the product side. Similar solutions covering the same principles have also been developed by a number of companies. There are a range of other pipe couplings and process connections. In the US, couplings according to ISO 2852 / DIN 32676 are the most widely used hygienic connections. EHEDG established a position paper of EHEDG-approved couplings and process connections. The EHEDG guideline No. 16 also provides a great deal of insight into static sealing[1].

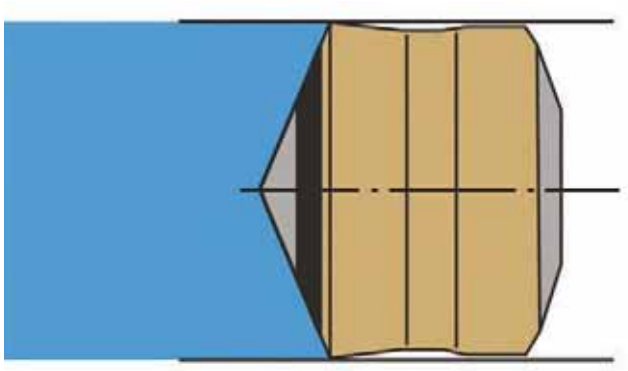

Figure 7: Hygienic dosing piston

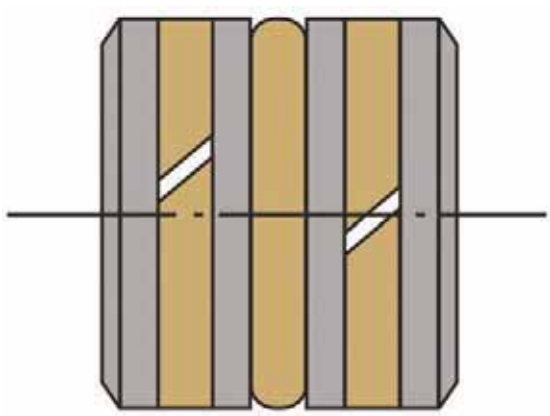

Figure 8: Hydraulic piston (non-hygienic)

Piston seals and rod seals

Piston seals and rod seals fall under the category of 'linear dynamic seals'. They are used to seal, for example, a plunger piston in a homogeniser or a piston in a dosing unit for viscous products in a filling machine. Rod seals are necessary to seal e.g. the rods of dosing pistons or valve stems against the atmospheric drive side. Rod seals are usually mounted in the housing whereas piston seals are mounted on the moving piston.

Seals shall form a barrier between either the product or the hygienic zone against the atmosphere. Such seals are available in hygienic design. See **Figure 5** (page 43) and **Figure 6** for examples for rod seals. A piston seal would look similar, just inside out with the dynamic sealing lip on the outside. For aseptic sealing, which requires hygienic design plus being impermeable to microorganisms, a suitable double seal arrangement or a hermetic sealing with bellows or diaphragms is required to avoid microorganisms and spores being drawn from the environment into the product area.

From a hygienic point of view, it's easier to design a hygienic rod seal than a hygienic piston seal, because for rod seal arrangements, split

groove designs and clamping devices can be located outside of the product area. An optimum in hygienic design for a piston can be achieved by e.g. a metal-rubber-plastic bonded part with neither crevices nor hollow spaces and bearing area incorporated (see **Figure 7**, page 44). **Figure 8** (page 44) shows a standard hydraulic piston with countless crevices in comparison.

Shaft seals

Shaft seals are sealing rotary movements. The majority of shaft seals in food equipment are probably mechanical meals, which seal axially by two hard carbon or ceramic rings, one of them being axially spring loaded. Mechanical seals are an own class of sealing systems and are not covered by this article. Centrifugal pumps usually use such sealing systems, because they've got a long wear life potential and can cope with high sliding speeds.

Radial oil seals, which are commonly used in combustion engines and gearboxes to seal the rotary shafts usually cannot be used in product contact because the garter spring that keeps the sealing lip in contact with the shaft is difficult to clean and does not meet hygienic design principles.

Shaft sealing with rubber or plastic seals is a difficult task in food equipment where the product is often not lubricating or even abrasive, containing fruit pips, fibers or crystallising sugar. Also, the sealing lip is always running at the same place. This can cause problems with friction heat but also wear marks on the shaft, which is usually made of stainless steel. So, a matching system of seal material and shaft hardening or coating needs to be engineered and its suitability validated by testing.

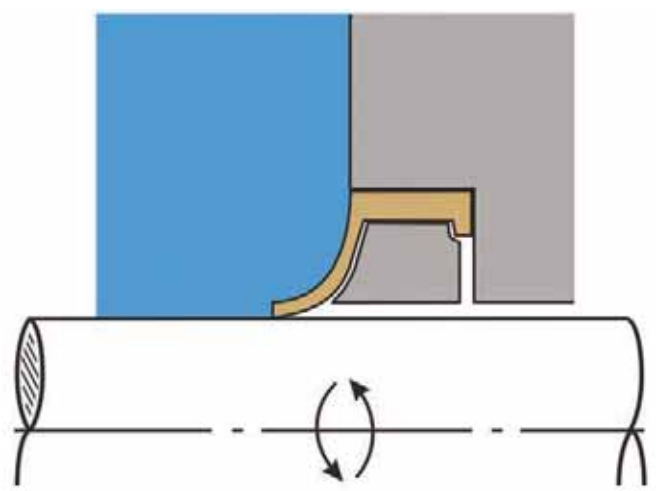

Figure 9: Hygienic thermoplastic shaft lipseal

When it comes to hygienic applications, two different types of seals are used. For low and atmospheric pressures and higher sliding speeds of up to several meters/second, thin rubber or plastic (often PTFE-compounds) without any energising elements are used (**Figure 9**). Such seals create – when properly designed – low contact forces and low heat generation between the sealing lip and the shaft. However, they cannot cope with higher pressures. For many designs, 0.2 MPa is already the upper limit. To be well cleanable, a front-flush design, as shown in **Figure 9**, is preferable.

The other type of seal has a sealing lip that is pressed to the shaft surface by means of a preloaded rubber element, e.g. O-ring or moulded

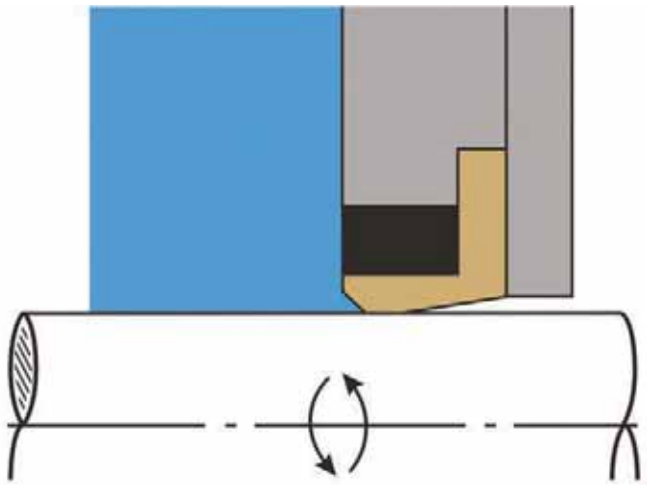

Figure 10: Flanged front-flush shaft seal

part (**Figure 10**). Metal springs are also common, but they are not suitable for hygienic applications if the spring is inserted into the seal groove on the product side. Special solutions are available on the market, where the springs are inserted from the back side. A metal spring offers the advantage that temperature limits, chemical and physical compatibility with product and cleaning fluids, flavour transfer and the like are only dependent on the plastic material and the rubber material is out of the equation. Preloaded thermoplastic seals can cope with higher pressures up to several dozen MPa, depending on design and speed. The seal manufacturer often gives MPa x m/s (PV) limits. The higher the speed, the lower the acceptable pressure.

There are a lot of other seal challenges in hygienic design like 'mechanical force seals' in valve plugs, butterfly valve seals, personal access port gaskets and others. But covering all aspects of hygienic design sealing is simply impossible because of the amount of pages required. For readers who are interested further in this subject, EHEDG guideline number 16 or the upcoming new seal guideline will offer a great deal more information.

Legend for figures

Reference

1. http://www.ehedg.org/uploads/EHEDG_pp_connections.pdf

About the Author

Ferdinand Schwabe works as a Consultant primarily for equipment manufacturers for food equipment. His roles include carrying out third party inspections for equipment certification according to 3-A Sanitary Standards and evaluation of the hygienic design of equipment. Ferdinand also runs internal training courses covering preparation for 3-A TPV inspection; hygienic design using EHEDG training material; and sealing technology. Ferdinand has more than 30 years' experience within the food equipment and seals industry and has various professional qualifications, including Technician in Mechanical Engineering; Certified QMB and Internal Auditor for Quality Management Systems; Certified 3-A Conformance Evaluator; and Authorised EHEDG Trainer for Hygienic Design Courses.

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pH development in meat

The change of pH during slaughter is thought to be the most important factor for the development of meat quality factors like juiciness, tenderness, taste, colour and drip loss. Little quantitative knowlege existed on how pH evolves with time and temperature. Wageningen UR (University and Research Centre) has developed a predictive theory of pH during slaughter. The model is based on post-mortem fysiology and the physics of carcass cooling. The model validation is done based on experiments performed with a prototype pH sensor. The predictive model is a promising tool to adjust the cooling strategy to initial meat quality, or to assess novel cooling methods.

pH and meat quality management

After slaughter there is still a lot of fysiological activity, which is mainly fuelled by the glycolysis. The primary function of the glycolysis is to generate metabolic energy in the form of ATP, via conversion of glycogen into lactate and CO_2. Due to the accumulation of lactate the pH will change, which imparts much of the fysiological activity, and thus meat quality. Certain combinations of pH and temperature will give rise to toughening of meat via cold or heat shortening. Hence, it is important to keep pH within certain bounds when carcasses are being cooled in the slaughterhouse. To this end Meat Standards Australia (MSA) has developed a pH-temperature window, stating that at rigour conditions (pH=6) the carcass temperature should be between 15 and 35°C, and more preferably between 15 and 20°C. This window is nicely illustrated with the pH/T state diagram, displayed in **Figure 1** (page 50). In this diagram the regions of cold/heat shortening are indicated, together with other important fysiological events – which are explained in more detail below. One can clearly observe that the pH/T window proposed by MSA is to prevent either cold or heat shortening.

The pH/T window as proposed by MSA would be a very practical tool, if either pH and temperature can be recorded throughout the process, or if they can be predicted using a few simple initial measurements. Both the prediction and the registration of the state of meat carcasses in slaughterhouses, in terms of pH and T, have been the subject of research by Wageningen UR within the European Pasteur project.

Fysiology

The post-mortem fysiology is very complex, as illustrated by the metabolic network displayed in **Figure 2** (page 50). In our fysiological model all these reactions are incorporated. The main driver behind the postmortem metabolism is the glycolysis, which produces ATP from

the glycogen reserves in the muscle. ATP is consumed (hydrolysed) for muscle contraction (via the motor action of myosin ATPase) and sequestering of calcium ions (Ca^{2+}) in the sarcoplasmic reticulim (SER) via the action of the SERCA enzyme. During hydrolysis of ATP, ADP, inorganic phosphate and hydrogen ions (H^+) is produced. In the early stage of post-mortem phase, ADP can regenerated into ATP using Creatine Phosphate via the action of Creatine Kinase. Throughout the whole post-mortem phase, 2 ADP can be regenerated into 1 ATP and 1 AMP, using the myokinase enzyme. The inorganic phosphate of AMP will be recycled, as it can be used by the glycolysis.

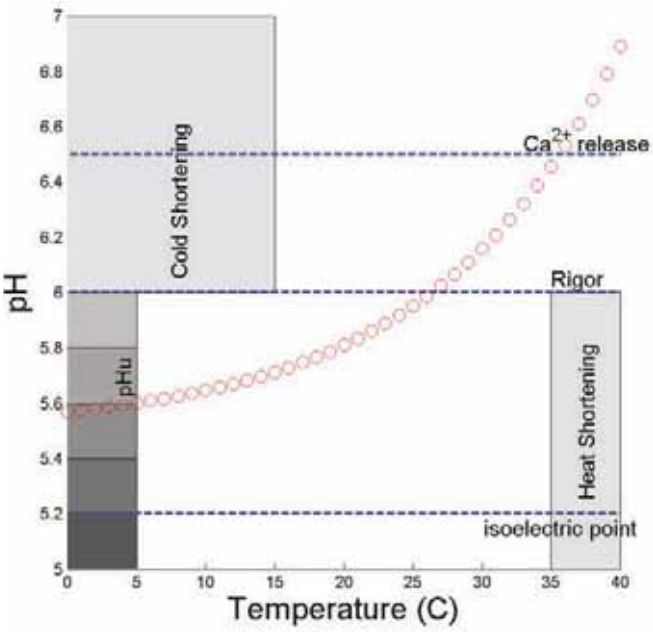

Figure 1: pH/temperature state diagram showing desired temperature window at rigor, and danger zones for cold/heat shortening. The dots indicate the preferred path of a carcass through the pH/T state diagram.

Next to ATP, glycolysis also produces lactate and hydrogen ions (H+), which will lower the pH – which has strong effects on the whole post-mortem muscle fysiology. Important fysiological events affected by pH are: a) the release of calcium from vescicles into cytoplasm at pH=6.5; b) rigour, which occurs at pH=6, where the muscle motor proteins irreversibly bind to each other; and c) neutralisation of muscle proteins at the isoelectric point of pH=5.2. All these critical points have been indicated in the pH/T diagram of Figure 1.

The release of calcium from SER enables the contraction/shortening of muscle, but also stimulates the action of enzymes (calpain) breaking down the meat structure – this so-called aging process is important for the development of meat tenderness. Rigour induces toughening of the meat, but is counteracted by the meat aging process. At the isoelectric

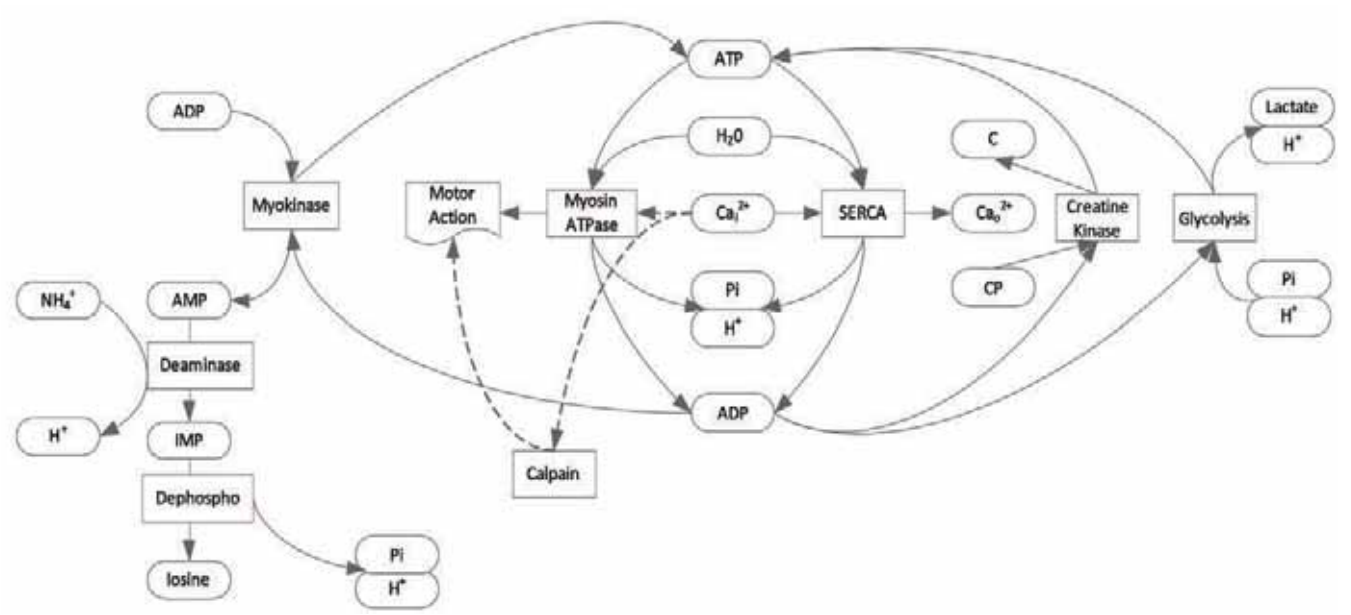

Figure 2: Metabolic network of fysiological reactions occurring in the post-mortem phase of meat. Fysiological model builds upon earlier work of Vetharaniam *et al*. (2010)

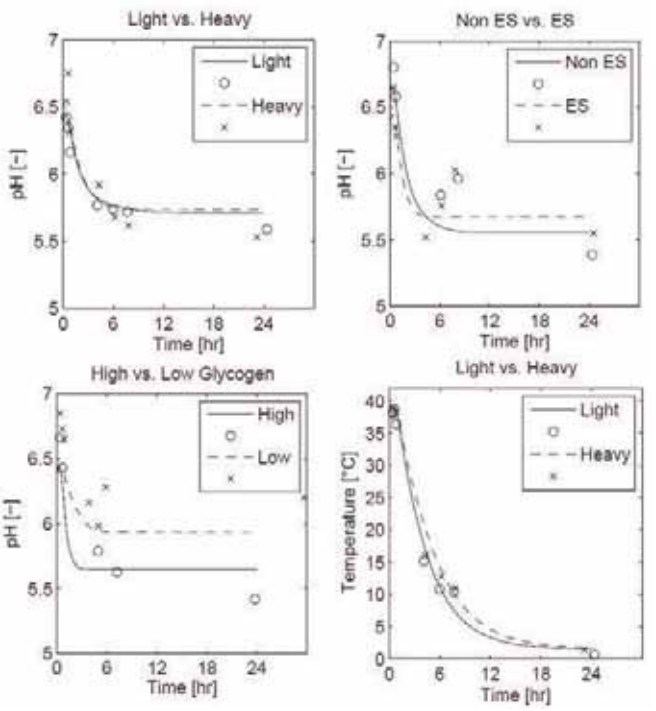

Figure 3: Model predictions of pH and temperature as function of time for some representative carcasses (lines), compared to experimental data (symbols)

point of pH=5.2 the meat proteins have a minimum in water holding capacity, which is adverse for the meat juiciness.

To prevent cold-shortening it is desired that the ultimate pH (pHu) is below pH=6. However, to have juicy meat the pH must stay well above the isoelectric point. Furthermore, for meat tenderness it is important to balance rigour and the aging process, which is achieved by keeping the meat temperature within a certain window at rigour conditions (pH=6). All these considerations are the essence behind the pH/T window of MSA.

Model development

Our model for predicting pH in the postmortem phase builds upon earlier work of Vetharaniam[1]. This earlier model has already modelled all biochemical reactions, as depicted in **Figure 2** (page 50). However, in this earlier model it is assumed that temperature is constant. For prediction of pH in slaughterhouses it is required to know the change of the carcass temperature with time, and how temperature affects the biochemical reaction rates. These two items we have included in the model of Vetharaniam. For the temperature dependency of the reaction rates, we have used the rule of Van't Hoff. How the meat temperature evolves in time is computed using a simple energy balance, taking into account the rate of heat transfer at the carcass surface, the weight and size of the carcass, and the (changing) ambient temperature. Furthermore, our model can also deal with the application of electrostimulation and the initial amount of glycogen in the muscle. Both these aspects were also not present in the earlier model by Vetharaniam. More details can be found in our scientific paper[2].

Model predictions

The validity of the model predictions has been tested using experiments performed in a Dutch slaughterhouse producing veal. Here, after killing the meat cools down during the slaughter process and active cooling to moderate temperature of approximately 20-15°C within a few hours, and is subsequently stored in a cold room at 2°C, where the carcass cools further down in about 20 hours. We have monitored the change in pH,

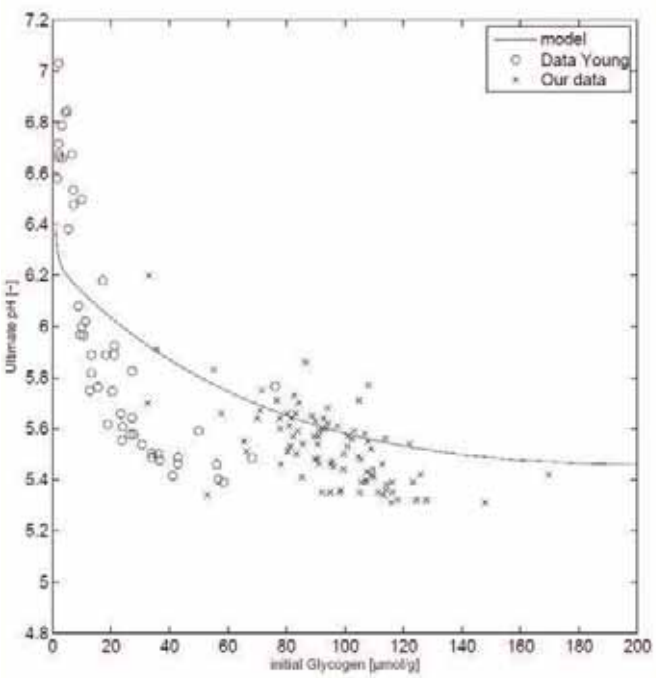

Figure 4: Ultimate pH as function of initial glycogen content, as follows from our model (line) and experiments (symbols)

meat and ambient temperature in a number of points in the production chain. As an indication of initial meat quality we have measured glycogen content at half hour post-mortem with a disposable sensor measuring the glycemic index of juice expressed for a meat sample.

Figure 3 (page 51) shows some representative predictions of the course of pH and meat temperature in time. We observe that the model can predict both temperature and pH quite accurately. An even better indication of the model accuracy is the fact that it quite well reproduces the correlation between initial glycogen content and ultimate pH at the end of the production chain. This relation is depicted in Figure 4, where the model predictions of the ultimate pH (pHu) are compared to all our experimental data – and data from other sources from literature. This graph shows that the model is also applicable to other types of meat, like beef, chicken and sheep.

The effect of the applied carcass temperature management on meat quality is conveniently evaluated via plotting the model predictions in the pH/T diagram, as shown in Figure 5. The solid line shows the path of a representative carcass through the pH/T diagram. The model predictions show that it nicely avoids the danger zones, related to cold/heat shortening, and with a pHu well above the isoelectric point. In this diagram we show also the experimental data points showing all carcasses have passed nicely through the temperature window 15 < T < 35°C at rigour (pH=6).

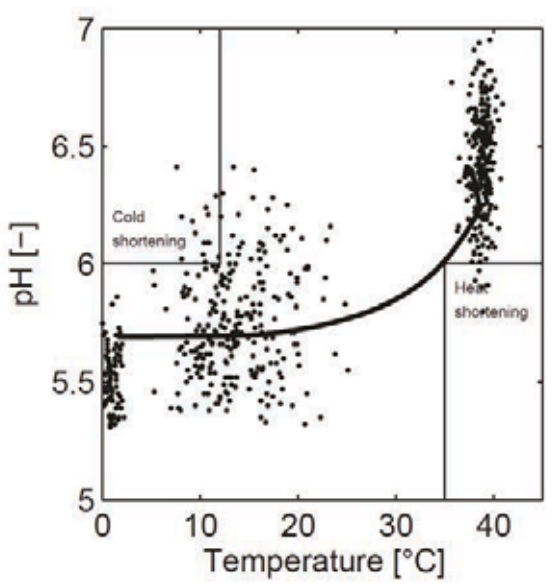

Figure 5: Assessment of meat quality via plotting model predictions (line) or experimental sensor data (symbols) in the pH/T state diagram

Continuous pH monitoring

As an alternative to the model predictions we have used a wireless pH/temperature sensor, which is coupled to a RFID. The prototype of the sensor is shown in Figure 6, which is developed by several partners within the Pasteur project in which Food & Biobased Research participated, and is led by NXP Semiconductors. This small sensor can be inserted into the carcass or a piece of it, in the early stage of the slaugther process. The sensor will continuously log the pH and temperature of the carcass, and will store it in the on-chip memory. The memory can be read wirelessly using the RFID device. Of course, the RFID-sensor can also be used in the logistic chain from the slaughterhouse to the distribution centre or supermarket.

Figure 6: The prototype sensor which can be inserted into the carcass (left black piece, right processing unit covered with container during operation), and records temperature and pH during the processing in the slaughterhouse

Using smartphone technology the recorded data of multiple carcasses can be displayed in the pH/T diagram, similar our experimental data as shown in Figure 5. The user can directly observe whether the processed batch of carcasses has passed safely the pH/T window, as defined by MSA. The developed concept of monitoring the status of food in the logistic chain with a wireless RFID-sensor, which translates the recordings into a product quality parameter, has recently been given the Food Valley Award 2013.

Practical applications

Our predictive model and/or RFID sensor technology can be useful for the meat industry in a number of ways. First, it allows slaughterhouses to adjust their carcass temperature management (cooling strategies)

depending on initial meat quality. For instance, if animals have experienced stress before slaughter, the glycogen content in their muscle is lowered, which can have an effect on their ultimate pH, which can be close to pH=6, as shown in **Figure 4** (page 52). This enhances the chance that the meat enters the danger zones of cold shortening. This can be prevented by proper adjustment of the cooling rates, which can be obtained with the use of the model.

The second use of the model is the assessment of a) novel or more sustainable cooling strategies; b) novel cooling equipment with improved heat transfer rates; and c) design of carcass temperature management for novel carcass weights/sizes or for new sources of meat, like buffalo or goat. For a representative carcass one can compute the path through the pH/T diagram given the novel cooling strategy. This assessment can be done already before having the cooling equipment installed. Of course, the assessment can be made broader via taking into account constraints concerning microbial safety, weight loss, etc. For example, there is currently an increased interest in rapid chilling strategies – which are beneficial for microbial safety and minimising weight loss due to evaporation. However, there is an increased risk of cold-shortening. The model would provide insight for a good balance between the advantages and disadvantages of the rapid chilling strategy.

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Acknowledgements

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Lene Meinert and Hardy Christensen Danish Meat Research Institute

How to determine shelf life of chill-stored fresh meat

The increased demand for chill-stored fresh meat with a long shelf life poses a huge challenge to the meat industry. Predicting the shelf life of chill-stored fresh meat is important in order to ensure optimal and flexible retail distribution and to ensure good eating quality at the use-by date. It is the responsibility of the meat producers to determine the use-by date. Chill-stored fresh meat will deteriorate, primarily due to microbial spoilage and lipid oxidation. However, during long-term chill storage using deep chill, protein oxidation or degradation may also significantly decrease the quality of the meat. The work presented in this paper is the result of several years of research at DMRI focusing on shelf life and the development of the mathematical tool for prediction of shelf life, DMRIpredict.

The importance of chill storage

Chill storage has been used for decades, since it has a significant effect on prolonging the shelf life of fresh and perishable products, such as meat and meat products. Nowadays, fresh meat is exported from one country to another across most of the world, and this is only possible due to efficient cold chains during transport and distribution. A preferred method for chill storage of fresh meat is the so-called deep chill storage, where the temperature is very close to the freezing point of the meat and the meat is typically vacuum-packed.

Important factors for shelf life

Overall, there are three factors of significance for shelf life of chill-stored fresh meat:

1. Temperature

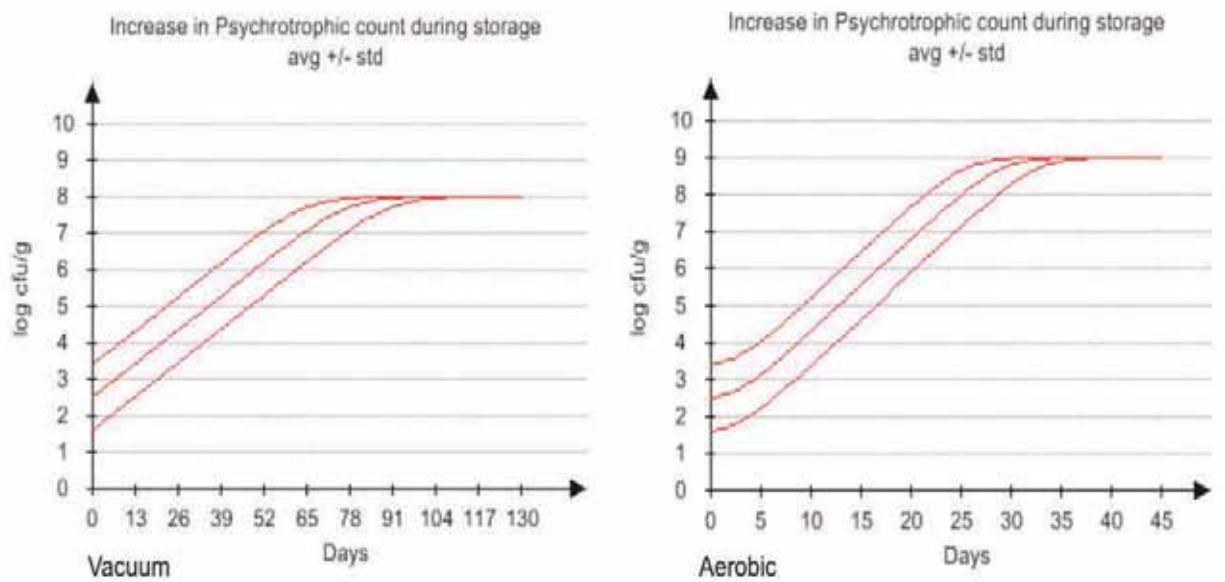

Figure 1: Growth curves of bacteria on pork cuts packed in vacuum and in wrap at -1°C. Aerobic: open meat boxes or wrap using oxygen permeable film; Vacuum: no oxygen, non-permeable film.

2. Packaging method
3. Number of microorganisms at the time of packaging.

Temperature is without comparison the single most important factor for shelf life of chill-stored fresh meat, and this applies to beef, pork and chicken. As a rule, the lower the temperature, the longer the shelf life (see Table 1).

The second most important factor is the packaging method. In most cases, vacuum pack and skin pack provide the longest shelf life at any chill temperature, compared with aerobic packaging (e.g. open meat boxes used in the slaughterhouse or a wrap with oxygen permeable film used in retail) or solutions with high-oxygen modified atmospheres (MAP).

Furthermore, the advantage of vacuum pack or skin pack are very clear at low temperatures, with shelf life increasing by many days compared with both aerobic packaging and MAP. Actual examples of the shelf life of pork cuts are shown in Table 1.

The third most important factor for shelf life is the bacterial count at the time of packaging. In this context, this only concerns the naturally occurring bacteria that are present on the surface of the meat at all times. Table 2 shows the shelf life of pork cuts at 4°C using aerobic packaging or vacuum, with varying bacterial counts.

The number of bacteria represents a very low level (1 log), a normal and average level (2.5 log) and 'old' meat in the context of packaging (4 log).

The number of bacteria on the meat at the time of packaging affects shelf life. However, the significance of this effect is dependent on the chosen packaging method. This can be seen in Table 2, as only two days of shelf life are gained by reducing the number of bacteria (psychrotrophic flora) on the meat by 3 log. However, when using vacuum pack or skin pack, the difference is eight days.

In summary, the longest shelf life is obtained at low storage temperatures and low bacterial counts combined with a packaging solution that promotes shelf life, which, in most cases, is vacuum and skin pack.

Table 1: Average shelf life of pork cuts as a combination of temperature and packaging method. Shelf life is here defined as the time at which 50 per cent of the packages are unacceptable. For comparison, the number of psychrotrophic bacteria is set at 2.5 log cfu/cm^2 at the time of packaging. Aerobic: open meat boxes or wrap using oxygen permeable film; MAP: high-oxygen modified atmosphere (70 per cent O_2/30 per cent CO_2); VAC/SKIN: no oxygen, non-permeable film.

	AEROBIC	MAP	VAC/SKIN
Shelf life at +7°C	7	8	11
Shelf life at +4°C	10	14	21
Shelf life at -1°C	21	36	57

Table 2: Average shelf life of pork cuts at 4°C in wrap and vacuum/skin pack as a function of different bacterial counts (psychrotrophic flora) at the time of packaging. Shelf life is here defined as the time at which 50 per cent of the packages are unacceptable. Aerobic: open meat boxes or wrap using oxygen permeable film; VAC/SKIN: no oxygen, non-permeable film.

No. of bacteria	AEROBIC	VAC/SKIN
1 log cfu/cm^2	11	25
2.5 log cfu/cm^2	10	21
4 log cfu/cm^2	9	17

Deterioration during chill storage

The two dominant processes of deterioration of fresh meat during chill storage are microbial spoilage and lipid oxidation. In general, both processes are slowed down by lowering the temperature. However, neither of the two processes is terminated, not even during deep chill storage.

Microbial spoilage

After slaughter, bacteria belonging to many genera can be found in fresh meat. The packaging method (especially the level of oxygen) and temperature will determine which of the bacteria present on the meat will multiply during storage. For instance, bacteria belonging to the genus Pseudomonas will normally be a dominant part of the flora after

storage under conditions that allow exposure to air, whereas they are not found after storage in vacuum pack.

The growth of psychrotrophs is independent of the surface type (rind or meat) and the origin of the meat (cuts, plant and country). Furthermore, changes from 'high' to 'low' temperatures and changes from 'low' to 'high' temperatures result in the same shelf life. The growth rate of the bacteria is a function of temperature with further influence from the packaging method. **Figure 1** (page 55) shows the growth of bacteria on pork cuts in vacuum pack and in wrap at -1°C (deep chill). Therefore, over time, the number of bacteria will reach a high level, and the meat will rot.

Total bacterial count and shelf life

One of the results from the shelf life tests showed that there is no direct link between shelf life and the number of bacteria at the end of shelf life. The expected average number of bacteria at the end of shelf life differs with temperature and packaging method. An example is shown in **Figure 2**.

Furthermore, not all of the bacteria present on the meat will lead to spoilage. Therefore, it cannot be stated that, at a certain number of bacteria (e.g. 6 log), the shelf life is at a given stage, since this will depend on the flora composition present on the meat. This is one of the reasons why meat from different commercial plants was included in our research.

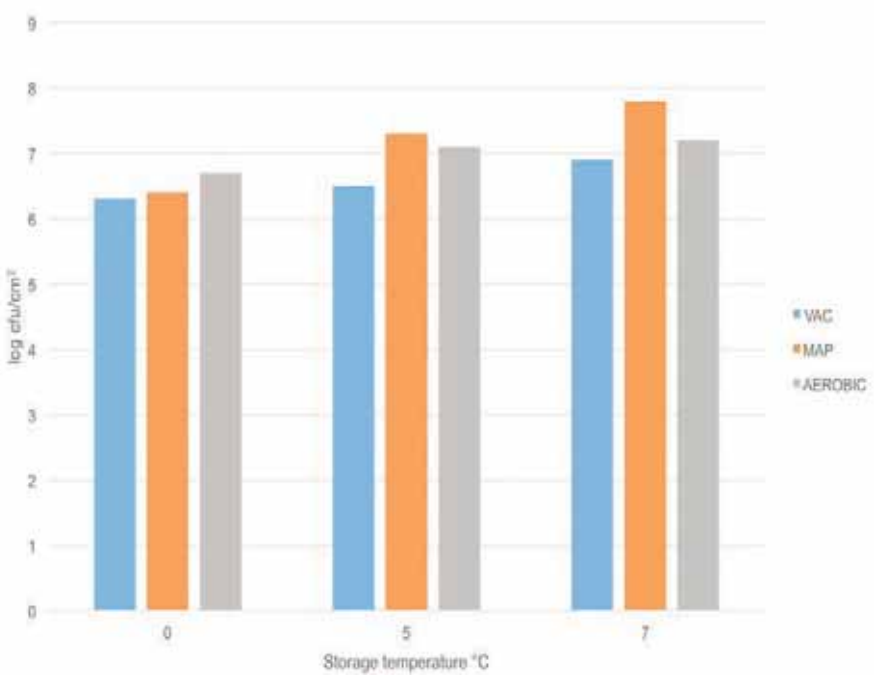

Figure 2: The expected average count at the end of shelf life (50 per cent of packages are unacceptable) for pork packed in wrap, MAP and VAC stored at three different temperatures, and with an initial number of psychrotrophic bacteria estimated at 2 log cfu/cm². WRAP: Aerobic packaging in oxygen permeable film; MAP: high-oxygen modified atmosphere (70 per cent O_2/30 per cent CO_2); VAC/SKIN: no oxygen, non-permeable film.

Lipid oxidation

The fat composition, or fat quality, has a significant influence on shelf life, and therefore also on the storage time, since the unsaturated fat content is prone to oxidation. Oxidation progresses in several stages, each stage generating oxidation products with different properties. In the early stages, the oxidation products (primary oxidation products) do not generate noticeable changes in the meat. However, later on in the process, when the secondary oxidation products form, the rancid taste and odour will become apparent. This change in odour is used to determine the shelf life of fresh meat cuts.

How to determine shelf life

Irrespective of whether the limiting process for shelf life is microbial spoilage or lipid oxidation, both processes induce odour changes in the meat, sometimes also in combination with changes in appearance. Common odours associated with microbial spoilage are 'putrid', 'sour' and 'rotten', while odours associated with lipid oxidation include 'chemical', 'rancid', 'butter-like' and 'cardboard'.

In several studies, it has been observed, and subsequently validated, that the odour is indeed the first sensory attribute to change with regard to decrease in shelf life. Everyone can use this approach, for example meat packing companies in connection with raw material control or consumers who want to check a pack of meat from the fridge.

What is the maximum shelf life of fresh pork?

If all known factors affecting shelf life are controlled, what then is the maximum shelf life? To determine this, an experiment using pork filets was conducted. The filets, which contain only one per cent fat (approximately), were cut under sterile conditions, so the number of bacteria was close to zero. The filets were packed in vacuum and stored at -1°C. Throughout the shelf life, the number of bacteria was at a minimum, so no growth occurred. The meat odour did not change markedly, though it was less fresh at the end of the experiment. However, the odour did not indicate either spoilage or oxidation. The filets were

cooked and then assessed by a trained panel, who detected a clear change in flavour at approx. day 65, at which point the meat tasted bitter and 'old'. This was linked to measures of protein degradation (free nitrogen). This finding suggests that enzyme activity that degrades protein may also be a limiting factor for shelf life.

Development of reliable shelf life models

One of the cornerstones of our work with DMRIpredict was to include as much natural variation in the models as possible in order for the models to be reliable and robust.

Large natural variations were seen when modelling changes in raw meat odour, and this is a reflection of a real life situation. These variations occurred despite the fact that the studies were standardised and controlled in relation to a number of factors (time of slaughter, cuts, packaging method, storage temperature, etc.). Many cuts (e.g. 90-100 loins in a single study) were used in each study to ensure the inclusion of natural variation. Therefore, the variations express reality and reflect what is seen in the retail sector, and they cannot be related to single factors such as pH, which has been investigated (data not shown), cross-breed, rearing, feed, commercial plant, etc. The selection of different cuts from different commercial plants was made in order to include as much natural variation as possible and to ensure the robustness of the models. Therefore, the backbone of the models is the large number of individual storage experiments using meat produced for retail.

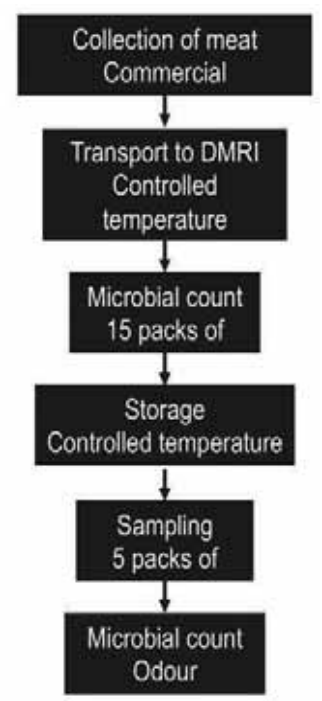

Figure 3: The overall experimental design for the execution of storage experiments. The lower the storage temperature, the greater the number of packs will be needed.

Storage experiments

A significant number of individual storage experiments were conducted in order to generate data for each model. An overall template for the execution of the storage studies was developed (see **Figure 3**).

Uniquely, all analyses (microbiological and sensory) were conducted on the same individual sample and could thus be directly linked to the development of spoilage. The order of analysis was: 1) psychrotrophic colony count; and 2) raw meat odour assessment. For experiments with meat packed in MAP, gas composition was measured first.

Prediction of shelf life with DMRIpredict

It is the responsibility of the meat producers to determine the use-by date. This can be both a difficult and resource-consuming task. Therefore, the Nordic meat industry's need for a tool to determine the optimal use-by date for various pork cuts led to the development of mathematical models for prediction of shelf life. During the last 10 years, DMRI has developed several shelf life models for beef, pork and chicken. Each model is based on the following three factors of significance for shelf life: temperature, packaging method and bacterial count at the time of packaging. All models can be accessed free of charge from: http://DMRIpredict.dk

About the Authors

Dr. Lene Meinert is a senior consultant in the Department of Meat Quality at the Danish Meat Research Institute. She is currently involved in several R&D projects, including the development of a mathematical model for shelf life prediction of frozen pork, and boar taint-related challenges including analytical methodology and the use of tainted meat. Lene has extensive experience in the coordination of research projects involving both universities and industry.

Hardy Christensen is a senior consultant in the Department of Hygiene and Preservation at the Danish Meat Research Institute. Hardy has a broad knowledge of and insight into slaughterhouse processes related to hygiene and food safety. He is currently involved in R&D projects regarding the development of shelf life models for fresh, frozen and cured pork along with consultancy work for the meat industry throughout Europe.

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Advancing analytical microbiology in the dairy industry

Today's consumers have greater expectations than ever before regarding food. They expect not only safe, good quality and value-based products but also a real commitment of the food company toward social responsibility to the community, e.g. regarding nutritional education, sustainable development and adaptation to local geographical specifications. Those expectations are symbolised by a consumer needs pyramid: the basic requirement being consumer safety, the over consideration being product conformity to bring consumer satisfaction and, at the top, product superiority that brings consumer loyalty[1].

Innovation is a key contributor for product superiority. Among innovations in the dairy industry, the study of fermenting microorganisms takes an important part as they are essential ingredients in product manufacturing. Fermenting microorganisms are often used as a mix of species composing a beneficial microflora in the final dairy product for (i) texture and organoleptic properties; (ii) for product preservation against pathogens; (iii) for health benefit properties. All these beneficial aspects are mostly driven by fermenting microorganisms bringing added value to the products. Among beneficial microbes, probiotic used in dairy products brings health

benefits through its consumption. Hence, an increasing interest in the commercial exploitation of selected lactic acid bacteria (LAB) and probiotics in the food industry gives rise to many new products launched each year (**Figure 1**).

Sales of yoghurt and yoghurt-related products including Greek yoghurt, fermented milk product associated to functionality, or traditional products like kefir have increased 40 per cent since 2008 and are projected to increase between five to seven per cent per year between now and 2017 (source: Mintel). Growth of sales is also associated with a necessity to characterize more and more complex dairy products; e.g. fermented milk associated with probiotics often contains two more species than usually used in yoghurt, and traditional products like kefir or cheese contain a genuine microflora composed of several species of bacteria, yeasts and moulds, whose complete diversity is not well known. In parallel, to guarantee quality of some dairy food categories or dairy ingredients, CODEX and WHO/FAO have provided definitions of yoghurt and probiotic categories, respectively[2,3]. Both definitions state that microorganisms have to be alive and in a sufficient number in final products. Therefore, dairy microbiology needs methods with high discriminatory power to quantify probiotics in more and more complex dairy products, with information related to their ability to survive along the shelf life.

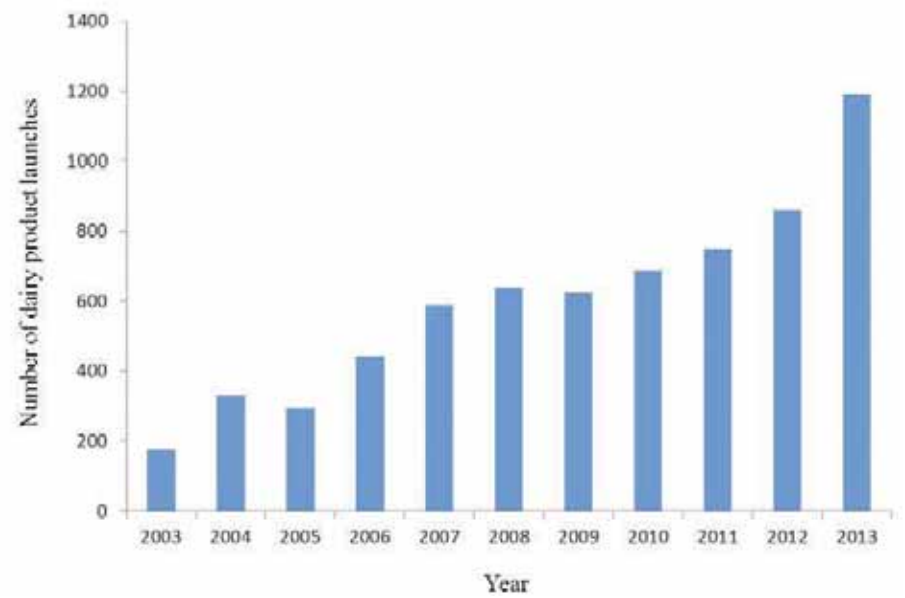

Figure 1: The number of dairy products launched from 2003-2013

Some standardised methods based on culture media are available to qualify these beneficial bacteria and are widely used to monitor enumeration of fermenting microorganisms or probiotics in the final product as recommended by standardisation committees. Selective culture methods rely on cultivability of microorganism but it's actually a narrow way to represent only a part of the bacterial population present in the product. Indeed, viable but not cultivated (VBNC) bacteria are not taken into account by these methods as well as their metabolic activities. Additionally, these culture-dependent methods are time-consuming, labour-intensive and show poor discriminatory power. Therefore, the dairy industry requires new, alternative methods to perform qualitative and quantitative measurements of fermented milk products and this represents the stakes of analytical microbiology today. We would like to shed light here that this challenge can be faced with alternative methods based on molecular biology or flow cytometry, which could offer new analytical solutions to dairy microbiology.

Fuel product superiority with bioanalytic management: From conception to standardisation

Before going deeper into technologies, we would like to introduce our analytical management system (**Figure 2**, page 62). For us, innovation in bioanalytics is a key priority to leverage performance of methods. The performance is represented by the combination of basic analytical criteria (specificity, sensitivity, repeatability, reproducibility, linearity, robustness with the uncertainty value,

accuracy, precision), as well as the cost and speed of analysis to meet the industrial demands.

- Firstly, bioanalytics requires to be connected to cutting-edge technologies and should select the best one in this 'Analytical Cloud' to translate it into a performing method that could be applied in food industry. Selection of technologies that will produce the methods of tomorrow could be fostered with pre-competitive research through cooperative ways of working with academic and private partners.
- The second phase is the 'Proof of Concept' that corresponds to the first results that we can get from testing a new idea with the selected technology into our domain of application. Here we expect to demonstrate the idea feasibility and to verify its potential to be used in R&D or quality control.
- The third phase, called 'Proof of Performance', aims to optimise the analytical criteria cited above to reach the highest performance. The performance of methods is the key driver, as it warrants the delivery of reliable analytical results at an industrial scale later.
- The last phase is to work on standardisation and it requires qualifying the performance of the methods that were already optimised. It generally implies contribution of an analytical network of laboratories for intra- and/or inter-laboratories testing in order to know result variability using the same method. This step is necessary to industrialise method use in multiple laboratories. The International Organization for Standardization (ISO) and International Dairy Federation (IDF) are particularly key partners in delivering standards in the dairy industry.
- For the final industry application of the method, a constructive way to perform analysis for long-term needs is to work with partners that can provide commercialised instruments and ready-to-use kits produced in a standard way.

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The system should be managed by a balanced portfolio to deliver a continuum of methods from conception to standardisation. Overall, these different inter-connected phases are thus integrated in an analytical management process that continually feeds innovation, quality and superiority of dairy products.

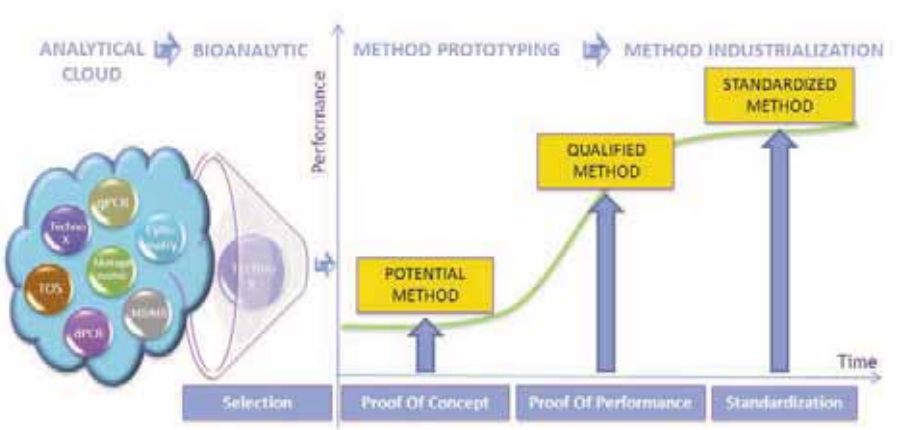

Figure 2: Analytical Management System

Deciphering the complexity of dairy products with molecular tools

A growing scientific literature showed that enumerating fermenting microbes or probiotics in dairy products could be successfully performed with molecular methods. Recently, we published a review showing the new analytical opportunities of using PCR-based methods in the dairy industry[4]. The recent advances in quantitative PCR (qPCR) applications offer a faster and reliable analytical tool to perform experiments with high throughput/automation analyses and thus could subsequently reduce costs per assay, increase reliability of results and meet industrial demands. The strength of the molecular technics is its high discriminatory power to target multiple microorganisms in samples. Moreover, the boom of genome sequencing provides a rich support of genetic information for designing a specific molecular biomarker. We emphasise that the dairy industry cannot settle for basic qPCR, one of whose major drawbacks is that qPCR counts could not be directly associated with cell viability as samples are often composed of dead/viable cell mixtures. Fortunately, viability PCR (v-PCR), a new kind of PCR, has been developed for several years to overcome this limitation using impermeable nucleic binding dye like ethidium monoaside bromide (EMA) or propidium monoaside (PMA) prior DNA extraction and qPCR.

In this technique, the membrane integrity is used as a viability marker of the microorganism so that a compromised membrane can be permeable to the dye which can penetrate only into dead cells, intercalate with DNA and subsequently inhibit DNA amplification, and thus allow discrimination between viable cells, including VBNC, and dead cells. Proof of concept of this new method and its reliability was attested on dairy products to quantify viable probiotic strains of *L. acidophilus, L. casei* and *B. lactis* in fermented milk and *B. animalis, L. rhamnosus* and *L. helveticus* species in Cheddar cheese[5]. V-PCR method was also applied to quantify viable probiotics in faecal samples from people having ingested fermented milk products[6]. This method could therefore be used for evaluating probiotic's ability to resist stressful conditions during their transit through the gastrointestinal tract. The increasing popularity of v-PCR produces many promising results but more and more different protocols were developed without proper evaluation of the robustness.

Standardisation of real-time PCR methods is growing with sets of guidelines like MIQE that describe the minimum information necessary to evaluate qPCR experiments

Standardisation of real-time PCR methods is growing with sets of guidelines like MIQE that describe the minimum information necessary to evaluate qPCR experiments[7], or ISO22119:2011[8] guidelines that define requirements to detect food-borne pathogens in foodstuffs by PCR and qPCR. The recent recommendations for better use of qPCR in the food industry should be used to develop alternative methods based on qPCR for quantification of dairy microorganisms[9,10]. Digital PCR or droplet digital PCR that are based on the principle of the most probable number for target quantification appears to be a new generation of PCR that could facilitate standardisation of PCR. Indeed, it provides absolute quantification without the need of setting up standard curves and also produces data with better accuracy than qPCR. Furthermore, this system opens great opportunities for developing multiple testing in a single analysis, which increases throughput of analysis. No application has yet been described for microbe's quantification in food. However, good performances of dPCR from proof of concept studies in clinical application and the recent availability of guidelines for warrant delivery of dPCR data of quality, i.e. dMIQE11, suggest

that dPCR or even v-dPCR could bring new analytical benefits in food microbiology in the future.

Complementary to these targeted molecular approaches, new opportunities for bioanalytic have appeared with the great development of untargeted approaches. They are classically referred to as the 'omics' approaches and are able to provide a more complete picture of product biological composition. Metagenomic, thanks to the boom of high throughput DNA sequencing, is a very helpful tool to draw the taxonomic description of microorganisms present in a sample. Usually performed to study microbial diversity in complex environments like gut microbiota or environmental samples, this technology has been recently applied to complex dairy foodstuffs, such as raw milk[12], cheese[13] and kefir[14]. Most of the time, metagenomic reveals presence of taxa not traditionally identified by culture-based approaches. Moreover, viability dye can be associated with the metagenomic to provide information related to the viable community present in sample[12]. Thus, a food v-metagenomic approach could be very powerful to optimise and control manufacturing of complex dairy products like cheese or kefir that contains diverse bacteria, yeasts and moulds. However, metagenomic is associated with sillico analyses and therefore requires a development of expertise in bioinformatic to treat the high quantity of data. This untargeted screening shows a great potential to be used in the future in routine product testing to check product compliance in a real time single analysis, both for safety control consideration and for conformity regarding specification of beneficial bacteria count. But this interesting perspective would be reached only if the performance of the untargeted screening with food metagenomic is confirmed, hence moving from a proof of concept phase to a well-standardised method.

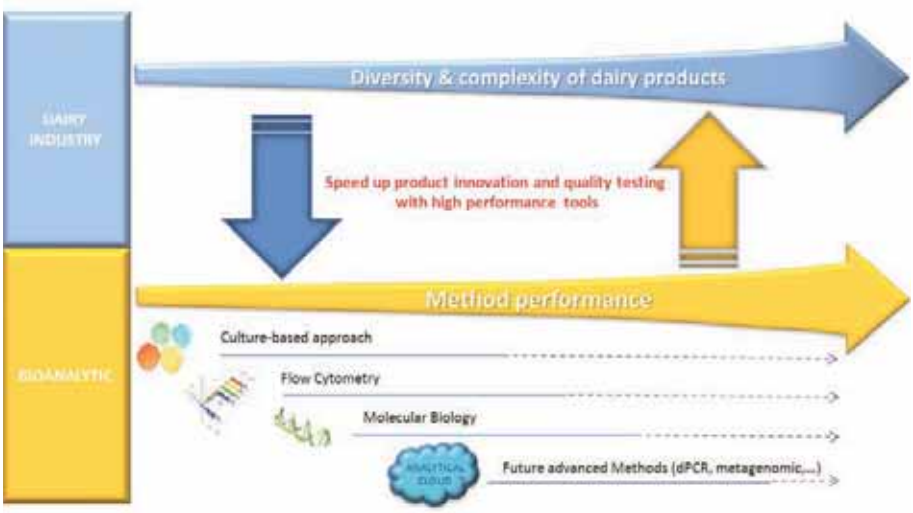

Figure 3: The exponential growth of probiotic products in the dairy market

Flow cytometry: Technology without border

Flow cytometry (FCM) is not a new technology. FCM in combination with fluorescent techniques have been widely used in clinical diagnosis, pharmaceutical application and fundamental research for the multiparameter analysis of cell populations. This technique was introduced 10 years ago in dairy industry for the evaluation of somatic cell load and total bacteria count in raw milk, which has revolutionised the grading of raw milk, producing rapid and accurate results[15].

Later on, FCM was recognised as an ideal tool to evaluate the metabolic activity of the LAB and probiotic starter cultures. It has been successfully used to predict LAB physiology during strain propagation for ferment manufacturing. FCM has also been applied to assess the viability of probiotic starter cultures through storage and to monitor cell damage after stress treatments[16]. There is a range of fluorescent probes associated with various cellular functions (e.g. membrane integrity, membrane potential, intracellular pH, enzymatic activity, intracellular ions), making the fine characterisation of the cell physiology possible. Meanwhile, the viable population could be enumerated in a near real-time analysis (<1h). Quantification result by FCM are generally more than that found on the plate, which reveals that there is a considerable amount of VBNC population in the dairy starter culture during processing, storage and stress treatment[10].

The future of analytical methods for the dairy industry will be 'on-line', 'real-time' and 'all-in-one', with advancing analytical technologies that should show a total picture of every microorganism in the products

FCM has been proposed as a means to enumerate viable probiotic populations in dairy products. However, while a whole population can be characterised, distinction between near genera or species remains difficult. Recently at Danone, we developed a specific FCM method to enumerate viable *Bifidobacteria* in commercial products by using double staining of antibody and viability probe[17]. With the development of custom antibodies, specific FCM enumeration of probiotics will be accessible to characterise more and more complex dairy products. Beyond the simple enumeration, FCM provide higher knowledge about microbial fitness in the products from production until the end of shelf life.

FCM appears as a very promising tool for analyses of raw milk, starter culture and final products in the dairy industry. The implantation of this technology as routine analysis requires an automated system and standardised method. Today, some automated FCM are accessible in the market, while the commercialised kits are rather dedicated to the detection of microbiology contaminants. Since 2012, an ISO standardised FCM method is being set-up under coordination of the IDF, for enumeration of LAB in starter cultures and their applications[18].With the development of appropriate kits and validation of the ISO method, FCM will be a prospective microbiology analytical solution for tomorrow's challenge.

Conclusion

Analytical methods to detect food-borne pathogens have been well developed and standardised for safety control. Meanwhile, methods with high performance to qualify beneficial bacteria are still missing in spite of the exponential growth of probiotic products in the dairy market (**Figure 3**, page 63).

Today, culture-based methods are validated as a reference in food microbiology but only three ISO/IDF culture-based methods for the enumeration of fermenting bacteria and probiotics are available. Moreover, these methods are facing great challenges to analyse more and more complex dairy products. Innovation on culture medium as chromogenic media might overcome some limitations of these widely routine used methods. The promising flow cytometry methods offer more rapid and accurate enumeration, with fine metabolic characterisations of each bacterium in the products. With the publication of IDF/ISO method and development of appropriate kits for the automation

system that already exist in the dairy industry, it will again revolutionise analysis food. Alternative methods based on molecular biology like v-PCR have shown high discrimination power on complex products and rapid time-to-result in many proof of concept studies. Evaluation of the performance through method standardisation and commercialised automation kits are still needed to enhance further application. Fast developments of untargeted approaches as metagenomic also bring new opportunities to analyse very complex dairy products.

The future of analytical methods for the dairy industry will be 'on-line', 'real-time' and 'all-in-one', with advancing analytical technologies that should show a total picture of every microorganism in the products; it will provide information from pathogen identification to probiotics fitness in one analysis. The targeted analyses will be replaced by untargeted screening, which meets all the needs from safety to superiority aspects.

The key points for the management of the analytical system are the stimulation of continued innovation and the promotion of win-win collaborations with different partners in each phase of the system.

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Enzymatic detoxification of mycotoxins for healthy food

Mycotoxins are poisonous fungal metabolites that occur in food commodities colonised with filamentous fungi and in food products contaminated during processing and storage. Intake of mycotoxins with food poses a health risk to the consumer and legal limits for maximum levels of major mycotoxins in food have therefore been established worldwide. Compliance with these limits poses a challenge to food industry because good integrated plant protection, adequate storage and good manufacturing practices are often insufficient to keep mycotoxin levels below the limits.

New options for mycotoxin management with the help of biotechnology rely on enzymatic activities that detoxify mycotoxins enzymatically. Crop plants can be engineered to detoxify mycotoxins in the field. Purified detoxification enzymes or genetically engineered microorganisms (GMOs) producing such enzymes can detoxify mycotoxins during storage and processing of raw materials in food production. GMOs do not have to be declared on food products in the EU when the microorganisms were removed from the product or the enzymes synthesised by GMOs were used merely as processing aid. The situation is similar to numerous consumer goods on the market produced with the help of purified vitamins and enzymes produced by GMOs. Enzymatic detoxification of mycotoxins offers an opportunity to make food healthier with the help of genetic technology without jeopardising market share in spite of the adverse attitude of European consumers toward GMOs.

Chemical composition of agricultural commodities used in food production seldom matches the demands on healthy and balanced nutrition. The vast majority of undesirable compounds in foods originate from biosynthetic pathways of crop plants and microorganisms associated with living plants and stored plant products. Food pollutants consisting of manmade chemicals introduced into the food chain deliberately (e.g. pesticide) or unintentionally (e.g. contaminants released by packaging materials) or generated by chemical reactions during food processing (e.g. acrylamide) received prominent media attention but the potential health damage they may cause is not nearly as relevant as the health risk caused by contaminants of natural origin. Natural products that occur as contaminants of food include metabolites of plants, bacteria and fungi. Fungal toxic metabolites, called mycotoxins, have been reported to contaminate food since the Middle Ages. While acute and even lethal poisoning by mycotoxins occasionally occurs, major concerns regarding mycotoxin exposure today are long-term effects of chronic exposure such as distortion of hormone balance, suppression of the immune system, and the ability of certain mycotoxin to cause cancer.

In order to reduce the exposure of consumers to mycotoxins, legislatures limiting the amount of mycotoxins in raw materials and food products have been established worldwide. In Europe, assessments of risks posed by individual mycotoxins to human health are generated by the European Food Safety Authority (EFSA). The results of the assessments, published as opinions, are the basis of legislative control of mycotoxin levels in the European Union.

Legal limits for mycotoxin levels in food protect the consumer as long as they are observed. Full control of mycotoxin levels along the food chain is not feasible. In contrast to the control of synthetic contaminants, which can be achieved by enforcing good manufacturing practices and hygiene standards, only indirect means of the control of mycotoxin accumulation in food commodities are available. The efficiency of these measures is limited. Mycotoxin prevention during plant production has focused on the elimination of fungal inoculum, suppression of plant infection and inhibition of fungal growth by agronomic practices such as tillage and seed coating, growing resistant cultivars and using fungicides in the field. Optimised storage conditions and chemical preservatives protect stored commodities from spoilage. All these measures are effective to some extent, particularly when used in combination. Under strong infection pressure, however, growers fail to meet legal

requirements for mycotoxin levels. Grain that exceeds maximal legal levels is unfit for food and feed manufacturing. Even when mycotoxin levels meet legal limits, further reduction is desirable from the public health perspective, particularly regarding mycotoxins that are suspected or proven carcinogens.

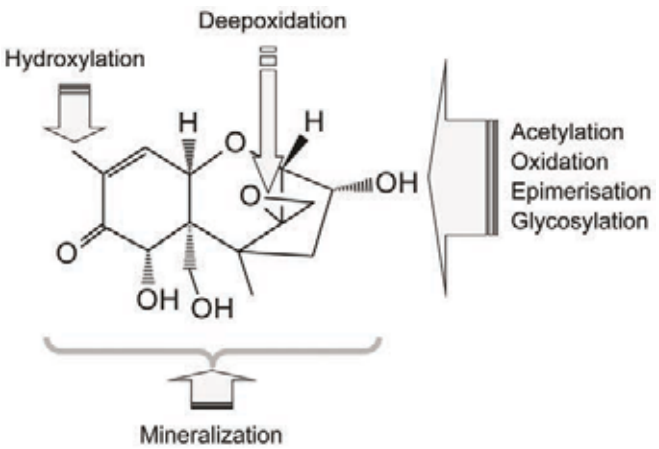

Figure 1: Enzymatic detoxification of trichothecenes

A promising strategy for the reduction of mycotoxin content in food products is biological detoxification, which is defined as enzymatic degradation of mycotoxins or modification of their structure that leads to less toxic products[1]. Organisms that possess detoxification activities can rarely be used for detoxification of mycotoxins in food commodities directly but they serve as a source of genes encoding suitable enzymes that can be expressed in other microorganisms or in crop plants. Enzymes prepared from genetically modified organisms and used in food processing do not have to be declared as 'GM component' on the final product in the European Union. Such enzymes are used on a large scale in food products sold on European markets without raising concerns about GM organisms, for example in bakery products and cheeses.

The following will describe enzymatic activities suitable for the detoxification of major mycotoxins in food, depict related industrial efforts and outline possible future developments.

Detoxification of trichothecenes

Trichothecenes are mycotoxins produced by a number of phytopathogenic *Fusarium* species, by *Trichoderma* species used in the biological control of plant diseases and by further fungal genera not relevant for food production. Major trichothecenes in cereals and maize are deoxynivalenol, nivalenol and their acetylated derivatives 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol and 4-acetyl-nivalenol, known as fusarenon X. The search for trichothecene-detoxifying microorganisms, which began more than 30 years ago and involved numerous academic laboratories and defence-related research projects, was marked by failures and setbacks[1]. In the last 15 years, a series of remarkable discoveries invigorated the field[2]. Detoxification activities for trichothecenes described so far are summarised in **Figure 1**, which shows deoxynivalenol as major trichothecene in cereal grains in temperate climate.

Complete mineralisation of trichothecenes by a pure bacterial culture was described already in 1983 but the activity was lost[3]. Very recently, the laboratory of Seiya Tsushima in the National Institute for Agro-Environmental Sciences in Ibaraki, Japan, isolated new several bacterial strains that appear to completely demineralise deoxynivalenol[4]. Full details of the discovery are yet to be disclosed.

The epoxide group of trichothecenes is their key toxicity determinant. Detoxification of trichothecenes by intestinal and ruminal microflora due to reductive deepoxidation was established in the 1980s and has been studied extensively since[2]. It took 15 years until the first pure bacterial strain capable of reductive deepoxidation of trichothecenes under anaerobic conditions was isolated[5]. The enzyme(s) and gene(s) involved in the process remain unknown. The intact bacterium was used as a component of a feed additive but its application in food industry appears unlikely.

Search for detoxification of trichothecenes intensified in the last decade. Various environments were used as sources of microflora for enrichment cultures, such as chicken intestine, fish digesta and most recently even human faecal microflora[6]. Many studies were successful in achieving biotransformation of deoxynivalenol by mixed bacterial cultures. The most recent and surprising result was the isolation of pure bacterial cultures, assigned to six Gram-positive and Gram-negative genera, that were capable of detoxifying deoxynivalenol by reductive deepoxidation under aerobic conditions[7]. These strains are promising as a source of genes and enzymes for the detoxification of trichothecenes in food production.

A second toxicity determinant of trichothecenes is the hydroxyl group on C_3. All modifications of C_3-OH of deoxynivalenol studied so far lead to the reduction of toxicity. The modifications studied most extensively were acetylation, catalysed by the product of *Tri*101 gene of *Fusarium graminearum* and related *Fusarium* species, and glycosylation, catalysed by plant UDP-glycosyltransferases. Acetylation of trichothecenes on C_3-OH by trichothecene-producing fungi is believed to protect the fungi from their own products[8]. Glycosylation of trichothecenes is a component of the defence response of plants infected with pathogenic *Fusarium* species[9].

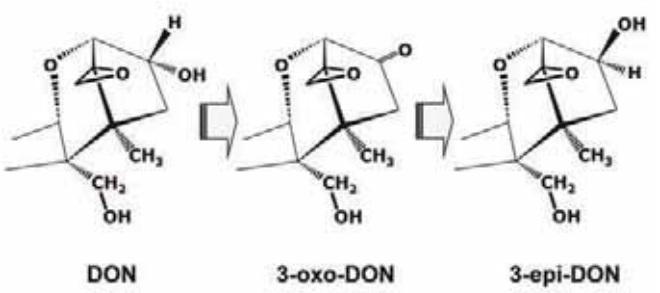

Figure 2: Epimerisation of trichothecenes

Epimerisation of hydroxyl on C_3-OH (**Figure 2**), which we hypothesise to proceed via an oxidised intermediate which is stereospecifically reducted[2], leads to 3-epi-deoxynivalenol, which is nontoxic (Gareis and Karlovsky, unpublished). The chemistry of the process is not understood and the enzymatic activities involved remain unknown.

Potential for trichothecene detoxification in food production

Expression of Tri101 in a genetically engineered wheat variety aiming at the reduction of deoxynivalenol content in flour was attempted by seed industry but the development was abandoned[2]. The use of an enzyme

with sub-optimal activity, encoded by a Tri101-homologue isolated from a *Fusarium* species that does not produce deoxynivalenol, may have been partly responsible for the failure, apart from animosity against genetically engineered crops that is commonplace in Europe[2]. As far as the author is aware, the strategy is not actively pursues by the industry in the moment.

Acetylation of trichothecenes in yeast expressing Tri101 is feasible[8]. Baker's yeast and brewer's yeast are example of organisms that can be enhanced by expressing Tri101 to detoxify deoxynivalenol, T-2 toxin and other toxic trichothecenes in dough, beer and other products. Labelling food products processed using enzymes extracted from genetically modified organisms or even living microbial strains containing genes from other organisms is not mandatory. For example, yeast used in beer brewing is regarded as 'processing aids'. If the yeast is removed from the product, as is the case with pale lager, pale draught beer and light beer, labelling of the beer as a product of genetically modified organisms is not be required by law. Even strict Bavarian Purity Law (in German 'Reihnheitsgebot'), which is voluntarily observed by most beer manufacturers in Germany, would not be violated by the use of a genetically modified yeast strain. Major problems of the strategy are firstly, the fact that trichothecenes acetylated at C_3-OH do not completely lose toxicity[10] and secondly, the possibility of hydrolysis of the acetyl group during passage through gastrointestinal tract[11,12].

Glycosylation of trichothecenes on C_3-OH likely prevents the uptake of these toxins by living cells; it is therefore regarded as detoxification[12]. A feeding study with rats showed that bioavailability of deoxynivalenol-3-O-glucoside is limited; the authors concluded that the derivative possesses considerably lower toxicological relevance than non-glycosylated deoxynivalenol[13]. Because glycosylation of trichothecenes is a component of defence response of crop plants to fungal infection, deoxynivalenol-3-O-glusocide occurs in cereal products[9]. Availability of yeast strains expressing trichothecene-3-O-glysosylase of plant origin[14] and yeast species which glycosylate trichothecenes naturally[15] opened the opportunity to use glycosylation of trichothecenes as a detoxification reaction in food processing.

Both acetylation and glycosylation of C_3-OH are potentially reversible. Irreversible detoxification reactions are known but suitable genes and enzymes are not available. Last year's re-discovery of bacterial activities that led to complete mineralisation of trichothecenes[4], which had been reported 30 years earlier[3] but irrevocably lost[1], as well as the recent discovery of reductive deepoxidation under aerobic conditions[7], has raised hopes that these activities will be available for food production in the future.

Enzymatic detoxification of mycotoxins: chances and hurdles for commercialisation

The feasibility of enzymatic detoxification of trichothecenes, which belong to most important mycotoxins in countries of the Northern temperate zone, has been established *in vitro* and demonstrated in genetically modified crops. Detoxification of further mycotoxins has been studied since the 1980s. Identification of enzymes detoxifying aflatoxins and zearalenone are significant achievements with potential impact on food production. The major obstacle for the implementation of the technology in crop production has been public opposition against genetic technology, nourished by ideologically and economically motivated lobbies in Europe and elsewhere. As a result, many food companies restricted the use of GMOs to manufacturing vitamins, enzymes and other processing aids, the origin of which does not have to be declared on food products. Tropical and subtropical countries which suffer from widespread mycotoxin contamination of food and whose markets are not indoctrinated by anti-GMO propaganda are likely to be the first to benefit from enzymatic detoxification of mycotoxins in crop production and food manufacturing. In the long term, however, even Europe cannot afford to ignore the potential of biotechnology in counteracting mycotoxins and other poisons of natural origin in food.

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