



RESEARCH AND INNOVATION FOR FOOD & BIOBASED PRODUCTS



Edition 2018

DIVISION OF SCIENCE
FOR FOOD AND BIOPRODUCT ENGINEERING





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Research & innovation for Food & Biobased Products

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FOREWORD

The production of fundamental knowledge to underpin innovation is central to INRA's missions and is thus also central to the activities of our CEPIA division (Science for Food and Bioproduct Engineering). Within the framework of this mission, our teams are developing research in several areas:

- ◆ Food science and engineering, with the aim to contribute to the development food items that are both tasty, nutritious, safe and sustainable.
- ◆ Biotechnology and green chemistry, two fields that supply key enabling technologies for the bioeconomy
- ◆ Knowledge engineering, artificial intelligence and multicriteria analyses, to support our research especially regarding the integration and use of data from heterogenous sources and the development of methodologies to appraise the sustainability of processes, products and value chains.

Since 2016, the collective strategic roadmap that is applied to our 22 research laboratories focuses on two key scientific challenge areas:

1. The design of food and biobased product quality
2. The development of efficient processes for the manufacture of sustainable products

Through our focus on these two areas we aim to provide a major contribution to several societal challenges. In this 2018 edition of our research results, you will find descriptions of work that contributes to renewal of food supply, considering the ever more stringent requirements in terms of safety and sustainability. As our results reveal, to achieve this goal it is necessary to acquire new knowledge (e.g. regarding cooking processes or digestive mechanisms), develop new analytical tools and, sometimes, propose new processes to make innovative products. You will also find herein results of work that contributes to the bioeconomy transition, for example the development of new technologies to produce biobased chemical intermediates and materials. Last but not least, you will find descriptions of work focused on the development of mathematical tools, which are essential for data treatment, building knowledge and to appraise the performance of life cycles (processes, products and value chains).

Michael O'Donohue

Head of division CEPIA



Bioresources and bioprocesses for the bioeconomy

While the primary role of the agro-industry is to meet the population's food requirements, agricultural raw materials can also be used to produce materials, chemicals, and energy. Therefore, rather than establishing parallel value chains for biobased raw materials and the processes needed to transform them, it is preferable to develop an integrated approach that is consistent with the concept of the bioeconomy. In this regard, the development of:

- ▶ the bioeconomy is largely enabled by facilitating technologies, such as biotechnology and green chemistry, technologies that are perfectly adapted to the complexity of biobased raw materials
- ▶ biobased materials will be key for the transition to a bioeconomy. Rationalizing the design of biobased materials and understanding where their functional properties come from—and ultimately how to control them—hinges on acquiring in-depth knowledge of structure science on a gradient of scales (from nm to mm).

Together these two focus areas form the basis of CEPIA's biobased research and are illustrated in this section.

Participants

- ♦ INRA-BIA / Plateforme BIBS, Nantes
 - ♦ USC INRA AFMB, Marseille
 - ♦ Université de York, Angleterre
- Projets Marie-Curie International Outgoing Fellowship “zyBiom” (7th European Community Framework Program - 328162) et Microbio-E A*MIDEX project (ANR-11-IDEX-0001-02)

Read more

Lytic xylan oxidases from wood-decay fungi unlock biomass degradation

(2018) Nature Chemical Biology
Couturier M *et al*

IPR knowledge management

- ♦ Processes for breaking cellulosic substrate fibrillation and making celluloses using a new family of fungal lytic polysaccharide monooxygenases (LPMO).
Brevet FR17/57422
- ♦ Polysaccharide-oxidizing composition and uses thereof.
Brevet FR16/306162

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Fungal Biodiversity and
Biotechnology (BBF)

Discovery of a family of oxidative enzymes that target the recalcitrant polysaccharides in wood

Lignocellulosic biomass is a pivotal resource for biorefinery process development, yet efforts to further upvalue this biomass resource are bottlenecked by its resistance to enzymatic degradation. Nature counts certain filamentous fungi whose complex and diverse enzymatic arsenal makes them efficient wood-decomposers. This enzymatic machinery is gaining increasing traction as an avenue for developing powerful yet sustainable biotech processes.

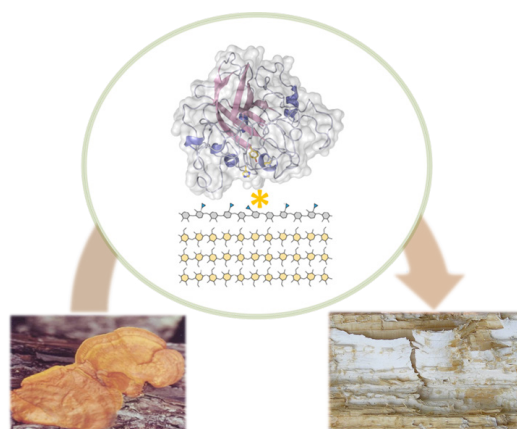
► RESULTS

Exploring the natural fungal enzymatic biodiversity in the CIRM-CF collection hosted at the BBF unit enabled us to pinpoint a family of enzymes that are only found in wood-rotting fungi (mainly Basidiomycetes). By engaging complementary approaches and several national and international research collaborations, we managed to elucidate the biological function of this new enzyme family. These enzymes are lytic polysaccharide monooxygenases (LPMO) that have a specific activity on a hemicellulose-family polysaccharide called xylan, which is associated with the cellulose chains in wood. Xylan is considered recalcitrant as it cannot be hydrolyzed by xylanase-type hydrolases, but it can, however, be oxidatively cleaved by these LPMO.

► FUTURE OUTLOOK

In terms of biotechnology potential, these enzymes could prove a disruptive new workhorse for bioenergy production processes. The first members of this new class of enzymes to be characterized significantly increase the saccharification of wood via oxidative cleavage of xylans coating cellulose fibers. These innovative new enzymes can also create early-stage breaks in paper-pulp cellulose fibers to drive the production of biobased materials.

The white-rot decomposer fungus Pycnoporus coccineus secretes an LPMO enzyme specific to xylans coating cellulose fibers in wood. This wood cellulose fiber-specific oxidative cleavage holds valuable potential for driving biorefinery processes





Participants

- INRA-BIA, Nantes
- CIRAD-UMR IATE, Montpellier
- plateforme CIRM-CF, Marseille
- Terres Inovia, Terres Univia, Paris

Research Project: Oléochampi 3 & 4 with Terres Inovia and Terres Univia (2015-2018).

Read more

*A two-step bioconversion process for canolol production from rapeseed meal combining an *Aspergillus niger* feruloyl esterase and the fungus *Neolentinus lepideus**

(2017) Microorganisms

Odinot E, Fine F, Sigoillot J-C, Navarro D, Laguna O, Bisotto A, Peyronnet C, Ginies C, Lecomte J, Faulds CB, Lomascolo A

Rapeseed and sunflower meal: a review on biotechnology status and challenge

(2012) Applied Microbiology and Biotechnology

Lomascolo A, Uzan-Boukhris E, Sigoillot J-C

Development of a fungal process for producing vinylphenols from oilseed meals

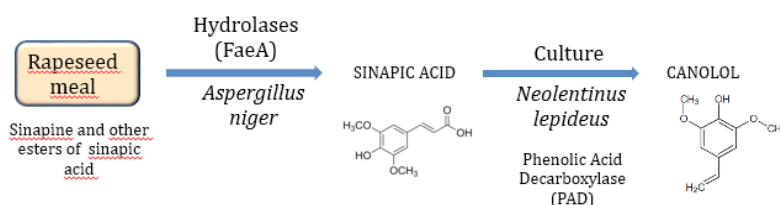
Oilseed meals, chiefly rapeseed and sunflower, are oilseed-industry byproducts that constitute a cheap (€200/tonne) and abundant potential feedstock for the development of biorefinery processes. They are particularly rich in phenolic compounds, especially sinapine (1–2% of press-cake DM content).

► RESULTS

By exploring the biodiversity within wood-decaying fungi and plant biomass, we found that *Neolentinus lepideus* is the only fungal species known to date that can naturally and efficiently convert sinapic acid into canolol (2,6-dimethoxy-4-vinylphenol) with a molar-percentage bioconversion yield of 80%. This bioconversion is driven by an enzyme (phenolic acid decarboxylase, PAD) whose sinapic acid-specific activity is novel and constitutes a breakthrough. In our work, we have developed and patented a two-step bioconversion process that combines the complementary action of two filamentous fungi: the micromycete *Aspergillus niger* and the basidiomycete *Neolentinus lepideus*. The first step of the process uses the type-A feruloyl esterase (AnFaeA) from *A. niger* to release the esterified forms of sinapic acid—mainly sinapine—from the raw meal, forming free sinapic acid. The second step of process uses the fungus *N. lepideus* to convert free sinapic acid into canolol by non-oxidative decarboxylation. Canolol is a potent antioxidant and anti-inflammatory agent. It can also serve as a platform chemical for the synthesis of bisphenol A substitutes and biobased polymers.

► FUTURE OUTLOOK

Our results demonstrate how a cheap agricultural byproduct can be converted using a microbial/enzyme-based process into vinylphenol, a high value-added 'green chemical'. While our work focused on ferulic acid, the bioconversion process will actually allow the conversion of any biomass-derived hydroxycinnamic acids (typically ferulic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid) or derivatives thereof into vinylphenols, such as vinylguaiacol, vinylphenol and vinylcatechol.



CONTACT

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Participants

Studies financed under the Futurol project 'Procéthol 2G' which is funded by BPI France, supported by OSEO, and sponsored by the Agro-Resources and Industries Competitiveness Cluster <https://www.projetfuturol.com/>

This same project funded postdoc research by Thomas Auxenfans

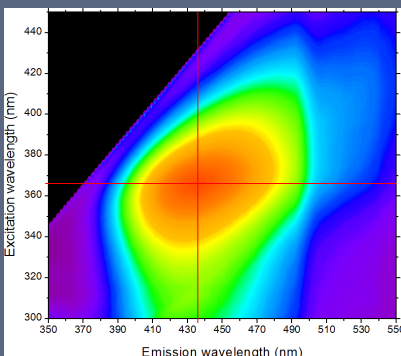
Read more

Exploring accessibility of pretreated poplar cell walls by measuring dynamics of fluorescent probes

(2017) Biotechnology for Biofuels
Paës G, Habrant A, Ossemond J, Chabbert B

Seeing biomass recalcitrance through fluorescence

(2017) Scientific Reports
Auxenfans T, Terryn C, Paës G



Autofluorescence of a biomass sample (measured fluorescence intensity plotted against fluorescence excitation and emission wavelengths).

CONTACT

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Fractionation of Agricultural
Resources & Environment
(FARE)

Mobilizing fluorescence methods to understand and predict the hydrolysis of lignocellulosic biomass

Biorefineries mobilize a series of unit-operations to convert biomass into commercial goods such as biofuels. In the Futurol project, the aim was to devise a process to produce fuel-ethanol from lignocellulosic biomass, using enzymes to drive a unit operation that hydrolyzes the plant cell wall polysaccharides and produces fermentable sugars.

It is well-established that enzyme activity depends on substrate accessibility at nanometric-scale, meaning that limited substrate accessibility results in low enzyme activity. In lignocellulosic biomass refining the hydrolysis of cell wall polysaccharides is hence largely dependent on their accessibility, which depends on the botanical origin of the biomass and also on the severity of the so-called pretreatment process, a unit operation that is designed to partially breakdown the biomass feedstock. Therefore, predicting the yield of enzymatic hydrolysis of lignocellulosic biomass is notoriously difficult. However, it is also essential since the time required to achieve this step is a key process parameter.

► RESULTS

In order to study lignocellulosic substrate accessibility, we have developed a FRAP (fluorescence recovery after photobleaching) -based method to measure the diffusion kinetics of fluorescence-labelled dextrans, whose sizes approximate to those of lignocellulose-degrading enzymes. Our results demonstrate that differences in accessibility are more related to the organization of the cell wall polymers (including polysaccharides) than their concentration. Particularly, pretreatments that strongly decrease lignin content do not enhance diffusion, probably due to the fact that the remaining lignin reorganizes and creates a more complex network. Alternatively, even a minor decreases in hemicellulose content will enhance diffusion.

Turning to the prediction of enzymatic hydrolysis, we demonstrated that there is a strong correlation between the initial fluorescence parameters of the pretreated biomass and its saccharification rate, and this correlation held on three different species of biomass. Better hydrolysis of the biomass samples is explained by the disappearance of a particular type of lignin bond (the β -aryl-ether bond) revealed by fluorescence spectroscopy, and which accounts for better accessibility of the cellulose.

► FUTURE OUTLOOK

Fluorescent enzyme probes will be used to measure both their accessibility and their interactions with lignocellulose in order to optimize their enzymatic activity, and we will also be assessing genericity for fluorescence-based prediction of saccharification rate on other pretreatments.

Participants

These findings were obtained as part of the PROBIO3 project that seeks to develop a microbial-driven production chain for specialty fatty acids based on renewable resources and industrial by-products.

PROBIO3 federates a consortium of 16 partners, including 9 INRA research units, INSA, CNRS, 4 Airbus Industries, EADS, Sofiproteol, Tereos and 3 ITERG Technical centers, CVG, CREOL

Read more

Production of Medium Chain Fatty Acids by Yarrowia lipolytica: Combining Molecular Design and TALEN to Engineer the Fatty Acid Synthase

(2017) ACS Synth. Biol.

Rigouin C, Gueroult M, Croux C, Dubois G, Borsenberger V, Barbe S, Marty A, Daboussi F, André I, Bordes F

Mutant yeast strain capable of producing medium chain fatty acids

(Brevet N°EP17305044)

Bordes F, Rigouin C, Gueroult M, Croux C, Percheron B., Barbe S, Marty A, Daboussi F, André I

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Biosystems and Process Engineering (LISBP)

Novel approach combining rational enzyme engineering with genome editing in the yeast *Yarrowia lipolytica* to produce the fatty acid precursors of biokerosene

The efficiency of an aviation fuel revolves around a number of factors, including the size and composition of the component alkanes. Alkanes are readily obtained from fatty acids, which makes the use of oil crop microorganisms a particularly attractive route. As the aircraft industry is turning to biofuels, the challenge is to be able to control the microorganism's lipid composition to meet the requirements for approved commercial use.

► RESULTS

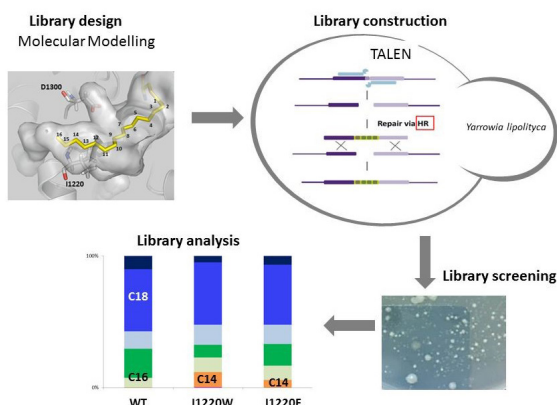
Our efforts were channelled into rationally engineering the lipid profile of *Yarrowia lipolytica* towards the synthesis of shorter fatty acid chains. However, the molecular determinants of chain length specificity in this yeast were still unknown. To address the challenge of remodelling lipid biosynthesis to shorten the chain length of the synthesized fatty acids, we posited that the ketoacyl synthase domain of the *Yarrowia lipolytica* fatty acid synthase is directly involved in chain length specificity. Working up from this starting point, and guided by molecular modelling, we followed a rational redesign approach based on unravelling structure–function relations and managed to design ketoacyl synthase mutants. We used an original new genome editing technology called TALEN (transcription activator-like effector nucleases) which facilitated the construction of new strains and proved an efficient tool for gene silencing and site-directed mutagenesis. This approach enabled us to pinpoint the key gene to mutate to shorten fatty acid chain length without compromising yeast growth performance.

► FUTURE OUTLOOK

This pioneering research opens up a whole new vision of understanding molecular-scale lipid biosynthesis and engineering non-conventional yeasts for the production of lipids custom-tailored to industrial

m i c r o b i o l o g y
r e q u i r e m e n t s .

Furthermore, the huge potential synergized by combining rational engineering techniques with genome editing opens up new pathways for leveraging use of the yeast *Yarrowia lipolytica*.





Participants

The green chemistry company Carbios has coordinated the large-scale cooperative project Thanaplast™ involving six partners, including INSA and INRA - as part of Toulouse White Bio-technology, CNRS, the University of Poitiers, the Déinove Company, and the Barbier and Limagrain groups.

Read more

Construction of a synthetic metabolic pathway for biosynthesis of the non-natural methionine precursor 2,4-dihydroxybutyric acid

(2017) Nature

Walther T, Topham CM, Irague R, Auriol C, Baylac A, Cordier H, Dressaire C, Lozano-Huguet L, Tarrat N, Martineau N, Stodel M, Malbert Y, Maestracci M, Huet R, André I, Remaud-Siméon M, François JM

Construction of a synthetic metabolic pathway for the production of 2,4-dihydroxybutyric acid from homoserine

(2017) Metabolic Engineering

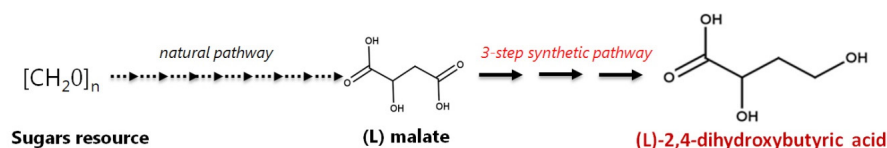
Walther T, Calvayrac F, Malbert Y, Alkim C, Dressaire C, Cordier H, François JM

A new stride forward in moving away from a petrochemical-based economy: Construction of a synthetic metabolic pathway in a bacterium for biosynthesis of the chemical synthon 2,4-dihydroxybutyric acid

One of today's big challenges for industrial biotechnology—or 'white biotech'—is to get microorganisms to produce chemicals from "renewable" carbon sources. If it can be done, then cellulose and hemicelluloses in wood and straw could advantageously replace fossil-fuel resources (petrol, gas) to produce certain key industrial compounds. This approach—replacing petrochemicals with biobased alternatives—would not only cut CO₂ emissions but also re-establish a normal terrestrial carbon cycle.

► RESULTS

The development of white biotechnology has a number of scientific and technical challenges to overcome. A team of researchers from the LISBP—Biosystem and Bioprocess engineering laboratory, working in collaboration with industry partner Adisseo and Toulouse White Biotechnology, have managed the breakthrough scientific feat of constructing an entirely new (synthetic-route) metabolic pathway that, when expressed in a bacterium, enables it to produce 2,4-dihydroxybutyric acid from glucose substrate. This versatile chemical synthon has huge technological potential, as it can be converted into methionine, an important essential amino acid supplement for animal feeds that is still produced exclusively by petroleum-based chemical synthesis.



► FUTURE OUTLOOK

The synthon can also serve as a building block for a wide panel of products with applications in other sectors of the chemicals industry, aerospace industry and pharmaceutical industry ready to mobilize a handful of known and readily-controllable chemical and/or biochemical reactions.

This research, which was largely financed by the French national research agency (ANR) under the first strategic S&T 'Investments for the Future' programme, was published in June 2017 in the prestigious high-impact scientific journal *Nature Communications*.

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Participants

This project was funded through an INRA–Region co-sponsored PhD project and was also partly financed by the CEPIA division's ANS MicroPro program.

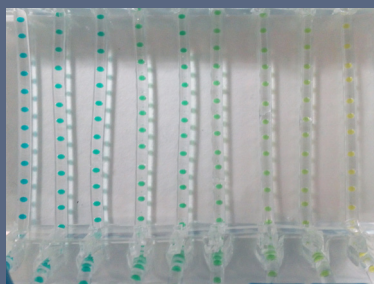
Read more

Millifluidique à gouttes : un outil pour le criblage des interactions entre biopolymères

(2017) Thèse de doctorat, Unité BIA
Amine C

Droplets-based millifluidic for the rapid determination of biopolymers phase diagram

(2017) Food Hydrocolloids
Amine C, Boire A, Davy J, Marquis M, Renard D



Droplet-based millifluidics tool for screening biopolymer–biopolymer interactions.

Droplet-based millifluidics repurposed for plant protein assembly

A regular dietary supply of plant proteins is widely recommended. However, their use as an ingredient is limited by the fact that they have an inherently low water solubility and a heavily aggregated fraction. To better understand and control this aggregation, we developed a medium-throughput screening tool working by droplet-based millifluidics.

► RESULTS

The experimental setup consists of an assembly of cheap and commercial off-the-shelf tubing and connecting (Fig. A) that can serve to map the phase diagram of single proteins or protein mixtures. This device (Fig. B), once coupled to an image data acquisition system, can serve to:

- ♦ generate a homogeneous proteins/buffer and/or proteins/biopolymers mixture in a flash (~s),
- ♦ vary the composition of the mixtures simply by adjusting the flowrates,
- ♦ determine droplet turbidity by greyscale analysis,
- ♦ control process temperature within a 4°C–40°C range.

The fitness-for-purpose of the device (mixing efficiency, turbidity calibration against greyscale level) was first gauged using colloidal dispersions of titanium dioxide (TiO₂) then validated by establishing a known phase diagram of a binary mixture of biopolymers, i.e. β -lactoglobulin–acacia gum. The device was then mobilized to probe a rapeseed napin–pectin assembly.

Using millifluidics made it possible to use ten times less material and run experiments five times faster than with a conventional-scale tubing-system approach, while at the same time being less expensive than microfluidics and requiring less expertise to deploy.

► FUTURE OUTLOOK

Potential further developments push in a number of directions—we are exploring synergies between plant proteins, animal proteins and other biopolymers to increase protein solubility and to generate smart new assemblies like microgels and capsules.

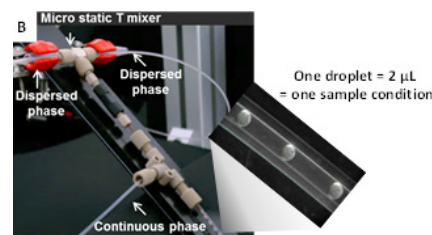


Figure A. Set of tubing, syringes and connecting used for millifluidics device design. ©Chloe Amine

Figure B. Set-up able to mix two solutions and generate droplets in a co-flow configuration. Each droplet represents a condition and can be assimilated to a reactor.

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- ♦ INRA Pays de la Loire, UR 1268 BIA, Nantes
- ♦ Synchrotron SOLEIL, Gif-sur-Yvette
- ♦ Université Clermont Auvergne, Université Blaise Pascal, Institut Pascal, Clermont-Ferrand
- ♦ INRA Auvergne-Rhône-Alpes, AgroResonance Platform, UR370 QuaPA, Saint-Genes-Champagnelle

Read more

The impact of processing and aging on the oxidative potential, molecular structure and dissolution of gelatin (2017) Food Hydrocolloids
Duconseille A, Traikia M, Lagree M, Jousse C, Pages, G, Gatellier P, Astruc T, Sante-Lhoutellier V

Molecular and structural changes in gelatin evidenced by Raman microspectroscopy (2018) Food Hydrocolloids
Duconseille A, Gaillard C, Sante-Lhoutellier V, Astruc T

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Animal Product Quality
(QuaPA)

Molecular and structural characterization of pig skin gelatin to predict its dissolution stability

Gelatin obtained from collagen, a component of skin and bone, is the primary excipient ingredient of pharmaceutical capsules. The gelatin capsules are designed to progressively dissolve, leading to controlled release of the active pharmaceutical ingredient directly in the patient's digestive tract. The dissolution rate of the gelatin can become altered during storage. Drugmakers therefore apply a standard accelerated ageing protocol followed by a dissolution test to sort their production batches according to dissolution ability. The industry knows that the dissolution ability of gelatin varies between production areas, but nobody has yet been able to explain where this variation comes from.

► RESULTS

Here we led a study spanning three production sites—two in Europe and one in the USA—with two objectives: 1) to unravel the mechanisms underpinning variability in gelatin dissolution ability, 2) to identify potential 'markers' of gelatin dissolution in order to predict its properties during shelf-life ageing.

Circular dichroism analysis on different batches of 'fresh' gelatin makes it possible to predict their post-ageing dissolution ability. Artificially-aged gelatins that are not dissolution rate-compliant have a higher amorphous phase content than dissolution rate-compliant gelatins. Among other major findings, note the changes in intermolecular structure, with the formation of dityrosine, a potential marker of shelf-life ageing. The chemical composition of gelatin can be used to map its source based on the extent of dityrosine formation in the fresh and age-accelerated gelatins or the physical-chemical arginine environment. The oxidative potential of the co-extracted lipids and amine functions of the gelatin is thought to cause the molecular chains to cross-link. The decrease in dissolution ability has multifactorial causes—for instance, it only correlates with iron content for one of the three production sites.

► FUTURE OUTLOOK

Our findings offer lines of action for reducing the variability in gelatin dissolution rates: control and reduce the level of oxidation, and control and reduce lipid content.

Read more

Collagen type I from bovine bone. Effect of animal age, bone anatomy and drying methodology on extraction yield, self-assembly, thermal behaviour and electrokinetic potential

(2017) International Journal of Biological Macromolecules

Ferraro V, Gaillard-Martinie B, Sayd T, Chambon C, Anton M, Santé-Lhoutellier V

The “sisters” α -helices of collagen, elastin and keratin recovered from animal by-products: functionality, bioactivity and trends of application

(2016) Trends in Food Science & Technology

Ferraro V, Anton M, Santé-Lhoutellier V

Upvaluing animal biomass : structural fibrous proteins for engineering biobased material and bioactive compounds

The many opportunities for upvaluing animal biomass are underexploited in France, yet collagen, elastin and keratin all have properties that can be advantageously repurposed for a number of applications, typically replacing synthetic-sourced materials or producing bioactive components (for antioxidants, anti-hypertensives, anti-diabetics, anti-inflammatories, etc.).

► RESULTS

We investigated the extraction of fibrillar collagen from cattle bone (*Bos taurus*, type-1 collagen) factoring several parameters:

- age factor (young adult, i.e. 4 years old, and full adult, i.e. 7 years old) and anatomy factor (femur and tibia) to study collagen extractability according to variability in source material;
- drying-method factor (high-temperature or low-temperature) to study collagen assembly once extracted from the bone.

We found that bone age is negatively correlated with collagen extraction yield and positively correlated with minerals content (calcium and phosphorous) and proteoglycans content, which is important for collagen fibril assembly *in vivo* and probably *ex vivo* too. From an anatomical point of view, the tibia gave the highest yields and tibia collagen showed better stability in colloidal dispersion (zeta potential). Regardless of bone age and bone anatomy, the extracted collagen is able to self-assemble into 3-dimensional structures (fibrils or microcapsules). Circular dichroism studies led at the SOLEIL synchrotron facility enabled analysis of the denaturation-renaturation balance of collagen extracted under a temperature gradient. The thermodynamic parameters related to changes in protein secondary structure were thereby determined.

We learned that collagen from an adult-cow tibia has the most denaturation-resistant structure.

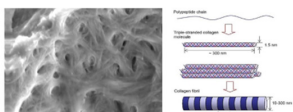
► FUTURE OUTLOOK

As a corollary of this finding, we conducted a review of the literature on potential applications for structural fibrous collagen, elastin and keratin proteins from animal byproducts. Published in *Trends in Food Science & Technology*, our paper is the very first publication by a French research institute on the use of animal biomass waste to recover valuable structural fibrous proteins.

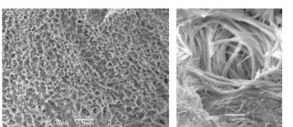
Biomedicine and Pharmaceutics

Animal by-products carry a pool of functional and bioactive proteins

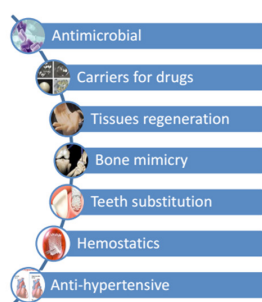
An example....



Connective tissue in animal by-product (bone)

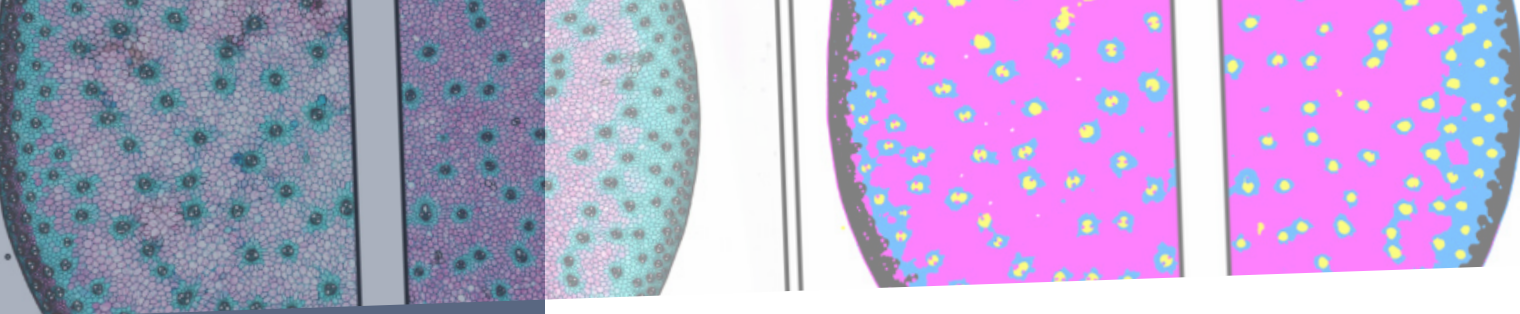


Connective tissue recovered from animal by-product



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Animal Product Quality
(QUAPA)



Participants

This research was national-fund grants coordinated by the French national research agency (ANR) under the S&T ‘Investments for the Future’ programme (ANR-11-BTBR-0006 BIOMASS FOR THE FUTURE), LabEx Saclay Plant Sciences (ANR-10-LABX-0040-SPS), and funding from the INRA’s CEPIA and BAP divisions.

The specimens were cultivated at Manguio in collaboration with the DIAPHEN platform.

Read more

Histological quantification of maize stem sections from FASGA-stained images

(2017) Plant Methods

Legland D, El Hage F, Méchin V, Reymond M

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Bourgin (IJPB)

Quantifying the histological profile of maize ear internodes to unravel plant response to hydric stress

Carefully controlled crop irrigation is crucial for sustainable agriculture. In this respect, the development of drought-adapted plant varieties is desirable, but only possible if knowledge is available regarding differential responses of plants exposed to water deficits of variable duration and intensity. Moreover, plant degradability and plant agronomic properties (such as drought-resistance) are both crucial factors that depend on plant-growth development and variations in plant cell-wall composition. Therefore, it is the diversity of these responses that can serve to identify genotypes combining stable agronomic performances with good degradability under varying environmental conditions.

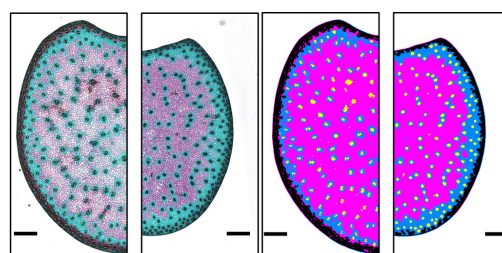
► RESULTS

We custom-developed an automated image processing and analysis procedure for quantifying the histological tissue-type pattern of maize stem sections using high-res colour-stained input images digitalized with whole-slide scanners. The method involves segmenting different tissue regions based on variations in the colour and morphology of the plant structures, which gives an anatomical ID of the section that can then be run through an automated analysis to quantify the colour and morphology of the tissue regions. The information output can be cross-correlated against information from other analytical methods (typically biochemistry and/or spectroscopy) to assess the relationships between the composition, histology and degradability of crop plants under contrasting water supply regimes. Plant response to water deficit can then be characterized and integrated at each of these levels.

► FUTURE OUTLOOK

Our custom-developed methodology has already served to study several maize genotypes that experienced variable cell-wall degradability and that were cropped under different irrigation regimes. The high throughput processability achieved makes it possible to characterize several hundred samples per week, which raises prospects for studying the basic genetics involved in biochemically and histologically variable phenotypes and their responses to hydric stress. The approaches developed here are transposable to other crop grasses, including major biomass-feedstocks plants like *Miscanthus*.

Illustrative example of sample-image acquisition of FASGA-stained sections from the same genotype cultivated under two different irrigation scenarios (with irrigation, at left, and without irrigation, at right), and output results of the image segmentation workflow on these same images segmented into distinct tissue-class regions.



Participants

Synchrotron Soleil, Gif sur Yvette

Read more

Structural proteomics: Topology and relative accessibility of plant lipid droplet associated proteins

(2017) Journal of Proteomics

Jolivet P, Aymé L, Giuliani A, Wien F, Chardot T, Gohon Y

SOLEIL shining on the solution-state structure of biomacromolecules by synchrotron X-ray footprinting at the Metrology beamline

(2017) Journal of Synchrotron Radiation

Baud A, Aymé L, Gonnet F, Salard I, Gohon Y et Al.

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Bourgin (IJPB)

Structural proteomics: topology of the proteins that stabilize lipid droplets in oilcrop seeds

The lipids in oilcrop seeds are stored in lipid droplets. Oleosins, which are proteins anchored at the droplet surface, help stabilize them, but also limit the extractability of the plant oil. Identifying how these proteins associate is a key challenge that needs to be resolved to understand the structure and dynamics of these bodies. Working in partnership with the SOLEIL facility, we developed Europe's first and only synchrotron X-ray footprinting technique. The technique employs X-ray-driven radiolysis of water to generate radicals capable of oxidizing near-vicinity molecules on millisecond timescales. This unique approach was able to confirm the model of S3 oleosin insertion.

► RESULTS

First, the solvent accessibility of the S3 oleosin was determined using a system that addresses the protein to the lipid droplets in yeasts. Second, a study of the oleosin found in lipid droplets of plant (*Arabidopsis thaliana*) seeds allowed us to observe changes in oxidation of amino acid residues according to the organism studied. Whether in the yeast or in the plant, oxidation is found on the same peptides, but it is not always the same amino acids that are oxidized. Likewise, solvent accessibility is more limited in the plant. In contrast to the yeast environment where S3 oleosin is the only protein found anchored on the droplet surface, in the plant environment there are four other oleosin isoforms present. The differences observed here almost certainly reflect interactions between S3 oleosin and its protein partners.

► FUTURE OUTLOOK

This research architects a whole new approach for the acquisition of structural data based on controlled protein oxidation using synchrotron X-ray-driven radiolysis of water coupled with proteomics analysis. Moving forward, these first structural proteomics studies raise prospects for soon identifying the protein-protein interactions at play at the surface of other intracellular organelles, and possibly even at whole-cell scale.

Participants

CNRS (GEPEA UMR-CNRS-6144) (E. Leroy), dans le cadre de la Fédération IBSM (Ingénierie des Biopolymères pour la Structuration des Matrices et des Matériaux; SFR-4202)

Read more

Rheology and structural changes of plasticized zeins in the molten state
(2017) Rheologica Acta

Chaunier L, Della Valle G, Dalgalarondo M, Marion D, Lourdin D, Leroy E

Fused deposition modeling of plant biopolymers: opportunities and challenges

(2018) Additive Manufacturing

Chaunier L, Guessasma S, Belhabib S, Della Valle G, Lourdin D, Leroy E

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Biopolymers, Interactions,
Assemblies (BIA)

Zein: a model material for 3D-printing biopolymer melts

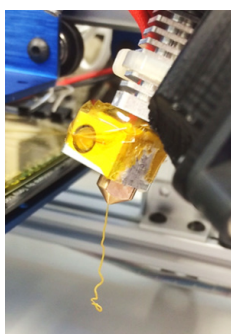
3D printing is an additive manufacturing technique that builds 3-dimensional objects based on a computer-file template. One of the most mainstream 3D printing processes is fused deposition modelling (FDM), which works by depositing a thermoplastic polymer filament layer-by-layer. To extend the range of FDM-process applications out to biomedical and pharmaceutical manufacturing, new thermoplastic materials need to be purpose-developed. All-natural biodegradable biopolymers, some biocompatible and potentially even edible, could make good candidate materials, but only with a purpose-tailored formulation.

► RESULTS

Our research has shown that zein, the seed storage protein found in maize, is suitable for 3D printing by deposition in the molten state. After hot melt extrusion with 20% added glycerol, zein protein offers thermomechanical properties that fit with FDM-process requirements, i.e. (i) high elastic modulus at room temperature ($E' > 1 \text{ GPa}$), and (ii) hot flowability once past its glass transition temperature ($T_g \approx 42^\circ\text{C}$). However, the hot reactivity of the plasticized zein due to the interplay of noncovalent interactions with the formation of disulphide bridges causes the melts to stiffen, which could bottleneck its workability in FDM processes. We found that long thermomechanical treatments (extrusion for 10 minutes at 130°C) lead to gelling. This reactivity stems from in-melt protein unfolding that further exposes protein sites involved in aggregation and crosslinking reactions. However, we also showed that filaments obtained via a shorter thermomechanical treatment (extrusion for around just one minute at 130°C) or by adding reducing agents that minimize the heat-driven aggregation of zein protein facilitates its melt-phase workability.

► FUTURE OUTLOOK

We intend to consolidate these findings by studying the addition of ionic liquids to zein-based materials, as they can act as both plasticizers and as active pharmaceutical ingredients (e.g. [lidocaine][ibuprofenate]) to target the pharmaceutical applications market.



Extrusion of a plasticized zein filament at the 3D-printer (FDM-process) extrusion-head nozzle outflow



Participants

- ♦ INRA URH,
- ♦ Herbipôle,
- ♦ INRA BIA

Funding from:

- ♦ ANR projects Agilait (ANR-06-PNRA-012),
- ♦ Valobab (ANR-11-ALID-007),
- ♦ CNIEL

Read more

Butter serums and buttermilks as sources of bioactive lipids from the milk fat globule membrane: Differences in their lipid composition and potentialities of cow diet to increase n-3 PUFA

(2017) Food Research International
Lopez C. *et al.*

The miscibility of milk sphingomyelin and cholesterol is affected by temperature and surface pressure in mixed Langmuir monolayers

(2017) Food Chemistry
Cheng K. *et al.*

Lipid droplets coated with milk fat globule membrane fragments: Microstructure and functional properties as a function of pH

(2017) Food Research International
Lopez C. *et al.*

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Milk & Egg (STLO)

High milk polar lipid content = the treasure of gold!

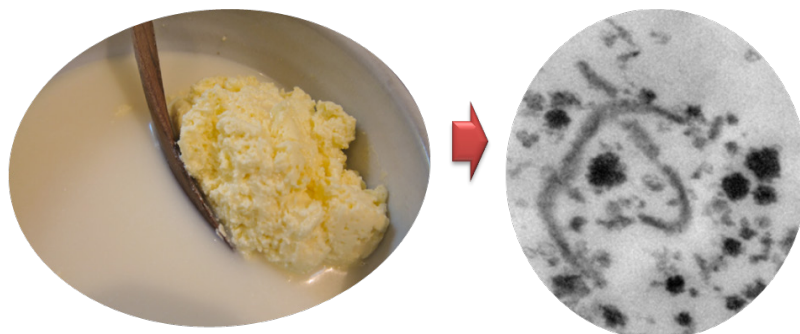
Polar lipids from various sources, such as soy lecithin, are widely used as food emulsifiers. However, polar lipids derived from the biological membrane that surrounds milk fat globules are still underexploited, despite their potentially huge nutritional and function value. Butter-industry by-products (buttermilk and butter serum) are good sources of these potentially valuable polar lipids and fragments of milk fat globule membrane.

► RESULTS

In-depth polar lipid composition analysis on a number of different buttermilks and butter serum revealed quantitative and qualitative variations that can be correlated to processing technology and dairy cattle diet. Milk polar lipids are a good source of important health-promoting bioactive lipid components (e.g. omega-3 polyunsaturated fatty acids, and sphingomyelin). The attractational interactions between milk-derived sphingomyelin and cholesterol were quantified, and the findings provided key insight into how lipid domains form in the milk fat globule membrane. Based on these results, the Dairy Science Platform in Rennes has developed a process technology that is able to prepare an ingredient with a 32% milk polar lipids content, which is much higher than that found in other commercially-available ingredients. This new ingredient also demonstrates outstanding emulsification properties.

► FUTURE OUTLOOK

This new milk polar lipid-rich ingredient could be used to produce emulsion droplets biomimetic of milk fat globules, typically in infant formula. Further research is needed to better understand how the functional and nutritional properties of milk polar lipids and milk fat globule membrane fragments are related to their high sphingomyelin content.



Buttermilk: a good source of potentially valuable milk polar lipids and fat globule membrane fragments

Ecodesign and process sustainability

Pressing environmental issues argue for a profound rethink of food value chains. Material and energy efficiency must be improved and waste and the generation of pollutants along the value chains must be reduced. Ecodesign approaches constitute a way to move forward, redesigning the way biobased raw materials are farmed, processed, and ultimately disposed of. The ecodesign philosophy for designing and redesigning products and processes involves internalizing the product's whole environmental footprint, and thus developing a multicriteria approach spanning the whole end-to-end product lifecycle.

This section illustrates how CEPIA researchers are embracing ecodesign principles to develop new methods, design new processes and develop new multi-criteria decision support systems to underpin innovative design strategies.

Participants

The research on the production of higher alcohols (isopropanol, isobutanol) was carried out under collaborative projects with the MIT (MIT-France Seed Fund and the CNRS-led France-MIT Bioenergy programme).

The research on the production of hydrocarbons was carried out as part of the S&T 'Investments for the Future' ProBio3 project.

The bioreactor engineering research was carried out with international industry partners engaged in building bioreactor systems (Pharyx, USA; HEL, UK).

The microbial electrosynthesis process is explored in partnership with the DECHEMA-Forschungsinstitut research team (Germany).

Read more

Over expression of GroESL in Cupriavidus necator for heterotrophic and autotrophic isopropanol production

(2017) Metabolic Engineering

Marc J, Grousseau E, Lombard E, Sinskey AJ, Gorret N, Guillouet SE

Metabolic engineering of Cupriavidus necator for heterotrophic and autotrophic alka(e)ne production

(2016) Metabolic Engineering

Crepin L, Lombard E, Guillouet SE

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Microbial conversion of CO₂ into commodity compounds of interest: the dream becomes reality

The development of biosynthesis processes to turn CO₂ into commodity compounds is gaining increasing attraction as a way to cut greenhouse gas emissions and establish a low-carbon bioeconomy. We implemented an integrated iterative approach coupling metabolic engineering on a chemolithotrophic bacterium—i.e. a bacterium able to grow using CO₂ and H₂ gas substrates—with fermentation engineering. We managed to produce commodity compounds, like higher alcohols (biobased solvents) and hydrocarbons, not naturally synthesized by this bacterium, at a yield scale of a few grams per litre from CO₂ as sole carbon source.

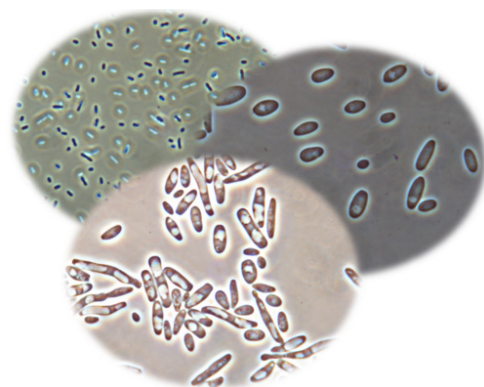
► RESULTS

The metabolic engineering on this bacterium reprogrammed its key carbon pathway away from the natural product polyhydroxybutyrate (PHB) towards the synthesis of compounds of interest. Tools and toolkits borrowed from synthetic biology enabled us to express a synthetic pathway of isopropanol production yielding 20 g/L of isopropanol from sugar. Then, coupled with bioreactor engineering, this strain was able to produce isopropanol at titres of up to 3 g/L solely from CO₂, H₂ and air gas mixtures.

This same strategy has also been leveraged to successfully obtain strains producing hydrocarbons (alkanes/alkenes, fatty aldehydes, and others) from CO₂.

► FUTURE OUTLOOK

These results provide the proof-of-concept demonstrating that it is effectively possible to produce commodity compounds of interest from CO₂ using chemolithoautotrophic bacteria. The ability to successfully produce compounds at g/L-scale raises prospects for the near-future development of CO₂-based bioprocesses.



Ralstonia Bacteria



Participants

- ♦ ONIRIS,
- ♦ Science without Borders (Brazil)

Read more

A novel method of oil encapsulation in core-shell alginate microcapsules by inverse gelation technique

(2017) Reactive and Functional Polymers

Martins E, Poncelet S, Renard D

Monodisperse core-shell alginate (micro-)capsules with oil core generated from droplets millifluidic

(2017) Food Hydrocolloids

Martins E, Poncelet S, Marquis M, Davy J, Renard D

Use of micro/millicapsules in several industrial applications

©Thesis Evandro Martins (2015)
Université de Nantes

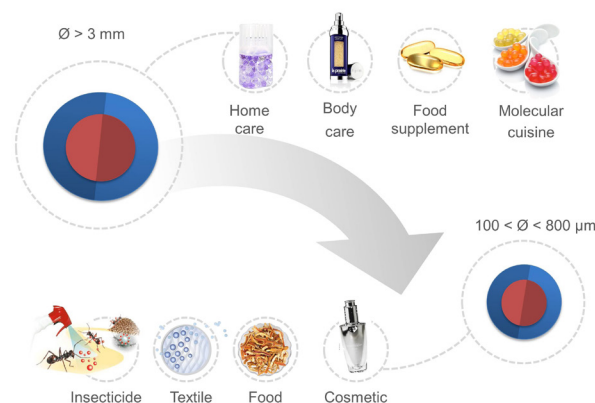
Innovative processes for oil encapsulation

Encapsulation of active compounds is already a mainstream process in all kinds of industries, where it is used to shield often-fragile compounds like oils from manufacturing/storage process-induced degradation and decay. We have produced microcapsules whose size is such that they minimize these process interferences with the texture or appearance of the final product.

► RESULTS

Oil encapsulation by inverse gelation works by dropwise addition of a calcium chloride/oil emulsion into an alginate bath. The calcium ions inside the emulsion then migrate out towards the bath and cross-link the alginate chains (inverse gelation). This technique can thus form millimetre-scale capsules (3–7 mm) with core-shell morphology. However, sub-millimetre-size capsules had never before been produced by inverse gelation. In an effort to get smaller-sized capsules, we developed a process that consists of forming droplets by dispersing

the emulsion in a stirred alginate bath, and that works with either oil-in-water emulsion or water-in-oil emulsion. Alginate crosslinking to form the membrane then produced microcapsules at sizes in the range of 370 to 500 μm . The dispersion protocol



was then re-engineered for a millifluidics process in order to control microcapsule size. We thus managed to produce monodisperse capsules size-bracketed to within diameters of 140 μm up to 1.4 mm.

► FUTURE OUTLOOK

Moving forward from this successfully engineered process, we still have to optimize the release of calcium ions from the capsule core so as to increase membrane thickness —because a thicker membrane will offer greater mechanical strength after drying while at the same time delivering near-90% encapsulation rates for the dry capsules. The capsule sizes generated also pave the way to applications in non-food sectors (textiles, cosmetics).

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Assemblies (BIA)

Participants

This study was led in partnership with Lactalis Research and Development Group, at Retiers, France.

Funding source: ANRT CIFRE 548/2009

Read more

Investigating semi-hard cheese aroma: relationship between sensory profiles and gas-chromatography-olfactometry data

(2012) International Dairy Journal
Thomsen M, Martin C, Mercier E, Tournayre P, Berdagué J-L, Thomas-Danguin T, Guichard E

Combination of odour-stimulation tools and surface response methodology for odour recombination studies

(2017) Flavour Fragrance Journal
Thomsen M, Dosne T, Beno N, Chabanet C, Guichard E, Thomas-Danguin T

Experimental apparatus coupling a multichannel dynamic dilution olfactometer delivering aroma compounds, alone or in mixture, to the sniffing port of a gas-chromatography system delivering odour-active components at given times

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Behaviour (CSGA)

Olfactometer–Olfactoscan coupling to dissect cheese aroma

The aroma of a foodstuff results from the perception of a complex mixture of different odour-active compounds, each of which having its own specific odour-active traits and intensities. We used the Olfactoscan device to test the effect of adding odour-active compounds specific to the smell of certain semi-hard cheeses, in mixtures with a basic aroma formulation, on the smell specificity of cheeses. The Olfactoscan technique consists in to a multichannel dynamic dilution olfactometer delivering aroma compounds, alone or in mixture, coupled to the sniffing port of a gas-chromatography system delivering odour-active components at given times.

► RESULTS

A study on three unprocessed semi-hard cheeses retained 8 odour-active compounds that gave the cheeses their basic typical aroma (V1: butane-2,3-dione, acetic acid, V2: butanoic acid, 3-methylbutanoic acid, 3-methylbutan-1-ol, dimethyl trisulfide, V3: methional, V4: 1-octen-3-one) and 4 compounds contributing to the specificity of each cheese (2,3-dimethylpyrazine, benzaldehyde, methyl 2-methyl-3-furyl disulfide, and gamma-heptalactone). A first experiment using a 4-channel dynamic dilution olfactometer determined the optimal concentrations of the 8 compounds that, when mixed, most closely reproduced the aroma of each cheese. A second experiment then tested the added effect of the 4 specific compounds and the individual effect of the 4 compounds initially delivered via the olfactometer

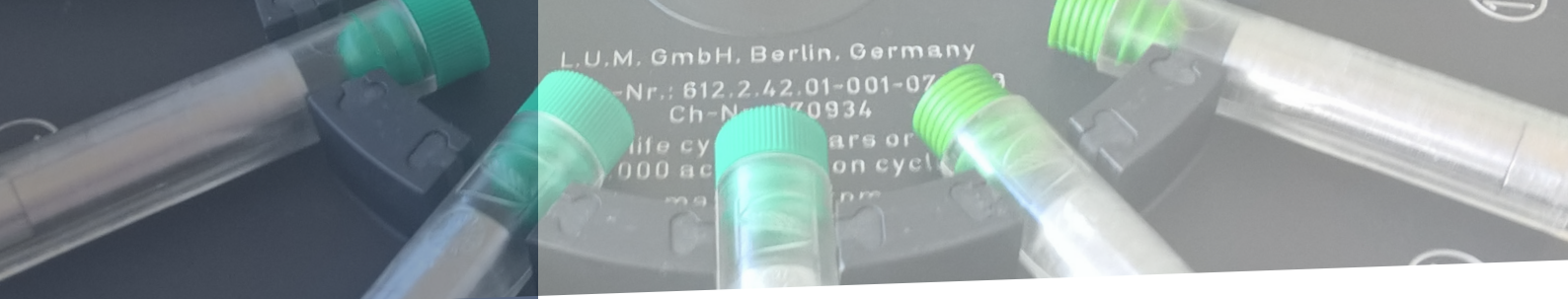


on the perception of the cheese odour. Each of these recombined mixtures was directly compared by a naive sensory panel to the real cheese odour delivered by the experimental set up.

This study ultimately unravelled the positive impact on the smell specificity of the cheeses tested of cabbage aroma (dimethyl trisulfide), foot aroma (butanoic acid) and coconut aroma (gamma-heptalactone), respectively, for each of the three cheeses, and the negative impact of bitter almond (benzaldehyde) and mushroom smells (1-octen-3-one), at certain concentrations, for one cheese.

► FUTURE OUTLOOK

This same approach can also be used to select odour-active compounds congruent to certain tastes, and it has recently been applied to test the potential of certain odour-active compounds in fruit juice to enhance perception of sweetness.



Ready-loaded analytical centrifuge

Participants

Project implemented in collaboration with:

- the ‘Technologies for Agro-Industry’ team from the University of Technology of Compiègne (France)
- analytical instrumentation developer for industry LUM GmbH (Germany)

Read more

Multistep centrifugal consolidation method for characterization of filterability of aggregated concentrated suspensions

(2017) Separation and Purification Technology

Loginov M, Zierau A, Kavianpour D, Lerche D, Vorobiev E, Gésan-Guiziou G, Mahnic-Kalamiza S, Sobisch T

Centrifugal ultrafiltration for determination of filter cake properties of colloids

(2017) Journal of Membrane Science

Loginov M, Samper F, Gésan-Guiziou G, Sobisch T, Lerche D, Vorobiev E

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Science & Technology of
Milk & Egg (STLO)

A new tool for studying the filterability of solutions and suspensions

Knowing the filterability of solutions and suspensions is critical to optimize filtration processes. A number of “high-throughput filtration” systems has been developed to speed up filterability analysis, but most of them have been custom-developed and only exist as single copies.

► RESULTS

Working in collaboration with the ‘Technologies for Agro-Industry’ team from the University of Technology of Compiègne (France) and analytical instrumentation developer for industry LUM GmbH (Germany), we developed a novel photocentrifuge–filtration system enabling rapid simultaneous multi-sample characterization based on commercially-available hardware, i.e. a LUMiSizer® analytical photocentrifuge.

Filterability is characterized based on analysis of the centrifuge filtration/consolidation curves obtained in continuous mode by the analytical photocentrifuge. This curve analysis allows:

- for centrifuge-based consolidation of suspensions: to obtain the concentration and pressure dependencies of specific cake resistance;
- for centrifuge-based ultrafiltration of solutions: to measure the resistance of the fouled membrane, to determine the compressibility of the cake formed at the membrane surface, and to evaluate the mechanism of filtration.

Measurements can be carried out in a wide range of solid pressure from 5 up to 500 kPa.

► FUTURE OUTLOOK

The tool as developed can be applied to quantitatively characterize filtration controlled by cake formation (filtration of concentrated suspensions or ultrafiltration of solutions). In addition, it could be used for characterization of membrane fouling (microfiltration of dilute suspensions, typically juices, wine, and other organic suspensions), although we still need to model and study centrifugal microfiltration, i.e. variable-pressure microfiltration assisted by sedimentation, and to identify the microfiltration membranes geared to analysis of small-volume samples.



Filtration cell with its reservoir

Participants

UMR Institut Pascal, Université
Clermont-Auvergne ; ADIV, IFIP
Institut du Porc

ANR Na- ANR-09-ALIA-013-01,
2010-2013

Read more

*Developing a multi-physical finite
element-based model that predicts
water and salt transfers, proteolysis
and water activity during the
salting and post-salting stages of
dry-cured ham process*

(2018) Journal of Food
Engineering

Harkouss R, Chevarin C, Daudin
JD, Sicard J, Mirade PS

Dry-cured ham: a process simulator can now define routes of manufacture that yield lower-salt products

Nutritional imperatives mean that makers of dry-cured ham need to reduce the amount of salt in their products. However, any over-strong drop in salt content will lead to excessive proteolysis, causing texture problems that can undermine slicing yields and create microbiological stability issues.

► RESULTS

By coupling physical heat and mass transfer models with statistical proteolysis rate quantification models, the 3D version of the ‘numerical ham’ model developed here can quantitatively predict the space-and-time course of proteolysis index, water content, salt content and water activity (a_w) and the kinetics of weight loss inside a real ham geometry. This simulator was built from a series of 181 X-ray computed tomography images, and the set of variables can be visualized as 3D maps, profiles, and even mean values calculated in each main group of muscles making up the ham.

The model has been tested at a big industry partner, where it served to estimate—after a 10h computation—the extra time needed at the low-temperature rest phase for two batches of hams produced at 25%-shorter and 33%-shorter salting-phase times to reach the same a_w values at the end of the rest phase as normally-salted hams. The model estimated this time as 3 weeks.

► FUTURE OUTLOOK

Work is currently underway to bring a number of improvements, chiefly integration of the strong drying-driven variation in ham volume (that can shrink 30%–40% of initial volume), in order to more accurately predict in-ham salt diffusion patterns.

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(QUAPA)

Participants

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- ♦ Chemical Engineering laboratory (LGC, Toulouse), Jean-Pierre Belaud

Read more

A decision support system for eco-efficient biorefinery process comparison using a semantic approach

(2016) Computers and Electronics in Agriculture
Lousteau-Cazalet C *et al.*

Sustainable design of biorefinery processes: existing practices and new methodology

(2017) Biofuels Bioprod Biorefining

Julio R, Albet J, Vialle C, Vaca-Garcia C, Sablayrolles C

Knowledge engineering and modelling/simulation to empower life cycle assessment: sustainable ecodesign of biorefinery processes

The depletion of fossil-fuel resources has fostered the creation of a whole new type of industry based on renewable biomass resources: the biorefinery. Nevertheless, the ecodesign of innovative processes remains a complex task due to the myriad possible configurations and the serious shortage of reliable and accurate data on these technologies.

► RESULTS

To break this bottleneck and populate the life cycle inventories, our research team has proposed two approaches:

- ♦ an approach coupling knowledge engineering (KE) methodology with life cycle assessment (LCA) methodology;
- ♦ an approach coupling process modelling/simulation with LCA;

The first approach is applied to the pretreatment of lignocellulosic biomass, and it proceeds in three steps: 1) definition of the purpose and limits of the study, 2) big data extraction from several heterogeneous data sources, and 3) environmental impact assessment. The goal here is to pool and consolidate the literature data to complete the foreground data, and then run the LCA to compare different pretreatment methods. This approach, and the allied toolkit, can help choose the right processes.

The second approach is applied to different biorefinery processes engaging different biomass feedstocks (by-products from wheat, wood, microalgae), and also proceeds in three steps: 1) model how the processes operate using mathematical or semi-empirical models, 2) use these functional models to simulate the processes and get in-detail mass and energy balances, 3) then use these mass and energy balances to carry out an environmental impact assessment on the cradle to grave process lifecycle. By iteration of the approach, it becomes possible to optimize the environmental performance of a process by determining the operating conditions and optimal sequence of unit operations.

► FUTURE OUTLOOK

On top of environmental performance, it is equally possible to assess social performance and economic performance, and to work on improving production yields, which will give a more all-encompassing triple-bottom-line vision of the entire end-to-end process lifecycle, and thus serve to develop biorefineries built on all three pillars of sustainability.

These two approaches can prove precious support to lifecycle thinking-based decisionmaking.

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- ♦ LITIS University of Havre,
- ♦ CSTB Grenoble,
- ♦ LIST Luxembourg

Read more

Framework and computational tool for the consideration of time dependency in Life Cycle Inventory: proof of concept

(2016) Journal of Cleaner Production

Tiruta-Barna L, Pigné Y, Navarrete Gutiérrez T, Benetto E

Operational integration of time dependent toxicity impact category in dynamic LCA

(2017) Science of the Total Environment

Shimako AH, Tiruta-Barna L, Ahmadi A

DyPLCA : development of a novel method of Dynamic Life Cycle Assessment— Integrating the time dimension in the environmental impact assessment

Life cycle assessment (LCA) is the most widely used method today for assessing the environmental impact of human activities, and its assets and advantages need no introduction. However, the method as it stands, grounded in randomly-assigned time horizons or steady-state conditions, fails to consider dynamic processes. This prompted us to develop an operational method for integrating the time factor into LCA, which integrates models for the two big steps: the life cycle inventory phase and the life cycle impact assessment phase.

► RESULTS

Our approach is based on dynamic modelling of the process networks that intermesh into the life cycle of a product, coupled with dynamic representation of major environmental impacts, i.e. climate change, human toxicity and ecotoxicity. These models are implemented in software that was designed under the ANR-sponsored DyPLCA project.

The currently accessible DyPLCA software solution free-to-use online at <http://dyplca.pigne.org/> is capable of computing the dynamic life cycle inventory. A database containing the time-scaled parameters for ten-thousand processes has been developed by building on the traditional leading LCA database Ecoinvent. The models for the climate change, human toxicity and ecotoxicity indicators have been adapted to dynamic conditions by building on existing models and coupled with the DyPLCA tool for the dynamic inventory.

A few case studies have been carried out in chemical or biochemical process engineering where they helped identify limitations and improve the approach through systematic comparison of results against the now-classical regular

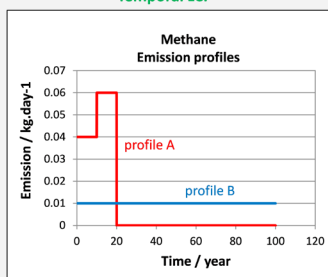
Conventional LCA

Climate change impact result = 25 kg CO₂-eq

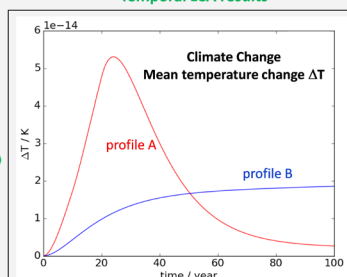
Example : Emission of 1kg CH₄ by a system

Dynamic LCA

Temporal LCI



Temporal LCA results



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Biosystems and Process Engineering (LISBP)

LCA method.

► FUTURE OUTLOOK

We are pressing ahead with further development of the methodology to capture its potential as a policy and decision support tool for organization-scale LCA researchers and regulators like the European Commission and its science and knowledge service the Joint Research Centre (JRC).



Research in data and knowledge engineering

Food and biobased product quality is a multifaceted concept, which allies intrinsic quality criteria with external quality traits such as sustainability.

Therefore, designing quality is a multicriteria problem that requires the development and use of decision support systems that need to be able to integrate and articulate a range of quite different knowledge-sets.

In the CEPIA division, we focus research on areas such as automated data mining, heterogeneous data integration and knowledge representation with the aim of generating high-value-added information, chiefly—as illustrated in this section—in the form of software, handbooks, and massive open online courses.

SpecOMS: software for exploring the protein universe

Participants

Laboratory of Digital Sciences—Nantes (LS2N) under the Griote programme, financed by the Pays de la Loire region.

Thesis by Matthieu David, under thesis supervisor Guillaume Fertin

Read more

SpecOMS: A Full Open Modification Search Method Performing All-to-All Spectra Comparisons within Minutes

(2017) Journal of Proteome Research

David M, Fertin G, Rogniaux H, Tessier D

SpecTrees: an efficient without a priori data structure for MS/MS spectra identification

(2016) 16th Workshop on Algorithms in Bioinformatics

David M, Fertin G, Tessier D

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Biopolymers, Interactions,
Assemblies (BIA)

The protein universe is still largely uncharted territory: 99.8% of the proteins referenced in the Uniprot database have been predicted *in silico*—and so not strictly identified—based on the genomic sequence data available. Tandem MS/MS-mode spectrometry is the go-to technique most widely used for protein characterization, but the quality of the results depends on the sample preparation protocol, on the analytical mass spectrometry expertise mobilized, and on the ability of the software to handle and interpret the tens of thousands of spectra generated. This interpretation of the spectra is a time-consuming step, and only around 25% of tandem spectra get successfully interpreted. This low rate is widely explained by the presence of modifications carried by proteins and that are *a priori* unknown. These modifications can reflect post-translational modifications that are essential for protein activity, or they can result from variants that could explain certain phenotypes or certain disease processes. Deciphering and unravelling these modifications, which are thought to be displayed on practically all proteins, is a major scientific challenge for progress in healthcare and biology.

► RESULTS

The SpecOMS software demonstrates that the fragment ion accuracy reachable with the latest generations of mass spectrometers paves the way to a new generation of spectra interpretation algorithms. By exploiting a reorganization of the spectral data at all-sample level in an appropriately-gearred data structure and with efficient data access modes, SpecOMS can handle pairwise comparisons of the tens of thousands of experimental spectra from tandem mass analysis against hundreds of thousands of spectra, such as the spectra corresponding to the human proteome. SpecOMS is currently the world's fastest mass spectral analysis software (taking just minutes on a standard desktop PC) and the least-memory intensive, which makes it easy to use on any and all mass spectrometry platforms. The method provides a profile of the modifications brought by the proteins in a sample, and can even reveal modifications that are simply impossible to get with the other approaches available.

► FUTURE OUTLOOK

SpecOMS meets a whole set of the science community's needs, as its rapid adoption in an array of laboratories goes to show. A handful of issues still need to be resolved to ensure the software can exhaustively meet increasingly complex proteomics needs (metaproteomics, foodomics).



Participants

- ♦ INRA UR BIA Nantes (Kamal Kansou et Estelle Bonnin)
- ♦ INRA UMR FARE Reims (Caroline Rémond, Gabriel Paës, Jean Tayeb)
- ♦ Informatics Institute of University of Amsterdam (Bert Bredeweg)

Read more

Testing scientific models using Qualitative Reasoning: Application to cellulose hydrolysis

(2017) Scientific reports

Kansou K, Rémond C, Paës G, Bonnin A, Tayeb J, Bredeweg B

Testing scientific theories using conceptual models: enzymatic degradation as a case-in-point example

The literature review, which fundamental to natural science, is getting more and more difficult for domain specialists due to the inexorable inflation of scientific papers. To facilitate the capture and computation of scientific domain knowledge, we proposed a method for assessing and integrating published scientific models. The method has been applied to understanding the mechanism limiting the enzymatic hydrolysis of cellulose, which is pivotal to research in a number of domains, including the production of biofuels.

► RESULTS

Our method consists of taking several concurrent scientific models and comparing them against a set of observations from other scientific publications. These observations describe target behaviours of the system that we want to first reproduce via simulation and then, ultimately, explain. These behaviours could for instance be the cellulose hydrolysis rate which first increases but then slows over the course of the reaction, or adding an enzyme mid-reaction to trigger an acceleration in the hydrolysis process. To reduce the context-dependent effect that reflects mainly into the quantitative measurements of experiments our method assumes the qualitative abstraction of the numerical values into a qualitative space and the mapping of scientific models into qualitative models. Simulations generated with the qualitative models are then compared against the target behaviours—if they prove coherent, then the model will convey a plausible scientific theory of that behaviour.

We applied our model to test assess two published kinetic models which are thought to explain the rate-limited hydrolysis of cellulose—one involving the processive saturation of enzyme-hydrolyzable cellulose surface, and one involving the presence of obstacles at the cellulose substrate surface disrupting and ‘stalling’ the enzymes. We showed that neither of the two models can fully explain all the target behaviours, whereas a third model integrating the first two is able to explain all the target behaviours.

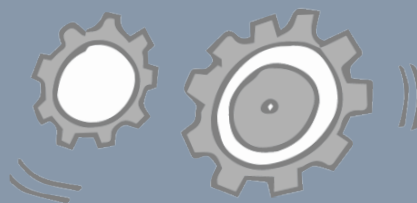
► FUTURE OUTLOOK

This research borrows techniques and tools from Artificial Intelligence to mine and model knowledge and aspects of scientific reasoning. Work on the method is moving forward into an effort to automate the procedure—and thus apply it, for instance, to screen qualitative models for target behaviours or to facilitate the extraction of salient data from a complex set of publications.

CONTACT

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Biopolymers, Interactions,
Assemblies (BIA)



Participants

Project funding in 2015–2016 came from Agropolis Foundation: ID-1401-005, via the Labex Agro S&T ‘Investments for the Future’ programme: ANR-10-LABX-0001-01, and in 2017 from INRA-FP.

The project has been coordinated by JC. Boulet (INRA-UMR SPO, PFP), initiated with JM. Roger (IRSTEA-UMR ITAP), supported by INRA and Agreenium, and engaged by the Universities of Montpellier, Lille, Bretagne Occidentale, Aix-Marseille, and Genève, along with big engineering schools (AgroParisTech, Oniris, SupAgro Montpellier), the association HelioSPIR near-infrared spectroscopy society, and industry partners Ondalys and Data_Frame. The ChemFlow software is hosted at the GENOTOUL platform.

Mobilization and impact

In 2016, CheMoocs and ChemFlow counted over 1500 and 750 students enrolled, respectively. At the end of 2017, the two MOOCs counted over 1800 and 1200 students enrolled, and ChemFlow counted over 1100 registered users.

CONTACTS

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Sciences for Enology (SPO)

ChemProject, democratizing chemometrics - principles and tools

The chemometrics training offer in France pales in comparison with the Scandinavian countries, prompting users to turn to self-education, and at the same time, the tools for processing near-infrared spectra are practically all commercial software.

► RESULTS

To address this issue, two massive open online courses on chemometrics (CheMoocs) have been put together. French language-sphere chemometrics experts produced the videos, written course material, exercises and quizzes, and a software (ChemFlow) was also developed to facilitate applied-side chemometrics data processing.

ChemProject (<http://chemproject.org>) plugs CheMoocs and ChemFlow together. It is a complete and comprehensive 100%-open 100%-free package that empowers users to independently lead applied multivariate (chemometrics) analysis, with an focus on near-infrared spectrometry.

– Both the French-language MOOCs are hosted by FUN – France Université Numérique (<https://www.fun-mooc.fr/>). In 22 lessons, they deliver core foundational knowledge on chemometrics tools, where and when to use them, and what their algorithms mean.

– The ChemFlow software is a 100%-open 100%-free web application (<https://vm-chemflow.toulouse.inra.fr/>). It gives users a platform to get hands-on data-analytic practice, to complete exercises set by the MOOCs, and to work on their own data.

► FUTURE OUTLOOK

In 2017, the two MOOCs counted 1800 and 1200 students enrolled, respectively. A good MOOC completion rate (10%–20%) and a surge in enrolments in 2017 show that ChemProject caters to both academic and industry demand. New method developments are in the pipeline, including an English-language version.

► READ MORE

Go to: <https://vm-chemflow.toulouse.inra.fr/>

Go to the following links to access CheMooc-Basic and CheMooc-Advanced:

<https://www.fun-mooc.fr/courses/course-v1:Agreenium+66002+session02B/about>

<https://www.fun-mooc.fr/courses/course-v1:Agreenium+66002+session02A/about>

<http://chemproject.org>



For safe and healthy food

Ensuring consumers get a safe supply of healthy food provisioned by a sustainable farm-to-fork supply chain requires an array of cross-science studies connected to the upstream production systems. Rationalized food quality engineering is a pivotal focus of research led by CEPIA—Science for Food and Bioproduct Engineering.

CEPIA researchers, as architects of food quality, lead research aimed at controlling the interplays between storage of produce, the processing of products, and the physical–chemical–organoleptic characteristics of foods. We also investigate the physiological effects of food structure by studying the mechanisms at work during food ingestion and digestion phases. CEPIA also addresses ultimate food safety, through an approach called ‘exposomics’ that aims to identify any and every kind of newly-formed compounds and markers of food contaminants.

Participants

Research work conducted as part of a thesis by Christelle Planche co-supervised by L. Debrauwer (UMR TOXALIM) and E. Engel (UR QuaPA) under the ANR-sponsored SOMEAT project (2013–2017, <http://www.so-meat.fr/>).

This work mobilized 5 INRA research units (UR 370 QuaPA, UMR1331 TOXALIM, UMR 518 MIA, UR1303 ALISS, UR0083A), two ANSES research units (Veterinary Drugs—Fougères, Food Safety—Maisons-Alfort) and one ONIRIS lab (LABERCA).

Read more

Effects of pan cooking on micropollutants in meat

(2017) Food Chemistry

Planche C *et al.*

In Exposure assessment for dioxin-like PCBs intake from organic and conventional meat integrating cooking and digestion effects

(2017) Chemical Toxicology

Tressou J, Ben Abdallah N, Planche C, Dervilly-Pinel G, Sans P, Engel E, Albert I

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Animal Product Quality
(QUAPA)

Effect of cooking on chemical contaminants in food. Meat as example

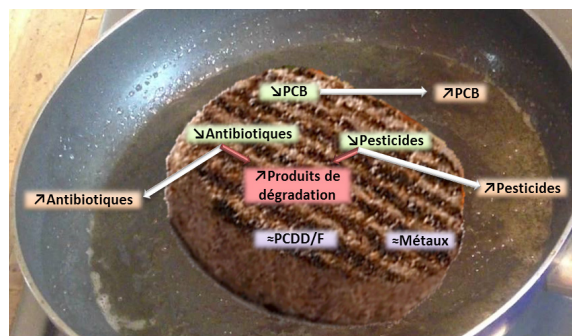
Recent chemistry and food toxicology research suggests that just knowing the level of contamination of a farmed product is not sufficient to assess its impact, as there are certain food processing operations, like cooking, that may significantly modulate the chemical risk to the end-consumer. We studied the effect of pan-frying on the main chemical contaminants potentially found in meat. Working on meat samples purposely infected with diverse contaminants, we profiled pre-cooking and post-cooking mass balances using multi-residue analytical methods run by the National Reference Laboratories for antibiotics and heavy metals and by the INRA Livestock Products Quality research unit for pesticides and organic environmental micropollutants. The phenomena observed were then validated on meat samples naturally contaminated during the animal's on-farm life.

► RESULTS

The study revealed distinct effects of cooking according to the types of chemical contaminants analyzed. Whereas pan-frying had no significant effect on in-meat dioxin and heavy metal contents, higher-intensity pan-frying led to higher losses of polychlorinated biphenyls (PCBs), antibiotics and pesticides, which could be attributed to the mechanical expelling of cooking juices and/or to thermal degradation processes.

To further investigate where these losses actually came from, heat-sensitive target contaminants were radiolabelled and spiked into the meat before cooking. We then tracked these compounds, along with any degradation products, which we also labelled, during the cooking process. A study led in collaboration with the TOXALIM unit and the ANSES [French food safety and food hygiene agency] at Fougères on two radiolabelled antibiotics showed that sulfamethoxazole thermally degraded into five degradation products, which were then structurally identified using nuclear magnetic resonance and mass spectrometry.

We also managed to model the modulatory effects of pan-frying on PCBs in order to assess and compare the risks tied to eating conventionally-farmed versus organically-farmed meat.



Modulatory effect of pan-frying on contaminants in meat



Participants

Conducted as part of the SOMEAT project (2013–2017, <http://www.so-meat.fr/>) funded by the ANR under the 2012 call for ALID ('sustainable food systems') projects.

Coordinated by the QuaPA research unit (E Engel), the SOMEAT project federated six other INRA units (UMR1331 TOXALIM, UMR 518 MIA, UMR210 ECOPUB, UR1303 ALISS, UMR1145 GENIAL, UR0083A), two ANSES research units (Veterinary Drugs—Fougères, Food Safety—Maisons-Alfort), one ONIRIS lab (LABERCA), and three technical institutes (IFIP, ITAVI, IDELE).

Read more

Micropollutants and chemical residues in organic and conventional meat

(2017) Food Chemistry
Dervilly-Pinel G *et al.*

In Exposure assessment for dioxin-like PCBs intake from organic and conventional meat integrating cooking and digestion effects

(2017) Chemical Toxicology
Tressou J *et al.*

Does organic meat really contain less chemical contaminants?

In a report published in 2011, the French Scientific Council for Organic Agriculture cited better healthfulness—and chiefly low content of chemical contaminants—as the major consumer rationale for buying organic food. However, no scientific study has clearly demonstrated that these presumed health benefit claims actually corroborate. The very first multidisciplinary study to get a state-of-the-science comparison between organic and conventional produce was carried out under the ANR SOMEAT project and used meat as model.

► RESULTS

Working up from a representative sample set of French-farmed poultry, beef and pork products, 256 key contaminants—including environmental micropollutants, mycotoxins, and veterinary drug and pest control product residues—were quantified using highly-sensitive highly-selective methods. Research led in parallel borrowed approaches from economics science to characterize national meat consumption patterns. All of this data was then pulled together to serve as the foundation for a chemical risk assessment using a Bayesian approach integrating cooking and digestion processes as factors modulating toxicological impact.

The project brought reassuring evidence that contamination levels are well below the European regulatory values for all of the targeted chemical contaminants, both in organically-farmed and conventionally-farmed meat. The absence of certain veterinary drug residues in organically-farmed meat also reassuringly confirmed that organic farms effectively observe the organic farming standards. However, while the project quantified environmental chemical contaminant levels below the regulatory thresholds in all the samples, it also showed significantly higher amounts of environmental contaminants in the organically-farmed meat. Organic farming standards stipulate that all livestock, whether poultry, pigs or cattle, is to be kept longer on-farm before slaughter and to be given continuous access to the outdoors, which may resultingly lead to a higher bioaccumulation of environmental pollutants in organic livestock systems.

► FUTURE OUTLOOK

These findings could ultimately lead to a recast of the production standards for organic food chains.

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Participants

This project was facilitated by the experimental systems mobilized under the ANR SOMEAT project (2013–2017, <http://www.so-meat.fr/>), notably experimental herds exposed or not to diets contaminated by different types of chemical contaminants.

The algorithm-enabled approach for systematic automated detection of volatile compounds in volatolomics signals was developed in partnership with CATI—DIISCO.

Read more

Solid-phase microextraction set-up for the analysis of liver volatolome to detect livestock exposure to micropollutants

(2017) Journal of Chromatography A Bouhlef J *et al.*

Marker discovery in volatolomics based on systematic alignment of GC-MS signals: Application to food authentication

(2017) Analytica Chimica Acta Abouelkaram S *et al.*

Comparison of Common Components Analysis with Principal Components Analysis and Independent Components Analysis: Application to SPME-GC-MS Volatolomic Signatures

(2018) Talanta Bouhlef J *et al.*

Tools for directly scrutinizing the animal volatolome and tracing food contaminants

The methods currently used to guarantee the chemical safety of animal-origin foods specifically target either the chemical contaminants or their residues. These methods may be powerful, but they are also terribly expensive and cannot viably serve for large-scale standard-procedure system-wide food safety tests. Volatolomics, a branch of chemistry that studies the volatile organic compounds emitted by a biological system, raises promising prospects for a much cheaper, high-throughput screening solution to detect food-chain exposures to chemical contaminants.

► RESULTS

In an effort to better exploit information captured in the volatolome, we pursued three objectives:

- 1) Minimize the distortions caused during the process of physically extracting volatiles from biological tissues of fluids by proposing a purpose-dedicated solid-phase microextraction method.
- 2) Mobilize a signal processing algorithm purpose-engineered for automatically accurately aligning volatolomics signals to systematically and automatically detect the hundreds of volatile compounds that make up the volatolome. This approach enables the most exhaustive characterization possible, without missing potential marker compounds.
- 3) Maximize a chemometrics method to extract marker proteins from this in-depth volatolome composition. The multi-matrix method selected for use minimizes the loss of information induced by more classical methods in which the 3D analytical signals have to be matricized in order to ‘average’ or flatten the volatolomics data.

► FUTURE OUTLOOK

These advances in methodology have confirmed the value of the volatolome as a tool for diagnosing livestock exposure to various chemical contaminants (environmental pollutants, pesticides, veterinary drugs). Work is underway to understand the biochemical mechanisms that generate the markers emerged and to test their robustness.

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Participants

This highlight came through CASDAR project #7106, in partnership with the URA and the ITAVI—Tours, UR AFPA/University of Lorraine—Vandoeuvre-lès-Nancy, and Oniris-LABERCA—Nantes.

Read more

Liver volatolomics to reveal poultry exposure to γ -hexabromocyclododecane (HBCD)

(2017) Chemosphere

Ratel J, Planche C, Mercier F, Blinet P, Kondjoyan N, Marchand P, Fournier A, Travel A, Jondreville C, Engel E

The hunt for hepatic volatile markers of livestock contamination by an emerging brominated flame retardant, γ -hexabromocyclododecane (HBCD)

Animal-derived products like eggs and meat may be accidentally contaminated by very high concentrations of γ -HBCD, a brominated flame retardant incorporated into certain construction-industry materials for insulating buildings—including livestock barns. As γ -HBCD is a consumer health hazard, it is currently assayed as part of food safety screening, but the analytical methods employed are difficult to generalize and wieldy to implement.

We have shown that examining the ‘volatile’ compounds of a biological system, or volatolomics, offers promising prospects for detecting exposure to certain contaminants. Coupling this approach with cheap new fast-throughput sensor technologies could enable large-scale detection of farm animal and animal-derived product contamination by critical chemical contaminants for consumer safety.

► RESULTS

Contamination settings were obtained via experimental poultry farms using laying hens contaminated or not with γ -HBCD at two different doses. Liver analysis on the slaughtered hens showed (i) that HBCD had bioaccumulated in the liver of the exposed hens, and (ii) that the liver volatolome profile was able to clearly discriminate the control-group hens from both dose-groups of HBCD-contaminated hens. The candidate volatile markers of HBCD exposure were essentially straight-chain or branched-chain alkanes, aldehydes, ketones, phenols and alkylbenzenes. Their link to exposure can be explained by oxidative stress and exposure-induced detoxification enzymes whose metabolic pathway closely modulates the concentrations of cell-system volatile compounds.

► FUTURE OUTLOOK

Coupled with appropriate purpose-adapted sensors, the livestock liver volatolome could ultimately be used in support of the existing chemical safety surveillance system on γ -HBCD risk in animal-derived foods.

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- ♦ National Institute of Health Sciences, Science food institute, Tokyo, Japan

Read more

Allergy to Deamidated Gluten in Patients Tolerant to Wheat: Specific Epitopes Linked to Deamidation

(2012) Allergy

Denery-Papini S *et al.*

A chimeric IgE that mimics IgE from patients allergic to acid-hydrolyzed wheat proteins is a novel tool for in vitro allergenicity assessment of functionalized glutens

(2017) Plos One

Tranquet O *et al.*

Mobilization and impact

The INRA-DG1 antibody and its chimeric version are currently protected under patent EP2961769 A1.

This chimeric IgE could ultimately be used as a lab-based substitute for patient sera for allergenicity assessments of functionalized glutens.

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Biopolymers, Interactions,
Assemblies (BIA)

A chimeric antibody for allergenicity assessment of functionalized glutens

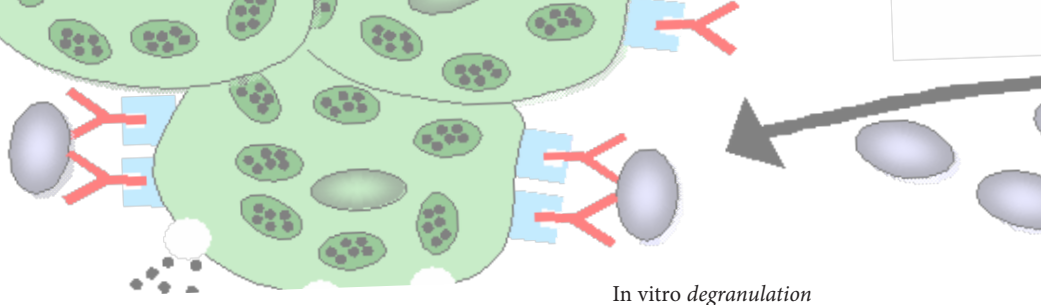
Since the 1990s, glutens have been functionalized by acid hydrolysis for use as ingredients in foods or cosmetics. However, these modified glutens turned out to be neo-allergenic, as the deamidated proteins induce the production of specific IgE-type antibodies that trigger an allergic reaction, and the first reports of allergies were described in Europe and Japan just a few years after commercial release of products containing deamidated gluten. We have produced a chimeric antibody specific to these deamidated glutens that can serve as a tool to characterize their allergenic potential.

► RESULTS

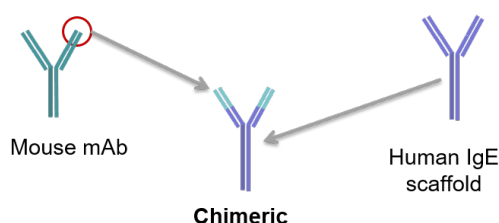
Analysis of 4 industrial samples of functionalized glutens implicated in allergy cases in Europe and Japan revealed that all 4 showed the same modified deamidation, but with different deamidation levels. An *in vitro* cell model mimicking the reaction triggered by exposure to sera from allergic patients showed that these deamidated glutens had variable allergenic potency. Using a mouse antibody (INRA-DG1) raised against the neo-epitopes recognized by the patients' antibodies, we found that the allergenicity of the deamidated products was mainly borne by epitopes with the most highly deamidated sequences. A recombinant chimeric (mouse/human) IgE-type antibody was produced by inserting the INRA-DG1 antibody binding site into a human IgE. This chimeric IgE, like the murine antibody, is able to detect the deamidated glutens, and it also has a similar biological activity to that of the patient IgE—the chimeric IgE-DG1, like the patients' IgE, induces a symptomatic reaction *in vitro*—and can thus serve to assess the allergenicity of functionalized glutens.

► FUTURE OUTLOOK

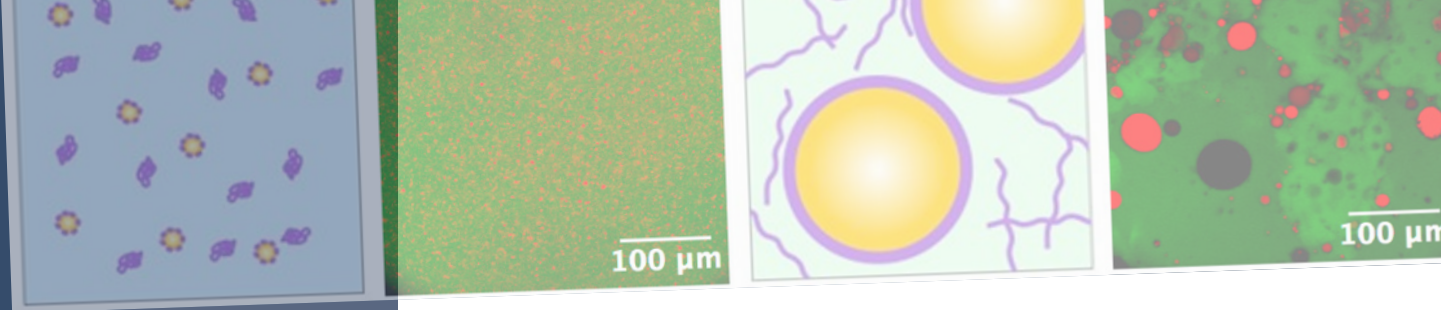
This research builds on patent WO2013/054063 protecting the INRA-DG1 antibody and its chimeric version. The chimeric IgE could ultimately be used as a lab-based substitute for patient sera to assess the allergenicity of deamidated glutens.



Principle of Chimeric IgE generation



Chimeric IgE



Participants

These results came from a collaborative project, funded by the ANR under the ALIAS [‘Food–Diet–Health’] research project (ANR-11-IDEX-0003-02) led by Paris-Saclay University, involving four research laboratories:

- ♦ UMR GMPA, AgroParisTech, INRA, Université Paris-Saclay, 78850, Thiverval-Grignon, France
- ♦ Institut MICALIS, AgroParisTech, INRA, Université Paris-Saclay, 78350 Jouy-en-Josas, France
- ♦ UMR PNCA, AgroParisTech, INRA, Université Paris-Saclay, 75005, Paris, France
- ♦ UMR GENIAL, AgroParisTech, INRA, Université Paris-Saclay, 91300, Massy, France

Read more

In vitro digestion of foods using pH-stat and the INFOGEST protocol: Impact of matrix structure on digestion kinetics of macronutrients, proteins and lipids

(2016) Food Research International Mat DJL *et al.*

Structure of protein emulsion in food impacts intestinal microbiota, caecal luminal content composition and distal intestine characteristics in rats

(2017) Molecular Nutrition & Food Research

Beaumont M *et al.*

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Physiological effects of food structures: when food processing shapes subsequent food digestion and gut microbiota composition

Foods are formed of various structures, and these structures result from the raw materials used and the food processes delivered (cooking, mixing, gelling, etc.). Could different food structures affect digestive physiology and intestinal microbiota composition?

► RESULTS

To tackle this question, we produced model foods with perfectly-defined structures by controlling the thermomechanical energy injected to mix and blend the protein and lipid fractions, and we also developed an *in vitro* method enabling real-time tracking of protein and lipid hydrolysis over the course of digestion.

Using the models and method together, we were able to rank-order a number of structure factors (oil droplet size, form of oil-droplet interface, soluble-state or gel-state proteins) as a function of their effect on rates of digestion. The results show that lipid–protein interactions are not only food-structure-dependent but also change over the course of digestion, which patently illustrates that the food needs to be considered as more than the sum of its nutrient parts.

Two contrasted model foods—one liquid, the other solid—were selected to serve for digestion experiments in the rat. We observed that the kinetics of intestinal transit and the amount of amino acid transporters and protein fermentation metabolites found in the caecum varied according to structure of the food. The composition and activity of the intestinal microbiota were also affected, as microbial species involved in protein fermentation came to dominate the microbiota when the liquid model food was given. These *in vivo* data suggest that the protein fraction not absorbed in the small bowel, *i.e.* the fraction available for the gut microbiota, was greater with the liquid food.

Taken together, these results demonstrate that food structure has clear effects on digestive enzyme reaction rates and on the allied *in-transit* kinetics, which leads to changes in markers of digestive physiology and microbiota composition.

► FUTURE OUTLOOK

These findings raise prospects for integrating structural parameters of foods as components of their nutritional properties, as well as offering lines of investigation to gain a deeper understanding of how diet connects to microbiota.

Participants

This project was financed by Arla Food Ingredients (AFI) and coordinated under joint Agrocampus Ouest/University of Copenhagen supervision.

Read more

Impurities enhance caking in lactose powder

(2017) Journal of Food Engineering

Carpin M, Bertelsen H, Dalberg A, Roiland C, Risbo J, Schuck P, Jeantet R

How does particle size influence caking in lactose powder?

(2017) Journal of Food Engineering

Carpin M, Bertelsen H, Dalberg A, Roiland C, Risbo J, Schuck P, Jeantet R

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Science & Technology of
Milk & Egg (STLO)

How to stop lactose powder caking?

Lactose, which by bulk is the biggest ingredient in breast milk, is also the main ingredient in powdered infant formula. However, the use of lactose powder to make infant formula is hampered by caking—the unwanted presence of lumps and clumps in the bulk powder. This study shows that the presence of impurities and fine particles enhances caking tendency, highlighting how firm control of washing grade and crystal size is critical to prevent caking.

► RESULTS

Powders containing residual impurities, i.e. non-lactose components (minerals, proteins, etc.) and presenting variable particle size distributions were produced at pilot scale and then subjected to different temperature and pressure conditions conducive to caking. This enabled us to identify a number of cues and clues to controlling caking:



Lactose powders

- ◆ Residual impurities content after the washing step has to be below 1%, as impurities will absorb ambient moisture more than lactose and thereby facilitate moisture-driven caking.

- ◆ Caking tests on various different sieved-grade fractions show a connection between caking and crystal size: the finest-grade particles with a mean diameter of less than 80µm contain more impurities and present a greater surface-to-volume ratio, thus increasing the potential for particle-to-particle contact points. Firm

control of the crystallization step and the pneumatic powder conveying steps, which affect crystal size distribution by growth and by attrition, respectively, is absolutely vital.

- ◆ Control of relative humidity during pneumatic powder conveying help keep the water activity of the powder at a sufficiently low level throughout the supply chain and through to the end-customer.

► FUTURE OUTLOOK

This research offers the industry prospects for reducing the caking tendency of lactose powders by adapting their process parameters to control crystal size grade and impurities content. It also lays the foundations for a predictive caking test that would enable production batches to be streamed into markets compatible with their degree of sensitivity to caking.



Participants

This study, funded by the CNIEL—French national joint trade organization for the dairy economy, as part of the ‘Code Poudre’ project (2013–2017; 2 theses and 1 PhD).

Read more

Heat-induced aggregation properties of whey proteins as affected by storage conditions of whey protein isolate powders

(2016) Food and Bioprocess Technology

Norwood EA, Chevallier M, Le Floch-Fouéré C, Schuck P, Jeantet R, Croguennec T

Investigation of secondary structure evolution of micellar casein powder upon ageing by FTIR and SRCD: Consequences on solubility

(2017) Journal of the Science of Food and Agriculture

Nasser S, Hédoux A, Giuliani A, Le Floch-Fouéré C, Santé-Lhoutellier V, de Waele I, Delaplace G

Browning of WPI, low-lactose WPI and NPC powder in different storage conditions [temperature/time in months]

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Materials and Transformations (UMET)

Milk protein powders change over time—but how?

Milk protein powders are high-value-added food ingredients thought to deliver optimal nutritional and functional value for up to 18 months of age. However, their functional properties (rehydration, heat stability, etc.) can alter as storage conditions change (temperature and water activity, a_w).

► RESULTS

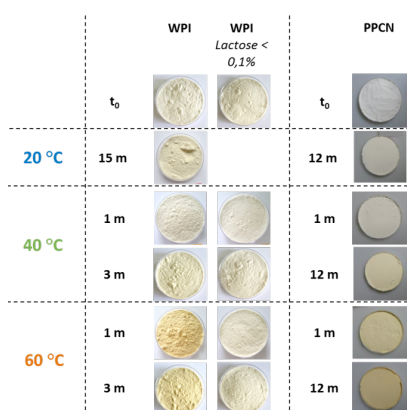
Experiments were set up to track and trend the time-course changes in structure and function of whey protein and micellar casein isolates over 15 months in controlled storage conditions (20°C, 40°C and 60°C; original a_w). Despite their low residual lactose content (1% p/p), both isolates showed browning.

At molecular level, the time-course change in structure of the whey protein isolates starts with lactose–protein interactions (lactosylation) increasing the reactivity of the proteins, some of which then aggregate. The time-course change in micellar protein isolate powders was characterized by changes in secondary structure, including loss of the α -helices.

These ageing-specific trajectories are positively correlated with temperature and time in storage, and we were able to establish time–temperature matches for homologous structural modifications. Nevertheless, these changes had contrasted patterns of impact on the functional properties:

◆ Whey protein isolates only showed altered heat-induced aggregation properties, caused by the molecular species formed by Maillard reactions.

◆ Micellar casein powder showed storage-induced aggregation of casein proteins on the particle surface, which resulted in substantially longer rehydration time. Cutting out the residual lactose content (<0.1% p/p) can minimize browning during storage although not stopping protein aggregation processes and the formation of insoluble proteins.



► FUTURE OUTLOOK

This study offers a solid foundation for defining accelerated aging protocols that can serve to read ahead and predict the structural changes affecting functional properties during storage of powdered whey protein isolates.

Participants

‘PROFIL’ inter-regional project (scientific coordinator: J. Léonil, UMR STLO) with joint funding from regional councils (Brittany, grant #13008651; Pays de la Loire, #2014-07081), INRA, Oniris, industry partners in public-interest research association BBA.

Read more

Aggregated whey proteins and trace of caseins synergistically improve the heat stability of whey protein-rich emulsions

(2016) Food hydrocolloids

Chevallier M, Riaublanc A, Lopez C, Hamon P, Rousseau F, Croguennec T

Increasing the heat-stability of whey protein-rich emulsions by combining the functional role of WPM and caseins

(2017) Food hydrocolloids

Chevallier M, Riaublanc A, Lopez C, Hamon P, Rousseau F, Thevenot J, Croguennec T

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Science & Technology of Milk
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Biopolymers, Interactions,
Assemblies (BIA)

How to improve the heat stability of high-whey-protein emulsions without using food additives?

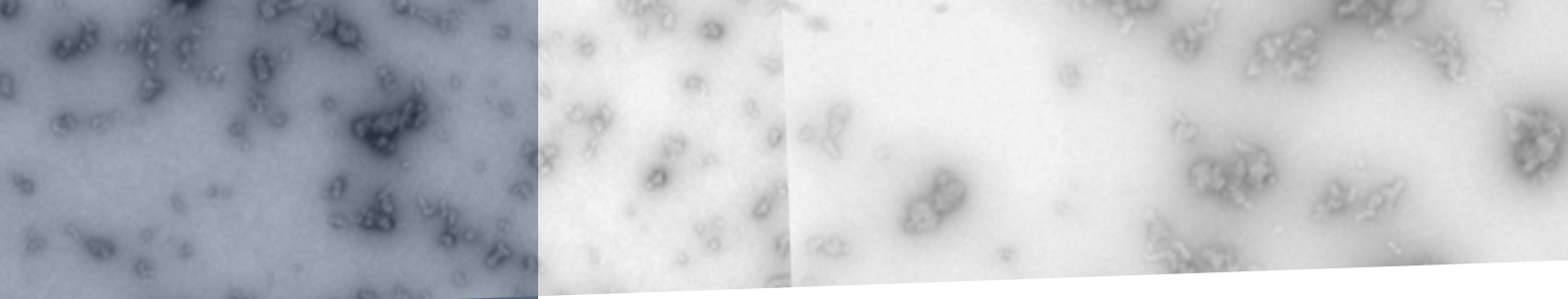
For consumers, there is strong demand for additive-free dairy products. For industry, however, creating heat-stable high-whey-protein emulsions without using additives remains a real challenge. We showed that it is possible to develop 100%-milk sterilization process-stable emulsions and in a large range of whey protein concentrations.

► RESULTS

The solutions of whey protein aggregates are heat-stable, but the presence of these aggregates coating the fat droplet surface induces destabilization of emulsions (gelation/phase separation), whereas emulsions formed with caseins coating the fat droplet surface are very heat-stable. We succeeded in controlling the stability of high-whey-protein emulsions throughout the various heat treatments by combining the functional roles of whey protein aggregates and caseins. Emulsions were prepared with whey protein aggregates and with different aggregate-to-casein ratios depending on the interfacial area to cover, and we then analyzed their structure at key scales (interface, fat droplets, emulsion) and studied their heat stability. The results show that heat stability of the emulsions can be modulated by controlling the ratios of whey protein aggregates and caseins between the fat globule surface and the continuous phase of the emulsion. Despite being additive-free, the emulsions stay stable throughout heat treatments when formed with the fat globule surface coated with caseins and with aggregated whey proteins in the continuous phase.

► FUTURE OUTLOOK

This research partly explains the mechanism that destabilizes food emulsions when they are made or used, and raises prospects for better stability control using an alternative to non-dairy food additives.



Comparison of fractal aggregates produced at pilot scale with tube heat exchanger or in an heated bath at laboratory scale

Participants

These results were obtained as part of a doctoral thesis sponsored with funding from the Pays de la Loire regional council: the 'PROFIL' project, led in collaboration with UMR STLO and dairy industry partners in the public-interest research association Bba

Read more

Characterization of heat-induced changes in skim milk using asymmetrical flow field-flow fractionation coupled with multiangle laser light scattering

(2010) Journal of Agricultural and Food Chemistry

Guyomarc'h E, Violleau F, Surel O, Famelart M-H

Determination of hydro-colloidal characteristics of milk protein aggregates using Asymmetrical Flow Field-Flow Fractionation coupled with Multiangle Laser Light Scattering and Differential Refractometer

(2018) Food Hydrocolloids

Loiseleux T, Rolland-Sabate A, Garnie C, Croguennec T, Guilois S, Anton M, Riaublanc A

Field-flow fractionation coupled with static light scattering—a powerful technique for characterizing mixed milk protein aggregates

During heat treatment, the soluble milk proteins get denatured and then aggregate together or at the surface of casein micelles to form mixed aggregates. These supramolecular aggregates possess potentially useful functional properties for replacing certain food additives, but these properties are sensitive to supramolecular structure and the presence of soluble proteins. We thus developed an innovative method for characterizing the mixed milk protein aggregates.

► RESULTS

Working up from separation system based on asymmetrical flow field-flow fractionation coupled with multiangle laser light-scattering and refractive index detectors (A4F-MALLS-DRI), we developed appropriate methods for separating different populations within heated solutions of milk proteins.

Heating a 50 g/L whey protein solution at pH7 to 80°C in the presence of 45 mM NaCl gave fractal aggregates whereas heating a 40 g/L whey protein solution at pH5.8 to 85°C gave dense spherical aggregates. Heating solutions containing casein micelles at pH6.3 to 80°C led to the formation of mixed aggregates featuring a casein core covered by fractal aggregates of soluble proteins.

Using this powerful new technique, we were able to show that:

- ◆ The fractal aggregates formed are not homogeneous: within the mixtures, the non-aggregated proteins, the small compact aggregates and the big branched aggregates assembled from smaller ones can all be separated.
- ◆ The spherical aggregates self-assemble to form large but less dense structures.
- ◆ The whey proteins preferentially aggregate on the large micelles to form mixed aggregates.

► FUTURE OUTLOOK

This technique, when applied on a complex mixture, can separate protein aggregates over a very broad size range (5nm–1µm), determine the size and apparent molar mass of the component structures, and quantify each population. Although developed for milk proteins, this novel technique should be extended to plant proteins, which are often aggregated, and lead to a better understanding of their functional properties.

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Biopolymers, Interactions,
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Encapsulation of bioactives in protein coacervates: protection and controlled release

Participants

♦ PROFIL Interregional Project (Bretagne, N°13008651 ; Pays de la Loire, N°2014-07081), INRA, Oniris, Industriels BBA.

♦ Faculté de Pharmacie, Centre de Recherche CHU du Québec, Université Laval, Québec, Canada.

Read more

Coacervates of whey proteins to protect and improve the oral delivery of bioactive molecule

(2017) Journal of Functional Foods

Chapeau A-L, Bertrand N, Briard-Bion V, Hamon P, Poncelet D, Bouhallab S

Scale-up production of vitamin loaded heteroprotein coacervates and their protective property

(2017) Journal of Food Engineering

Chapeau A-L, Hamon P, Rousseau F, Croguennec T, Poncelet D, Bouhallab S

Increasing the added value of proteins by exploring their plasticity and their multifunctional assemblies represents a major challenge. We previously showed that whey proteins have the capacity to assemble into microspheres called heteroprotein coacervates. Here we demonstrate that microspheres assembled by the complex coacervation of two whey proteins, i.e. lactoferrin (LF) and β -lactoglobulin (BLG), are efficient for the encapsulation, protection and controlled release of small bioactives like vitamin B9.

► RESULTS

By testing a range of concentrations and molar ratios of the components at different pH levels, we managed to establish the optimal set of coacervation conditions. Coacervation yield reached 55%, enabling 98% vitamin B9 entrapment, which translates into effective production of B9-loaded LF-BLG coacervates containing 4–5 mg of vitamin B9 per gram of coacervates. The process scaled up from lab (μ L) to bench (L) without loss of efficiency. Furthermore, the protein coacervates protect the native-form of the vitamin against oxidation, against UV light, and during freeze drying.

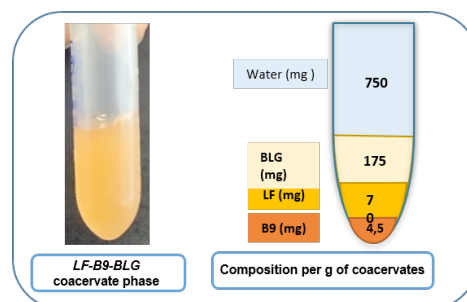
We compared the bioavailability of vitamin B9 after administered in various forms to rats *per os*. The protein coacervates significantly improved the bioavailability of the vitamin, providing higher plasma concentrations than after administration of the vitamin delivered alone or simply associated with either LF or BLG.

The coacervates therefore efficiently entrap the vitamin B9, which is made more stable as it is protected against degradation during processing and storage, and which is also better absorbed *in vivo*.

► FUTURE OUTLOOK

We have clearly shown that the protein coacervates make effective and efficient biocarriers for encapsulation and vectorization of vitamin

B9, creating a multifunctional product that is rich in bioactive proteins (e.g. LF and its biopeptides), iron and vitamin B9. This development opens up a whole new field of applications for assemblies of these proteins, both as an ingredient and as a food additive for targeted diet.



Encapsulation of vitamin B9 by complex coacervation of two whey proteins. The coacervate phase is rich in proteins, vitamin and iron sourced from natural lactoferrin (LF).

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Science & Technology of Milk & Egg (STLO)



Mobilization and impact

Patent application filed in France on 28 December 2015 under number FR 15 053762 and worldwide under number WO2016108024. Inventors: Garric G, Leonil J, Jeantet R, Lortal S, Schuck P, Gaucheron F

This process is proposed to various national and international milk-processing entities:

- ♦ small-scale entities, typically producer organizations or milk collection cooperatives that want to pool their milk, especially in periods of seasonal milk surplus, and produce basic but differentiated cheese products on an easy-to-use tool.
- ♦ businesses that already master the membrane technologies but want to optimize the uncoupling of texture production and aroma production
- ♦ big businesses expanding out into the overseas export market with differentiated texture and aroma matrices—whether prepared in France or in the target country of import.

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Science & Technology of
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Conceptually disruptive cheesemaking technology: how to get a ripe-aroma cheese in less than four 4 days

A patented process can make all kinds of cheeses that cost far less to produce and are far faster to make—under four days rather than several weeks for traditionally-made cheese. The process involves uncoupling the texture-development process and the aroma-development process by completely rethinking the substance and sequence of cheesemaking processes. The cheeses can be produced with textures ranging from spreadable to semi-hard and with design-on-demand aromas to meet market needs.

► RESULTS

The draining and acidification steps are uncoupled by using membrane filtration and centrifugation to fractionate a base-material milk and then reconstructing a texture matrix with fully-controlled protein-to-fat ratio, fat format, denaturation of whey proteins, mineralization and amount of available lactose. In parallel, an aromatic matrix is produced in less than four days according to an optimized interplay of three factors: type of bacteria (*Hafnia alvei*, *Yarrowia lipolytica*, *Propionibacterium freudenreichii* or a blend of Lactococci), environmental conditions, and base format. The texture matrix and the aroma matrix are then assembled together in a 90/10 (p/p) ratio, and the mix is given the required texture—essentially by adjusting the pH, temperature, and the amount of added NaCl and coagulant.

By replacing the draining step with membrane filtration, adding salt directly, and shortening ripening to a short-burst step in a fermenter, this resource-efficient concept makes it possible to independently control each and every step, substantially reduce the inputs (fluids and facilities), and standardize the process by-products. It is a fabulously flexible process—with the same set of material, it can produce practically any type of cheese and allied aroma on-demand for the next day, the end-product can be made nutritiously balanced by mixing yeast probiotics or prebiotics into the final cheese matrix.

► FUTURE OUTLOOK

Going forward, the next step is to optimize the process parameters in order to lend the curds targeted functional properties, from slicing to stretchability and meltability, as firm control of these properties is an essential prerequisite for crafting cheese-based food ingredients.

PARTICIPANTS

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Read more

Towards the use of biochemical indicators in the raw fruit for improved texture of pasteurized apricots

(2017) Food and Bioprocess Technology

Ribas-Agustí A, Gouble B, Bureau S, Maingonnat JM, Audergon JM, Renard CMGC

Apricot texture: variability as a function of cultivar, influence of maturity and impact of cooking

(2017) LWT-Food Science and Technology

Ayour J, Gouble B, Reling P, Ribas-Agustí A, Audergon JF, Maingonnat JF, Benichou M, Renard CMGC

Impact of canning and storage on apricot carotenoids and polyphenols

(2018) Food Chemistry

Le Bourvellec C, Gouble B, Bureau S, Reling P, Bott P, Ribas-Agustí A, Audergon JM, Renard CMGC

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Safety & Quality of Plant
Products (SQPOV)

Preserved apricot—how quality hinges on choosing the right variety

The apricot season is short and the harvested fruit does not preserve easily, so all the volumes not consumed fresh are absorbed by the processing industry (in preserves, compotes, jams or fruit juices). We studied the impact of cooking and storage of apricot halves in syrup on polyphenol and carotenoid concentrations and on ability to hold texture in different varieties.

► RESULTS

In response to thermal treatment, polyphenols and carotenoids remain relatively well preserved, but at different stages. In response to processing into apricot halves, there are polyphenol losses (amounting to 34% on average) due to thermal degradation and certain classes that diffuse into the syrup, like the flavan-3-ol monomers, hydroxycinnamic acids, flavonols and anthocyanins, whereas the flavan-3-ol polymers or procyanidins, which are bulk compounds in apricot, remain (at over 70% on average) in the apricot halves. After 2 months in storage, the polyphenol contents remain relatively stable. In contrast, carotenoids, and especially *cis*- β -carotene, show a net increase in apparent concentrations (+10% on average) after cooking that makes them more efficiently extractable. However, they then become degraded during storage (-16% on average), and this loss is thought to be dependent on their physical state, as *trans*- β -carotene (crystalline form) remains stable.

In terms of texture of the apricot halves, a fruit that is initially firm will not necessarily cook out firmer. We found that for similar initial firmness, the textures obtained after cooking showed huge differences between different varieties, varying up to four-fold between OrangeRed (the variety that cooks best), and Goldrich (the variety that cooks worst). We also found that maximum shear strength of the cooked apricots halves correlated negatively with the titratable acidity, ethylene production and β -galactosidase activity of the fresh apricots. The negative impact of fresh-fruit acidity could be due to pectin hydrolysis at low pH (<3.5).

► FUTURE OUTLOOK

Fresh-fruit acidity as a marker of unsuitability for cooking is currently being validated on a greater number of varieties.



OrangeRed before and after cooking



Participants

- ♦ UMR408 SQPOV “Safety & Quality of Plant Products”, INRA, University of Avignon, Avignon, France.
- ♦ UR370 Animal Product Quality, INRA, St Genès-Champanelle, France
- ♦ Human Nutrition Research Unit, Clermont Auvergne University, INRA, Clermont-Ferrand, France

Read more

The matrix of fruit & vegetables modulates the gastrointestinal bioaccessibility of polyphenols and their impact on dietary protein digestibility

(2018) Food Chemistry

Dufour C, Loonis M, Delosièrè M, Buffière C, Hafnaoui N, Santé-Lhoutellier V, Rémond D

Fruits, vegetables and their polyphenols protect dietary lipids from oxidation during gastric digestion

(2014) Food & Function

Gobert M, Rémond D, Loonis M, Buffière C, Santé-Lhoutellier V, Dufour C

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Safety & Quality of Plant Products (SQPOV)

Fruit & vegetable matrix modulates not just the gastric bioaccessibility of polyphenols but also protein digestibility

Polyphenols from fruit & vegetables (F&V) are the most abundant micronutrients in our diet. Once in the digestive tract, they can have a health benefit by limiting the formation of lipid oxidation compounds that are ultimately involved in the development of atherosclerosis and colon cancer. However, in order to be bioactive, polyphenols have to be bioaccessible, which means they have to first be released from the plant matrix in order to solubilize in the digesta. We thus investigated the gastric bioaccessibility of phenolic compounds derived from F&V as part of a western diet.

► RESULTS

We assessed (i) polyphenol bioaccessibility and (ii) protein digestion in minipigs fed a complete meal based on beef and sunflower oil with either cubed 'F&V' (apple, plum, artichoke) added, or the corresponding phenolic extract. The overall initial gastric bioaccessibility of the polyphenols was very low, at just 1.5% and 3.1% for the F&V and 'phenolic extract' meals, respectively. Strikingly, one class of polyphenols—the oligomeric flavonols—were not recovered in gastric digesta yet were found later in the ileon. These results point to a process of phenolic compound complexation by fibres and proteins during gastric digestion. We also showed that dietary protein digestion was far less efficient with the polyphenol extract than with the corresponding F&V, and that apparent ileal digestibility decreased and plasma amino acid concentration was lower over the 6h-long period of digestion.

Taken together, these results argue that a careful risk–benefit analysis is needed when taking food supplements, especially for malnourished subjects.

► FUTURE OUTLOOK

Further research is focusing on a family of polyphenols—the oligomeric flavonols—that appear to interact strongly with dietary fibres and proteins, and we will be assessing the impact of food processing (raw apple, purée, phenolic extract) on flavonol bioaccessibility, flavonol metabolism by microflora straight after the ileon, and complete-meal protein digestion.



Participants

This work was performed as part of doctoral research by Nadia Yacoubi (2014-2016), hosted within the Plant Cell Wall and Cell-Wall Polysaccharides team at the BIA unit under co-supervision with the Pathology, Bacteriology and Poultry Diseases department of Ghent University Faculty of Veterinary Medicine (Belgium).

This doctoral research was supported by a CIFRE [industrial research placement training] grant arranged between the ANRT and industry partner ADISSEO

Read more

Water-soluble fractions obtained by enzymatic treatment of wheat grains promote short chain fatty acids production by broiler cecal microbiota

(2016) Animal Feed Science and Technology

Yacoubi N, Van Immerseel F, Ducatelle R, Rhayat L, Bonnin E, Saulnier L

Short-chain arabinoxylans prepared from enzymatically treated wheat grain exert prebiotic effects during the broiler starter period

(2017) Poultry Science

Yacoubi N., Saulnier L., Bonnin E, Devillard E, Eeckhaut V, Rhayat L, Ducatelle R, Van Immerseel F

Carbohydrate-degrading enzymes to restore gut health in broilers

Gut health problems cause big economic losses in poultry farming, especially since the EU banned the use of antibiotics as growth promoters in 2006. Carbohydrate-degrading multi-enzyme preparations (MEP) have emerged as a widely-used alternative, particularly in broilers fed cereal-based diets. Their mode of action is multifactorial, but is generally explained by the fact that they produce prebiotic oligosaccharides in the digestive tract.

► RESULTS

To better understand their mode of action, we isolated water-soluble polymer fractions from wheat grain incubated with and without MEP. The MEP increased the amount of short-chain arabinoxylans (SC-AX) without producing oligosaccharides.

These fractions were then incorporated into wheat-based diet to feed broilers for two weeks after hatching, and their effects on broiler performances, gut health, short-chain fatty acid production and gut microbiota composition were studied. The results showed that the presence of SC-AX in the feed significantly increases chick weight gain and promotes the growth of butyrate-producing bacteria (*Lachnospiraceae* and *Ruminococcaceae*). Gut inflammation was also decreased, which may be connected to the increase in butyrate, which is known to have anti-inflammatory effects and stimulate the enteroendocrine L-cells that produce gastrointestinal hormones needed for good broiler gut health. Here we demonstrated that the hydrolysis of polysaccharides does not need to be driven as hard as previously thought in order to produce active health-promoting compounds. With a degree of polymerization at around 50, the SC-AX effectively increased production of butyrate, decreased T-cell infiltration in the caecal and ileal mucosa, and increased L-cell density in the ileal epithelium—all of which are processes that signal improved gut health.

► FUTURE OUTLOOK

These results suggest that the beneficial action of MEP on animal performances is linked to the partial depolymerization of wheat-grain cell-wall polysaccharides. This same kind of study now needs to be extended to other crude feedstuffs to check whether other partly-degraded cell-wall polysaccharides reproduce the same effect.

CONTACTS

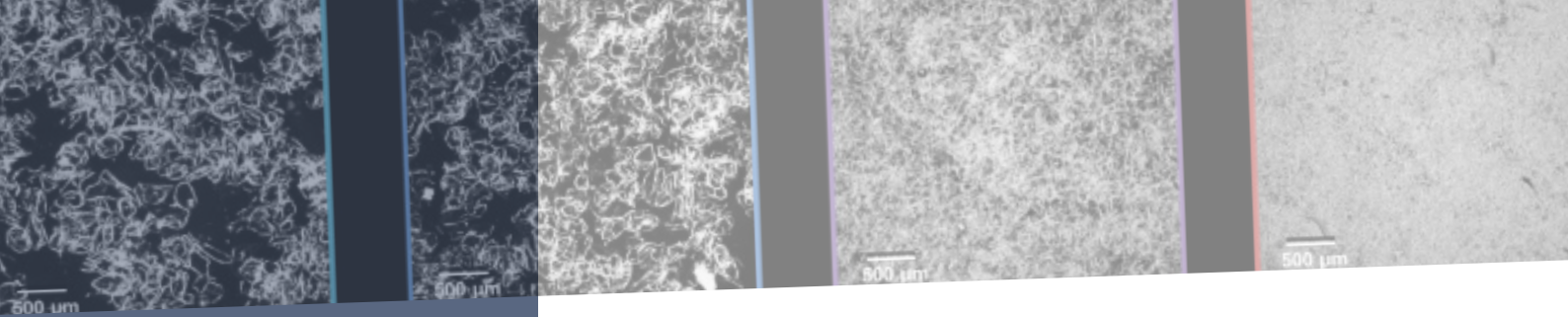
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Biopolymers, Interactions,
Assemblies (BIA)



Participants

This research was carried out as part of the European OPTIFEL project.

Read more

Design of Model Apple Cells Suspensions: Rheological Properties and Impact of the Continuous Phase

(2017) Food Biophysics

Leverrier C, Almeida G, Menut P, Cuvelier G

Influence of particle size and concentration on rheological behaviour of reconstructed apple purees

(2016) Food Biophysics

Leverrier C, Almeida G, Espinoza-Munoz L, Cuvelier G

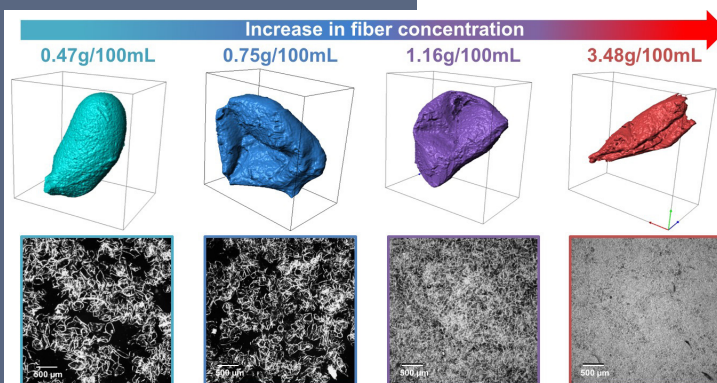
What underpins apple purées, or how do the cells pack down?

The texture of fruit and vegetables is a major quality parameter for consumers. We had previously shown that the texture of processed fruit and vegetables is essentially governed by the insoluble solids they contain. The scientific literature describes fruit or vegetable purées as suspensions of soft and compressible particles (cells or cellular aggregates), and although this theory is grounded in their (chiefly rheological) behaviour, the actual particle organization has never been directly observed.

► RESULTS

We have developed a method for extracting and selectively labelling the insoluble particles from apple purées. The method works to the principle of only using 10% of labelled particles (while extracting but not labelling the remaining 90%) in order to directly observe the labelled particles without all the noise and interference caused by the

crowded and densely packed purée matrix that normally makes it impossible to distinguish each individual cell. Working up from these particles, which here correspond to individual cells, we reconstructed a set of apple purées at different insoluble solids concentrations, i.e. 0.47g/100mL and 0.725g/100mL, which correspond to diluted purées, 1.16g/100mL which corresponds to the fibre-solids content of a standard purée, and 3.48g/100mL which corresponds to a dense insoluble solids-enriched purée.



Optical microscopy observation of 4 apple purées at different solids concentrations, and the corresponding confocal microscopy observations of the apple cells

We then observed the particles directly in the sample, using confocal microscopy. After 3D reconstruction of the microscopy images via Simpleware software, we were able to quantify the morphology of the cells. The results showed a significant volume shrinkage of the cells in the concentrated domain (at over 1.16g insoluble solids per 100mL of purée), as the cells became flattened in the densely packed matrix.

► FUTURE OUTLOOK

This study brings direct evidence, for the first time, that plant cells have the capacity to pack down when particles concentration gets too high. This change in volume occupied by the plant cells in the sample leads to a change in the sample's rheological behaviour, which in turn affects its texture. This same approach is generalizable to other crowded particulate systems.

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Food Process and Engineering (GENIAL)

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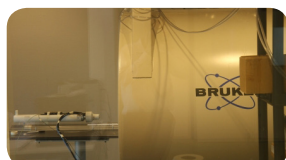


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<https://www6.montpellier.inra.fr/spo/Structures-collectives/Plate-forme-Polyphenols>

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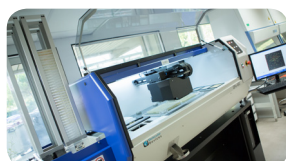


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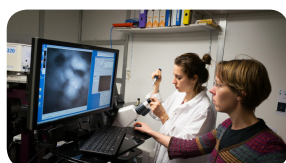


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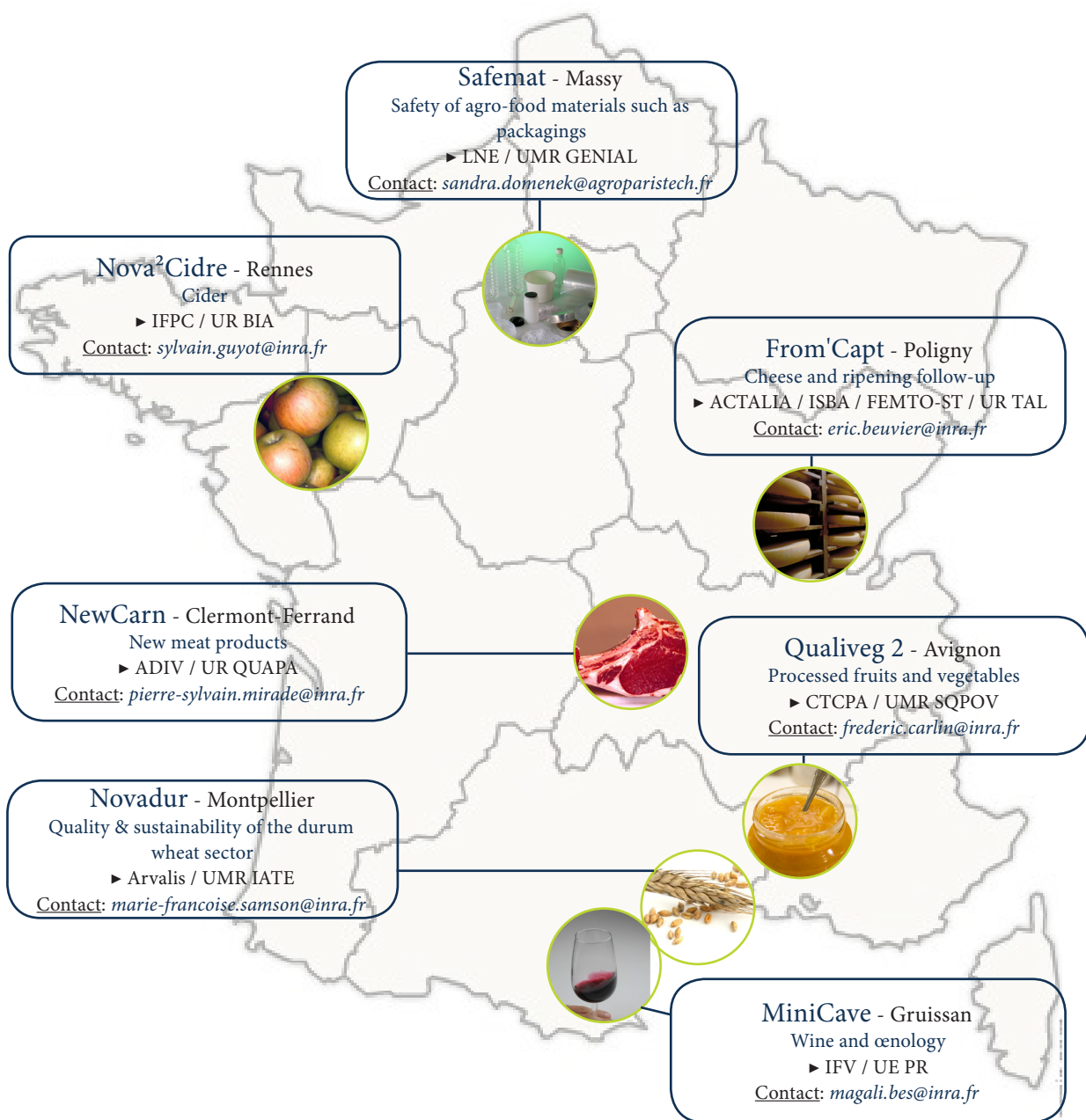
<https://www.synchrotron-soleil.fr/>

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JOINT TECHNOLOGY UNITS

UMTs—‘joint technology units’—are science platforms introduced by the Ministry for Agriculture in 2006 themed around an R&D project shared between research and development organizations.

In UMTs, partners co-build and co-conduct a national-scale-strategy R&D programme articulating upstream research work with downstream technology development. The UMT initiative thereby builds bridges between public agency-led and industry alliance-led research and help disintermediate methods and decompartmentalize mindsets to create fertile conditions for innovation.



All these ‘UMTs’ are online at:

<https://www.gis-reliance-agronomique.fr/GIS-UMT-RMT/Les-UMT>

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