

AGRO-FOOD



TNO innovation
for life

Today's challenges to the agro-food industry are to make their food production chains more efficient and sustainable without compromising on product safety. In addition, the agro-food industry has to meet the growing demand for healthy food products. Microbiology and Systems Biology (MSB) develops and delivers tools and technologies to help the agro-food industry address these challenges.

HEALTHY FOOD

Consumers increasingly demand food products that fit in a healthy life style. To address this need, many food companies promote their products by promising beneficial effects on health. The European Commission demands that such claims are supported by scientific data that have been evaluated by the European Food Safety Authority (EFSA).

MSB provides technology and service to facilitate health-claim assessment and substantiation. We are an internationally recognized partner for industry in applied nutrigenomics research. We have access to an extensive collection of *in vitro* and *in vivo* models of health and disease. We apply state of the art analytical platforms

to these models to measure and predict effects of food products on animal or human health. We are particularly experienced in consolidation of big data. We use advanced bio-informatics, statistics, computational modeling and machine learning to analyze and interpret the data to comprehensively and accurately assess our client's food products for beneficial effects on health.

TESTING FOOD PRODUCTS WITHIN THE RELEVANT DISEASE CONTEXT

Obesity and related cardiovascular and metabolic disorders are highly prevalent morbidities in high- and middle-income countries. Food manufacturers are increasingly developing products for people with these disorders. We have many mouse models of disease available to test food products for potential health benefits in specific disease contexts such as metabolic, cardiovascular and



inflammatory syndromes. In collaboration with food companies, we analyzed two food products with putative anti-inflammatory activity in humans and could confirm that these products targeted cellular processes involved in inflammation. Moreover, we found that these food products decreased key inflammatory markers and delayed onset of disease in a mouse model of human cardiovascular disease. Thus, our approach has helped our clients to characterize their food products within the relevant disease context.

TESTING FOOD PRODUCTS FOR SPECIFIC CONSUMER SEGMENTS

We have been diversifying this approach to test food products in specific categories of consumer segments. In a clinical study, we stratified individuals for body fat distribution and found that specific dietary compositions of fatty acid have beneficial effects in some overweight individuals, but potentially adverse effect in others. Finally, we are setting up a clinic, which aims to improve treatment of diabetes type 2 with personalized nutrition and life style advice. We will use specific metabolic markers to stratify newly diagnosed diabetics and subject each category to specific nutritional and life style interventions. Such study setups may also be used to test and optimize food products for beneficial effects in diabetics.

‘WE OPTIMIZE THE INNOVATIVE ABILITIES OF THE AGRO-FOOD INDUSTRY’

DEVELOPMENT OF A STANDARD TEST FOR HEALTH ASSESSMENT

The majority of health claims have not been approved by the EFSA on the grounds of insufficient scientific substantiation. The health assessment and substantiation of food products would benefit from having globally accepted standardized research methods to quantify optimal health. In collaboration with the food industry (Nestlé, DSM, DuPont, Friesland Campina and Abbott Nutrition) and academia, we are developing a new generation of biomarkers that assess health and can predict the tipping point when health turns into disease.

ASSESSING OPTIMAL HEALTH WITH CHALLENGE TESTS

We use a new innovative approach, called challenge tests, which defines health as the body's ability to show sufficient resilience to light stresses, such as a single consumption of a high-fat or sugar load, or a minor infection. The tests measure the body's capacity to respond to these challenges and how fast it recovers. As such, challenge tests will indicate whether people's health status is optimal or compromised. By using challenge tests, the effects of food products can be more easily detected and quantified. For example, we could detect the effect of a dietary supplement in overweight healthy men, challenged with a consumption of a high fat load.

INTERNATIONAL ADOPTION

We are leading two European research projects: Nutritech and PhenFlex, exploiting the challenge approach for health-claim substantiation. Nutritech will develop standardized research methods for global adoption in nutrition research, whereas The PhenFlex project aims to accelerate the food industry's application of the challenge approach. Their ongoing outcomes will be disseminated among stakeholders, including regulatory authorities, academia and the food industry.

RAPID TESTING OF ANIMAL FEED COMPOSITIONS

Poultry frequently suffer from infections of the gut, which lead to growth retardation and a consequent decline in poultry meat production. This accounts for significant revenue loss in the poultry industry. Animal feed manufacturers aim to develop feed compositions that improve animal health or prevent disease. Testing animal feed compositions for health benefits is currently a trial and error process and therefore time-consuming, ineffective and costly.

For two major animal feed companies, we developed a method to screen feed combinations for effect of animal health by analyzing the micro-organisms residing in the gut (gut microbiota). The gut microbiota is important for maintaining gut health, for example by preventing harmful micro-organisms in establishing infections. We developed a platform to screen hundreds of feed compositions for effect on gut microbiota in only one day. Our tool can be used to test and optimize animal feed. Our strategy can be generically applied to other animals or humans. For example, we have also used our approach to test animal feed in cats.

SAFE FOOD

Efficient risk assessment of food has become a challenging task. Changing consumer demands, raw materials of variable quality, new emerging food pathogens and increasingly complex products and production processes make the assessment of microbiological hazard difficult. We offer innovative detection methods for complex microbiological issues in food products and processing, saving the food industry time and costs in these important areas.



RAPID DETECTION OF FOOD PATHOGENS

Consumption of poultry meat contaminated with *Campylobacter* may cause gastrointestinal illnesses such as diarrhea and abdominal pain. As of 2011, the Dutch poultry slaughter industry is required by the Dutch Ministry of Public Health to reduce contamination of poultry meat by *Campylobacter*. Such contamination incidents can be reduced by improving hygiene during slaughter of poultry. However, methods to determine the effectiveness of hygienic procedures are currently lacking.

MSB developed a test, which can detect *Campylobacter* from a wide variety of samples, including swab samples, meat and faeces, without using time-consuming culturing steps. The *Campylobacter* test uses specific DNA markers that can be used for strain identification, tracking the source of contamination and determining sensitivity to antibiotics. The test is reliable, quantitative, fast and can be used to test a large number of samples. Our test could improve food safety by early detection of *Campylobacter* during the

slaughter process. Examples include testing caecal fluid or faeces from poultry, production-process environment and poultry meat.

EFFICIENT FOOD SAFETY MANAGEMENT

Currently used methods for detection of micro-organism are largely based on growth and do not assess viability. However, for the food-packaging industry it is important to know whether their sterilization methods are effective in eradicating common food pathogens such as *Listeria* or above mentioned *Campylobacter*. We have developed a real-time viability (RTV) assay, which measures the metabolic capacity of micro-organisms. The test provides robust results within minutes and can be used in automated fashion with a large number of samples. For a major food company we have used our RTV assay to screen for synergistic combination between high pressure treatments and common or natural preservatives. We have also used our RTV assay to assess quality of starter cultures of dairy products or probiotics.



SUSTAINABLE FOOD

The food and feed industry is actively looking for solutions to replace antibiotics or food preservatives with natural, sustainable ingredients. We have successfully developed tools to find such ingredients. Our technology can be used to find substitutes of antibiotics, preservatives such as E-numbers or can be used to reduce salt content.

FINDING NATURAL SUBSTITUTES OF CONSERVATIVES

Consumers increasingly demand use of natural food ingredients. Many chemically synthesized food ingredients are additives and preservatives known as E-numbers. A growing number of consumers perceive E-numbers as harmful. The food industry is thus interested to find natural alternatives of E-numbers. For Unilever we developed a method to find natural alternatives for an E-number that reduces food spoilage by inhibiting specific micro-organisms.

We analyzed the effect of this E-number on gene expression in these spoilage micro-organisms. We successfully identified highly specific markers for this E-number, which can be used to identify natural ingredients with similar activity. Such natural E-number mimetics could then replace this E-numbers in food products.

REDUCTION OF SALT

This strategy may also be used to find alternatives for other E-numbers or reduce salt concentrations. Similar to E-numbers, salt also functions as an antimicrobial. Consumers are increasingly interested in limiting their dietary salt intake as high salt intake is associated with medical risks such as high blood pressure. The food industry is therefore looking for solutions to reduce salt in their products. We have applied our technology to lower salt concentrations in pesto.

FINDING NATURAL SUBSTITUTES OF ANTIBIOTICS

Microbial infections in calf neonates account for a significant portion of cattle and calf losses in the beef industry. For example, upper airway infections is estimated conservatively to cost the UK cattle industry £60 million annually. Antibiotics have been commonly added to animal feed to prevent illness. However, the addition of antibiotics in animal feed is fully restricted by EU law since 2006 and the use of antibiotics in feed is being considered for elimination (or intense regulation) in other parts of the world. Moreover, consumers increasingly demand antibiotic-free meat. Therefore, feed companies are urgently searching for (natural) feed additives that can function as substitutes for antibiotics used in animal feed.

A major feed producing company asked us to identify potentially bio-active compounds that can inhibit the pathogens *Clostridium perfringens* and *Escherichia coli*. These pathogens commonly induce morbidity and death in calves by inducing gut infections. To find substitutes of antibiotics, we screened hundreds of natural ingredients for their ability to inhibit outgrowth of *Clostridium perfringens* and *Escherichia coli* in gut microbiota isolated from calves. We indeed identified ingredients which could inhibit *Clostridium perfringens*.



Our approach can be generically used to screen for inhibitors of other pathogens in animals or humans.

OUR EXPERTISE

In collaboration with other TNO expertise groups we offer:

- Nutritional intervention studies in *in vitro* models, mouse models of human disease, animals or humans: study design, execution of studies and interpretation of the data.
- Measurement and integrated analysis and interpretation of big data including clinical and physiological parameters, clinical chemistry markers, genomics, transcriptomics, metabolomics, proteomics data, obtained from different organs and/or body fluids.
- Rapid characterization and identification of micro-organisms independent of sample origin.
- Development of custom detection tests of micro-organisms (food spoilage, food pathogens).
- Fast and comprehensive identification of properties of micro-organisms (probiotics).
- Consultancy on food hygiene, food processing and food safety.

MICROBIOLOGY AND SYSTEMS BIOLOGY

Microbiology and Systems Biology is part of TNO, one of Europe's largest independent research institute for technological and strategic research and consultancy. By translating scientific knowledge into practice we optimize the innovative abilities of the industry and government. As a research partner, TNO works for various industries worldwide.

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