Food for Thought

Food science news, technology and developments from the Netherlands.

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Focus on Health

About Food for Thought

Written for food industry decision makers, *Food for Thought* reports periodically on agrofood activities and advances within the Netherlands. Its articles, overviews, and white papers provide an overall perspective on food technologies, innovations, and R&D taking place here.

Articles in this issue

New technologies for tracing and analyzing healthy food components in the body

Iso*Life*: the meaningful role of stable isotopes in health-related food research

Iso*Life*, founded in 2005 by Ton Gorissen and Ries de Visser, and located on Wageningen Campus, has developed a technology for the uniform enrichment of plants with the stable isotope ^{13}C that helps to tackle two major challenges:

- Improving the traceability of healthy nutrients through the use of new, traceable foods
- Increasing analytical efficiency through the use of non-interfering

Stable isotopes are playing an increasingly crucial role in state-of-the-art applications in health-related science and technology. This overview of Iso*Life*'s current research projects involving stable isotopes in food applications will explain this new development.

internal standards

Iso*Life* focuses on actual cases of health research in which stable isotopes like ^{13}C help improve the tracing and analysis of foods and their metabolites in the body, with enhanced efficiency, safety and comfort to the consumer and/or patient. Improvements in human health research are critical in view of the epidemic of health problems like overweight, obesity, metabolic syndrome, type 2 diabetes and intestinal cancers.

New technologies for tracing and analyzing healthy food components in the body are becoming increasingly available, and are finding use in routine diagnostics. We will illustrate four new applications of Iso*Life*'s unique, stable isotope products in ongoing health-related projects involving several consortia. These project cases will show how stable isotopes help to improve the monitoring of health effects, with better traceability and diagnostic analysis in health research and diagnostics.

Apart from enhancing analysis of human health, the new stable isotope technology fits nicely with strategies aimed at improving health by primary and secondary prevention. It will increase sustainability through its contribution to public health, thereby increasing economic productivity and lowering healthcare costs.

Today's modern sedentary lifestyle—often accompanied by an unlimited access to food—is known to be linked to an increased incidence of diseases like obesity, metabolic syndrome, type 2 diabetes, inflammatory diseases and cancers. Researchers have proposed various solutions to these problems, including changing lifestyles by introducing new, healthy foods.

De Visser explains: "For this purpose, Iso*Life* is collaborating with multidisciplinary research teams studying the fate of food in the human body. The goal is to increase our knowledge and support health claims. To gain insight into the fate of a particular food or food component in a living body—human or animal—we need to make the food component and its metabolites stand out against the background of endogenous metabolites, i.e., compounds already present in the body at the start of the experiment, originating from other sources, exogenous or endogenous."

A safe way of marking a food without affecting its digestion or metabolism is by enriching it with stable isotopes, like ^{13}C and ^{15}N, found in nature in small percentages (1.1% ^{13}C, 0.4% ^{15}N). These isotopes

Marcel de Groot/*Agency NL-NL Innovation*

USA

Allison Turner/*Netherlands Foreign Investment Agency*
Caroline Feitel/*Ministry of Economic Affairs, Agriculture and Innovation*
Karin Louzada/*Netherlands Office for Science and Technology*

Links to Dutch Food Industry Network

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Netherlands Office for Science and Technology
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may be increased in foods by up to 98%, are absolutely safe for humans and animals, and can be directly measured in the body (e.g., Lim et al., 2011). This is exactly what Iso*Life*'s new technology is all about: enriching food and feed with stable isotopes, to be used as tools in health research. The following cases will demonstrate this.

Starches preventing colon cancer

Colon cancer is the second-most common type of cancer in both men and women. One project deals with stable isotope-labeled starches from wheat and maize, which are used as tools in colon cancer prevention research. Much research into the risk factors for colon cancer has provided evidence for the protective role of starch fermentation. During this fermentation, short-chain fatty acids (SCFAs) are formed (Figure 1). According to Gorissen, "Increased production of these SCFAs from resistant starches—in particular butyrate—has proven to protect against colon cancer."

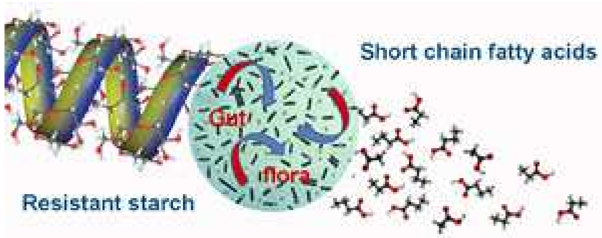


Figure 1. Fermentation of resistant starch and production of SCFA's by the gut microflora.

The mechanism of this protective action is not known, nor is it clear which SCFAs are formed by fermentation of different types of resistant starches originating from potato, maize or wheat, etc., and to what extent they contribute to the total protective action. In a feasibility study funded by the Dutch Government, the University Medical Center Groningen (UMCG) concluded that application of ^{13}C-labeled starches is of great value for research towards prevention of colon cancer."

Worldwide, Iso*Life* is the only commercial supplier of ^{13}C starches from food crops, thanks to a unique "closed-system" facility designed for the uniform labeling of higher plants. Using ^{13}C-labeled starches, researchers will be able to characterize the fermentation of starches, to determine the additional production of SCFAs from specific starches, to show which SCFAs are formed and to find the mechanism of uptake into the blood circulation. The objective of the research is to develop a diagnostic ^{13}C starch test for measuring the risk of development of colon cancer in humans. This requires detailed information on the fermentation properties of starches from different crops taking place within the colon.

During the past few years, researchers have cleared several bottlenecks in the production of ^{13}C-labeled corn and wheat starches. This opens up new avenues for developing a diagnostic ^{13}C starch test for colon health, for instance, by using the recently developed oral ileocolonic delivery "ColoPulse" device

(Schellekens et al., 2010) and the Stable Isotope Probing (SIP) technology for studying microbiological aspects of gut health (prize-winning paper by Kovatcheva-Datchary et al. 2009).

Such research fits within innovation programs on "Food and Health," "Disease prevention," "Biomarkers," and "Bio-ingredients and functional foods" for enhancing SCFA production in the colon. New starches may also be developed to help prevent type 2 diabetes by increasing the proportion of resistant starch in our daily nutrition. The economic perspective includes an increased market share of Dutch potato breeders for cultivars with increased resistant starch levels, a profitable diagnostic test for UMCG, new ^{13}C products and markets for Iso*Life*, and an increased health status and productivity for people. Within the EU association "Healthgrain Forum," further initiatives may be developed.

This project will lead to better diagnostics (supporting prevention of colon cancer) and better disease prevention through healthier foods with improved nutritional value.

Broccoli Cress, a vegetable with anti-cancer properties

Innovative food companies like Koppert Cress are pioneering the development of health claims for their new products. With Iso*Life,* they partner on a project on metabolome research of bioactive compounds in healthy humans for supporting health claims of new micro-vegetables such as Broccoli Cress (see photo). Glucosinolates are secondary plant metabolites in Brassica vegetables like kale and broccoli exhibiting various important nutritional properties. Glucosinolates have been gradually discovered to be phytochemicals exhibiting anti-cancer properties; Zhang, Talalay, Cho and Possner (1992) identified sulforaphane ($C_6H_{11}NOS_2$), originating from glucoraphanin in broccoli, as a strong inducer of quinine reductase, a phase II detoxification enzyme. In a recent review, researchers suggested that about three to five servings of broccoli per week seem to constitute a cancer preventive (Herra and Büchler, 2010).


Broccoli Cress

According to new EC legislation, it is no longer permitted to claim health benefits of a product simply because it *contains* "bioactive" compounds, without proving its *bioavailability* and *mechanism of action.* In collaboration with the world-renowned UMCG, researchers are conducting a project to deliver the required scientific proof. The evidence should not only verify the presence of the components in the product and the proven absorption of these components in the gut, but must also demonstrate the arrival of the components and their metabolites at the target location in the body where their health benefits are effectuated.

Gorissen: "In the current food innovation project, we investigate various aspects of ADME (adsorption, distribution, metabolism and excretion) that are required for underpinning health claims from the perspective of the consumer using the stable isotope ^{13}C." In addition to performing work regarding sensitive and relevant biomarkers, researchers are studying the effect of the food matrix on the bioavailability of components and their uptake in the bloodstream, as well as interactions between various components and inter-individual variation in ADME of bioactive compounds.

"The uniform ^{13}C-labeling of sulforaphane makes it an effective tracer for bioavailability studies. Preliminary data on ^{13}C-sulforaphane analysis in plasma indicate promising perspectives for oral administration of ^{13}C-enriched broccoli in the ADME studies," Gorissen explains.

"Concurrent with these ADME studies, we studied the source of glucoraphanin, the precursor of sulforaphane in broccoli seedlings or cress. Both ^{12}C (99 atom% ^{12}C) and ^{13}C (98 atom% ^{13}C) -enriched broccoli seeds were produced, and subsequently germinated and grown either in a $^{13}CO_2$ or a $^{12}CO_2$ environment. Afterwards, the ^{13}C enrichment of sulforaphane in seeds and in seedlings was determined by HPLC–MS. We found that sulforaphane originates exclusively from seed reserves and that *de novo* biosynthesis in broccoli seedlings was less than 1%. This information will help breeders and growers to produce healthier cresses." (Gorissen et al., 2011)

The use of ^{13}C-labeled vegetables and potatoes for demonstrating ADME of bioactive plant components in a quantitative way is a highly innovative approach. In the end, this should result in scientifically supported statements on the actual contribution to our health.

Macular degeneration—breeding new food crops that help prevent age-related macular degeneration (AMD) by increasing the intake and bioavailability of lutein and zeaxanthin

Picture source: http://medicineworld.org/

AMD is a major cause of blindness and visual impairment in older adults. It results in a loss of vision in the center of the visual field (the macula) because of damage to the retina.

The National Eye Institute and others have suggested vitamin supplements with high doses of antioxidants—like the carotenes lutein and zeaxanthin—to slow the progression of AMD and, in some patients, improve visual acuity (Tan et al., 2008). Lutein, zeaxanthin and β-carotene belong to a large group of health-promoting compounds, since they function as antioxidants and as precursors of vitamin A.

Synthesized in plants and some microorganisms, zeaxanthin is the pigment that gives bell peppers, corn, saffron, and many other plants and microbes their characteristic color.

Breeding new food crops can help prevent AMD by increasing the intake and bioavailability of the carotenoids lutein and zeaxanthin. Questions that still need to be answered are:

- Which foods contribute to the uptake of lutein and zeaxanthin by the gastrointestinal tract?
- How long does it take for vegetable carotenoids to reach the eye and how long do they stay there?
- What is the dose required for sustainable eye health?

Simply sampling blood plasma after consumption of the new high-carotenoid food does not yield reliable information on bioavailability since, once inside the body, the absorbed carotenes are mixed with carotenes already present and originating from other food sources. Again, ADME studies using ^{13}C-enriched foods can help deal with these challenges. The ^{13}C-enriched vegetables can be administered to volunteers as part of normal daily diets that may contain any other source of carotenes, since the ^{13}C-labeled carotenes can be easily distinguished from non-labeled forms in exo- or endogenous pools. After consumption of the experimental meals, plasma samples of the volunteers are taken during a long period (up to a month) and the carotenes are quantified using specific liquid chromatography–mass spectrometry techniques. The kinetics of the ^{13}C carotenes and their metabolites can thus be monitored in the blood circulation. Uptake efficiency of lutein and β-carotene was studied by these techniques using ^{13}C-enriched kale (*Brassica oleracea* var *acephala*) as vegetable (Kurilich et al., 2003; Novotny et al., 2005). Isotopic labeling of kale provided the opportunity for studying the kinetics of the consumed lutein and β-carotene without confounding background pools of these compounds in the body. Novotny et al. (2005) concluded that the bioavailability of lutein is much higher than that of β-carotene, although their absorption appeared to be similar. Iso*Life* is now performing a similar study with private and public partners.

Inborn Errors of Metabolism

Diagnostics of rare metabolic diseases used to be out-of-reach for modern analytical techniques, because of a lack of sensitive and specific assays that require stable isotopes as internal standard. De Visser: "At Radboud University Nijmegen Medical Centre (RUNMC), researchers are conducting a project funded by The Netherlands Organisation for Scientific Research (NWO) entitled 'High-resolution glycoprofiling in human medicine.' RUNMC studies an important group of orphan diseases with abnormal protein glycosylation. In this project, research and diagnosis has now been accelerated by new developments such as high-throughput analytical tools (innovative nanoLC-mass-spectrometry) that are much more efficient by enabling the detection of a complete profile of glycans in one single analytical run, leading to more efficient screening of young patients, especially newborn babies."

Picture source: http://www.fodsupport.org/

This technology forms an essential extension of the current infrastructure and enables highly sensitive and detailed fingerprinting of many different glycan classes that are present on proteins and lipids in body fluids or cells. Changes in the glycosylation of proteins and lipids are associated with a growing variety of disorders, including inherited glycosylation disorders. This type of structural information complements functional studies performed within the Nijmegen Glycobiology Platform.

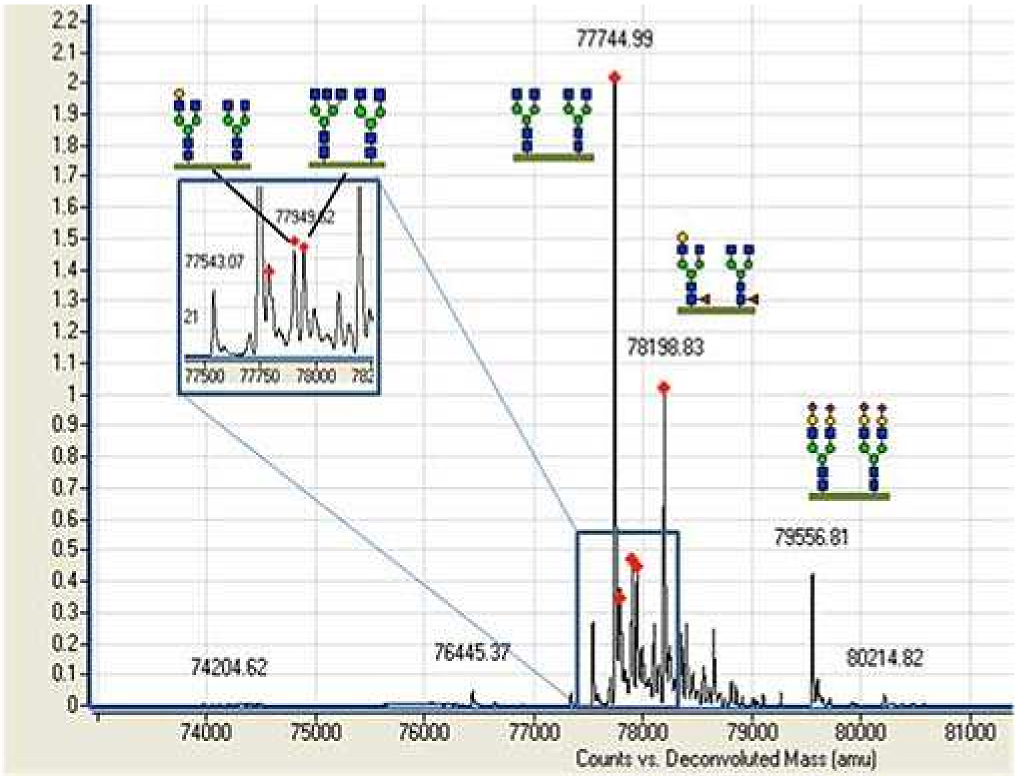


Figure 2. Mass spectrometry of intact serum transferring, showing a diversity of differently glycosylated protein isoforms. (Source: Dr. Dirk Lefeber, RUMC, Nijmegen Glycobiology Platform)

For this new technology to be reliable, stable isotope internal standards (SIIS) are required, which Iso*Life* is currently developing from food plants like chicory and potato. Similar stable isotope technology, using Iso*Life*'s internal standards, has been published and developed in the field of food science, e.g., at the University of Kaiserslautern (Germany), which, in this case, resulted in the improved analysis of polyphenols in fruit and human body fluids (Erk et al. 2009; Hagl et al. 2011).

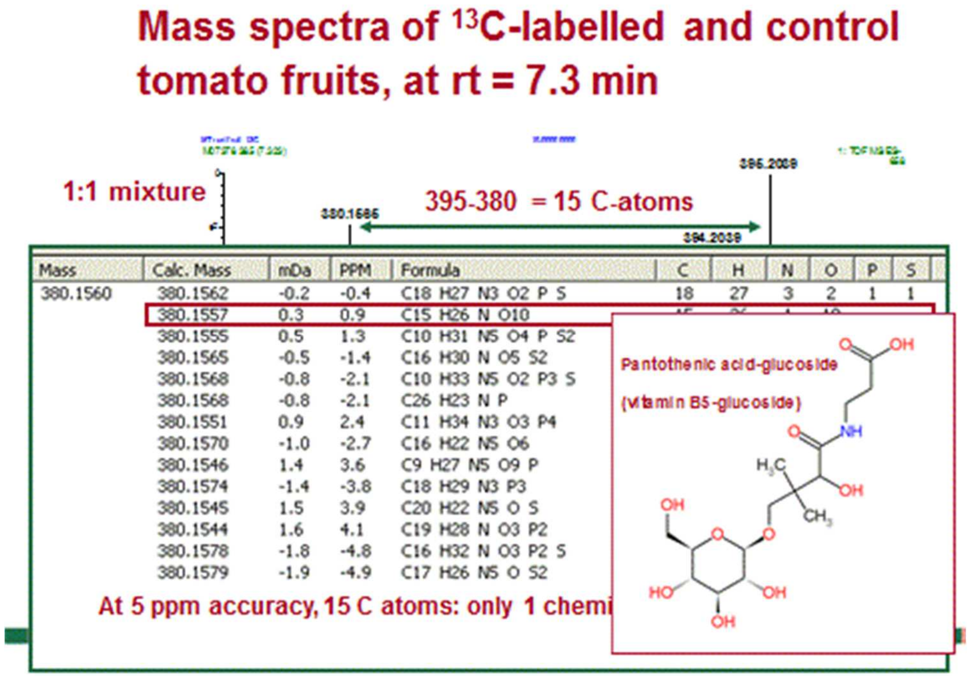
Additional applications include the use of isotopically enriched food oligosaccharides to study the systemic effects by analyses in serum and urine. In addition, the uptake of (isotopically labeled) food sugars and their conversion into protein linked glycans can finally be investigated in high resolution.

New stable isotope internal standards: applications in food and pharma research

NextGen Metabolomics Inc. (Michigan) (http://nextgenmetabolomics.com/) has developed an automated metabolic fingerprinting technology, IROA (Isotopic Ratio Outlier Analysis) for metabolic phenotyping of tissues or bio-fluids. The company is now applying Iso*Life*'s ^{13}C-enriched internal standards for reliable quantification.

De Visser: "IROA is a type of metabolomics assessment in which both the control sample and experimental samples are isotopically labeled so both may be easily found with absolute assurance of their identity, and any artifacts may be removed. This leads to very clean, high-resolution data sets that clearly define the biological response of a biological system. In the case of a phenotyping application, where it is not possible to isotopically label the biological sample, the sample is collected at natural abundance and is mixed with a fully predefined 'Internal Standard' that has been isotopically labeled using ^{13}C media or Iso*Life*'s technology. The ^{13}C-internal standards function as absolute yardsticks in the MS analyses. Degradation of metabolites during the analyses and variations in sample processing are accounted for by spiking the samples with U-^{13}C-labeled standards before processing."

De Visser: "The great advantages of ^{13}C-enriched internal standards are accurate quantification of your analytes, independent of time, place, sample composition, processing method, operator or apparatus used. They do not only enable accurate quantification, but also facilitate identification and quality control of metabolites in natural, non-labeled samples analyzed by LC-MS or GC-MS." (Figure 3)



Mass	Calc. Mass	mDa	PPM	Formula	C	H	N	O	P	S
380.1560	380.1562	-0.2	-0.4	C18 H27 N3 O2 P S	18	27	3	2	1	1
	380.1557	0.3	0.9	C15 H26 N O10						
	380.1555	0.5	1.3	C10 H31 N5 O4 P S2						
	380.1565	-0.5	-1.4	C16 H30 N O5 S2						
	380.1568	-0.8	-2.1	C10 H33 N5 O2 P3 S						
	380.1568	-0.8	-2.1	C26 H23 N P						
	380.1551	0.9	2.4	C11 H34 N3 O3 P4						
	380.1570	-1.0	-2.7	C16 H22 N5 O6						
	380.1546	1.4	3.6	C9 H27 N5 O9 P						
	380.1574	-1.4	-3.8	C18 H29 N3 P3						
	380.1545	1.5	3.9	C20 H22 N5 O S						
	380.1544	1.6	4.1	C19 H28 N O3 P2						
	380.1578	-1.8	-4.8	C16 H32 N O3 P2 S						
	380.1579	-1.9	-4.9	C17 H26 N5 O S2						

Figure 3: Identification of an unknown vitamin in tomato fruit by use of a ^{13}C plant extract as internal standard (Source: Dr. R. de Vos and Dr. B. Schippers, Plant Research International and CBSG, Wageningen)

The examples described in this article clearly show the potential of stable isotopes with respect to research in the "Nutrition and Health" domain. Stable isotopes will not only improve monitoring of health effects by enhancing traceability and diagnostic analysis, but will also contribute towards the substantiation of future health claims. If you are interested in collaboration with Iso*Life,* please contact Ries de Visser or Ton Gorissen.

Contact details:
Ries de Visser or Ton Gorissen
Email: info@isolife.nl

Website: http://www.isolife.eu

Links

http://www.umcn.nl/Research/Departments/metabolicandendocrinediseases/Pages/geneticsandmetabolism.aspx
http://www.glycobiologynijmegen.nl/
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http://nextgenmetabolomics.com/

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