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## Research and Innovation 2022. TRANSFORM - Division of Science for Food, Bioproducts and Waste TRANSFORM

Michael O'Donohue, Jean-Philippe Steyer, Mélanie Delclos, Laurence Fournaison, Rachel Boutrou, Carole Tournier, Olivier Tranquet, Olivier Vitrac, Catherine Garnier, Patrick Dabert, et al.

► **To cite this version:**

Michael O'Donohue, Jean-Philippe Steyer, Mélanie Delclos, Laurence Fournaison, Rachel Boutrou, et al.. Research and Innovation 2022. TRANSFORM - Division of Science for Food, Bioproducts and Waste TRANSFORM. [Research Report] INRAE; INRAE TRANSFORM. 2022. hal-03690422

**HAL Id: hal-03690422**

**<https://hal.inrae.fr/hal-03690422v1>**

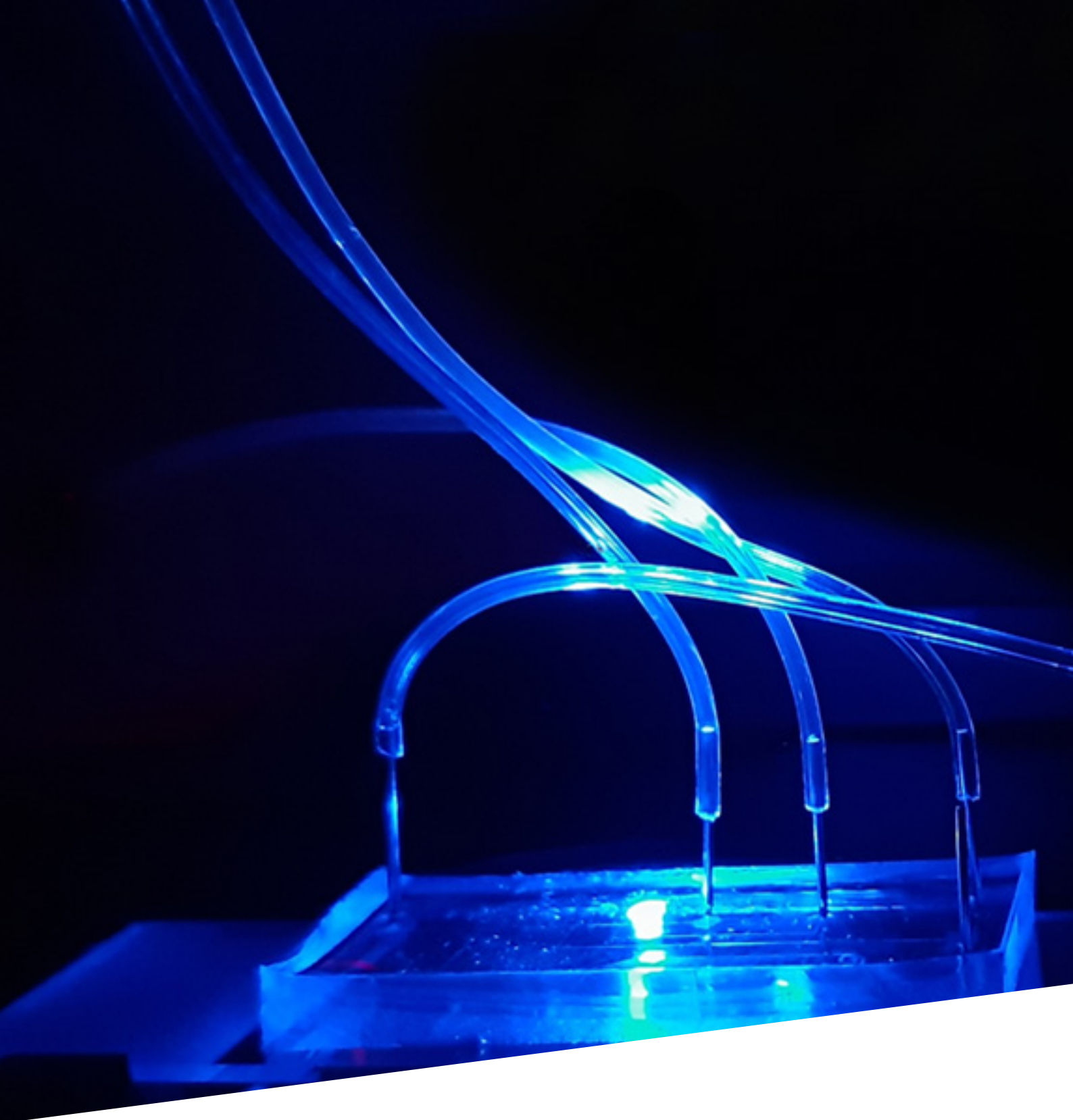
Submitted on 8 Jun 2022

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**INRAE**



## **Research and Innovation 2022 TRANSFORM**

**Division of Science for Food, Bioproducts and Waste  
TRANSFORM**

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**Design:** Mélanie Delclos

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O'Donohue, et al. (2022). Research and Innovation 2022

TRANSFORM Division of Science for Food, Bioproducts and Waste

TRANSFORM. <https://doi.org/10.17180/pfb8-qj03>



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# Foreword

Whether you are simply a curious reader or a connoisseur of TRANSFORM, the 2022 edition of our report offers you a collection of results that perfectly represent our theme areas and ambitions. Consistent with our 2021-2025 strategic roadmap, the Division's activities focus on the circular bioeconomy transition. In doing so, we aim to contribute to INRAE 2030 ambitions regarding food transition challenges, sustainable development and data empowered science.

We begin our report with data science topics. For several years now, our teams have been adopting modelling and simulation as central features of their research. In this edition, we illustrate the importance of these methods and tools for the circular bioeconomy transition, an economy that uses biobased resources as raw material for industry.

The second chapter of this report deals with two other major challenges for the circular bioeconomy, namely the development of sustainable processes that consume less energy and water and have less impact on the environment, and the development of biocatalysis as the technological core of a new industrial era. You will find in this chapter many examples of work combining the acquisition of fundamental knowledge and applied research. Finally, in the two last chapters, various illustrations of our work reveal two of the Division's defining themes, which are food and biobased materials research. As you peruse the pages of this report, you will realize that these two areas intermingle, because in the circular bioeconomy food manufacturing coproducts can become raw material for biobased materials.

I would like to finish this preface by wishing you an enjoyable read and by inviting you to follow our work on a regular basis by visiting our website (<https://www.inrae.fr/en/divisions/transform>).

**Michael O'Donohue**  
Head of TRANSFORM Division





## TRANSFORM collective scientific facilities



PROBE Platform for probing properties of food and bio-based products

### AGRORESONANCE - UR QUAPA

Nuclear magnetic resonance (NMR) spectra or images (MRI) are exploited for a broad range of applications to determine the chemical structure, to quantify the concentration, to understand the dynamic of small molecules, and to characterize the organization of various samples at different scales. It therefore gives access to valuable information on agro-resources, foods and living organisms.

The AgroResonance facility is part of the INRAE Clermont-Auvergne-Rhône-Alpes research centre. It hosts cutting-edge MRIs driven by highly skilled scientists able to develop original experimental design and then address key questions in food-farming, plant science, nutrition and health.

AgroResonance is ISO 9001 certified as well as granted by the INRAE infrastructure facility label. As founder member of IBiSA-sponsored regional multimodal imaging platform *In Vivo Imaging in Auvergne* (IVIA), it has also access to the vast majority of Clermont-Ferrand *in vivo* imaging modalities for animals and humans.

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PROBE Platform for probing properties of food and bio-based products

### BIORESOURCES : IMAGING, BIOCHEMISTRY & STRUCTURE (BIBS) - UR BIA

BIBS platform brings together expertise in various analytical fields and data processing to describe – by various means and on a range of scales from the millimetre to the nanometre – the structures and architectures of agriculture-based systems and to study the biopolymers that comprise them (polysaccharides, proteins, lipids).

The methods make it possible to i) characterise the structure of biopolymers (identification, quantification, modifications), their interactions, their organisation (local orders, mobility), and their location; ii) monitor their degradation or transformation; iii) screen collections of samples on chemical and structural criteria; iv) image the systems using different techniques and at different scales, to carry out correlative imaging; and v) address dynamic parameters (diffusion).

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### BIO2E PLATFORM - UR LBE

The Bio2E – Environmental Biotechnology and Biorefinery platform at the Laboratory of Environmental Biotechnology (LBE) conducts technological R&D activities to exploit bioresources and produce bioenergy, with expertise in anaerobic digestion, biomethanation, biohydrogen production, microalgae and physicochemical processes. The platform offers solutions for designing, implementing and optimising sustainable, efficient and reliable innovative bioprocesses for the recovery and/or treatment of various waste products from human activities. Bio2E hosts the INRAE-Transfert Environnement (IT-E) business unit, which provides services based on the knowledge produced by the LBE. The platform is able to support innovative projects from basic research, through proof of concept to demonstration (TRL 1 to 6).

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PROBE Platform for probing processes of food and bio-based products

## CHEMOSENS- UMR CSGA

ChemoSens is the platform for research and methodological development of the Centre for Taste and Feeding Behaviour (CSGA), located in Dijon, France. A unique aspect of this platform is the integration of expertise in chemistry and sensory analysis to develop new approaches to characterizing food and consumer behaviour.

Food flavour molecules are characterised through physicochemical analysis methods and monitored as they release active molecules during chewing. In addition, the platform has expertise in the analysis of lipids in food and in neurosensory tissues.

ChemoSens is renowned for developing sensometrics and sensory data acquisition techniques, including Temporal Dominance of Sensations (TDS). Through the platform, extensive databases have been built up, and TimeSens® has been developed as a web-based tool for the analysis of sensory data.

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## DAIRY PLATFORM (PFL) - UMR STLO

The Dairy Platform is an experimental facility where various technological dairy processes can be implemented in a single location, at different scales, and according to flexible and controlled processing technologies, including the fractionation of milk components, the production of more or less concentrated matrices, fermented or not (fresh products, cheese or concentrated milk) and the drying of dairy matrices. Other matrices (egg products, cereal- or pulse-based beverages) can also be studied (alone or combined). With a surface area of 800 square metres, it was completely refurbished in 2013 and has been ISO 9001-certified since 2009. The platform is open to academic and industrial partnerships. It deploys in-house expertise (technology, process engineering, biochemistry, microbiology, nutrition and eco-friendly design) implemented in academic or private research projects as well as in training programmes for future food industry executives.

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## PLANET- UMR IATE

The Processing of Plant Products with Emergent Technologies (PLANET) platform is a technology platform of the Joint Research Unit for Agropolymer Engineering and Emerging Technologies, certified as a collective scientific facility by INRAE since 2018. The PLANET platform's aim is to participate in research programmes and contribute to the development of methods and processes for the valorization of plant raw materials in various fields of application (food, biomaterials and bioenergy) and to develop scientific collaborations with external partners.

The activities of the PLANET platform revolve around five themes: fractionation of cereals and seeds, dry refining of lignocellulose, shaping of biosourced composites, food processing and structuring, and characterisation of raw materials and products.

The PLANET platform brings together an original set of instrumented equipments enabling the study of the main unitary operations of plant processing (sorting, milling, structuring, drying, etc.).

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## TOULOUSE WHITE BIOTECHNOLOGY (TWB)

As an expert in leading research and development (R&D) projects, TWB assists industry players in developing innovative and sustainable solutions for the benefit of people and the planet.

TWB sets up and runs R&D projects in the field of industrial biotechnology in collaboration with public laboratories and industrial players, supports the development of start-ups by offering them accommodation on its premises in a state-of-the-art scientific and technological environment and promotes the emergence of breakthrough innovations. By bringing together researchers, entrepreneurs, financiers, institutions and industrial players, TWB integrates and enhances all skills and areas of expertise and creates synergies while simplifying the contractual relationship.

Since its launch in 2012 under the triple supervision of INRAE, INSA and CNRS, and with a consortium of 49 private and public members as of 1 January 2022, TWB has contributed to the completion of nearly 260 collaborative R&D projects and to the growth of numerous start-ups that have raised a total of more than €250 million in funding.

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# RECOGNIZED INFRASTRUCTURES

## PROBE: an open infrastructure to characterise bioresources



**P**ROBE (Platform for profiling properties of food and bio-based products) is one of 14 distributed research infrastructures accredited by INRAE.

**A**gricultural resources are characterised by their complex and evolving structures, which greatly influences their properties in use. Thus, whether for food or non-food use, methods and concepts need to be developed to analyse these agricultural resources and to understand the structural modifications that take place during processing in relation to product functionalities.

**P**ROBE is a unique multiscale investigation platform of agricultural resources, from molecular structure to sensory-based characteristics. Its researchers, engineers and technicians use complementary cutting-edge technologies, such as mass spectrometry, NMR, MRI, microscopy, chemotyping, sensory analysis and food behaviour studies, and rely on innovative expertise in data processing to determine the physicochemical characteristics and properties of biomass, processed products and foods.

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## IBISBA



### IBISBA-EU

*Industrial Biotechnology Innovation and Synthetic Biology Accelerator - Sharing the way to innovation*

IBISBA EU, which was accredited by the European Strategy Forum on Research Infrastructures (ESFRI) in 2018, is a translational research infrastructure that aims to accelerate the development of industrial and environmental biotechnologies in Europe as a pivotal part of the circular bioeconomy. IBISBA brings together leading research infrastructures from nine European countries and combines the latest digital technologies to offer academic and industrial stakeholders unique access to integrated and innovative R&D&I services in biotechnology, to move into the era of 'Bioindustry 4.0'.

### IBISBA-FR

IBISBA FR is a French facility resulting from interorganisational cooperation of technology platforms offering services and training for biotechnology research and innovation, some of which are integrated into IBISBA EU. IBISBA FR was accredited as part of the French Ministry of Higher Education, Research and Innovation's 2016 roadmap (its accreditation was renewed in 2021) and strives to promote biotechnology development in France. Its next challenge will be to create the structure for and coordinate the national division of IBISBA EU.

**P**lus d'informations : [ibisba.eu](http://ibisba.eu) - [ibisba.fr](http://ibisba.fr)

**C**ontact: [ibisba-fr@ibisba.eu](mailto:ibisba-fr@ibisba.eu) - [network@ibisba.fr](mailto:network@ibisba.fr)

## Consumer – Food – Health (CALIS) infrastructure



**T**he CALIS research infrastructure is based on the national coordination of accredited platforms organised into three pillars: i) the 'Food' pillar, dedicated to food design and characterisation, ii) the 'Consumer' pillar, specialising in food choices and consumer behaviour, and iii) the 'Health' pillar, which focuses on the repercussions of food on human physiology and health (including the microbiota).

**C**ALIS offers services to both public and private scientific communities, based on technological developments, the provision

of data and experimental materials, and training activities. It supports a multidisciplinary approach to research questions on sustainable food for health by integrating the entire value chain from agricultural raw materials and their transformation into food through to the effect on human health.

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## Understanding how bioresources can be put to use



To make the best use of bioresources, the researchers at the TRANSFORM division are developing ways to study and simulate objects that are as diverse as wastewater and cooking oil. Increasing knowledge of the key mechanisms involved and their dynamics requires acquiring and processing a large amount of data to better exploit them.

**Page 8:** Increasing knowledge of mechanism dynamics, simulating, modelling

**Page 16:** Acquiring, structuring and exploiting data





©Vivien Dubois  
Infiltration area

# Characterising the hydraulic conductivity of soils applied to the infiltration of treated wastewater



## Read more

Rabouli S. *et al.*

Spatialization of saturated hydraulic conductivity using the Bayesian Maximum Entropy method: Application to wastewater infiltration areas

Water Research . 2021

<https://doi.org/10.1016/j.watres.2021.117607>

## Partnerships

- Société Iris Instruments

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## Context

In France, soil is increasingly being considered as a bioreactor to reduce pollutant concentrations in surface receiving environments (rivers) or as a natural water discharge for treated wastewater in the absence of a receiving environment. When designing the infiltration area to receive treated wastewater, the saturated hydraulic conductivity ( $K_s$ ) is the key measurement to assess the infiltration capacity of soil. Currently, there is no satisfactory technique for assessing the saturated hydraulic conductivity of a heterogeneous soil or its variability at the plot level (over the first two metres of soil). This lack of information can lead to malfunctions of the infiltration area (clogging, hydraulic short-circuiting). This research presents an innovative data fusion method that integrates geophysical and geotechnical measurements to better specify the spatial heterogeneity of the near-surface  $K_s$  value.

## Results

The study proposes a 2D representation of  $K_s$  using the Bayesian maximum entropy (BME) data fusion method that merges infiltration tests and electrical resistivity tomography. The results, based on numerical and field datasets, showed that BME is a powerful method: for the first time, researchers were able to produce  $K_s$

maps with a lower variance than all other methods tested in the literature. With only a few infiltration tests, this method makes it possible to detect anomalies that are not perceptible by traditional methods and thus to better design the infiltration area.

In some cases of very heterogeneous soils, these anomalies could have generated false estimates of the saturated hydraulic conductivity, leading to an error estimated at around 100 % in the designing of the volumes to be infiltrated.

## Future outlook

This research has enabled the development of a multi-method approach to combine geophysical and geotechnical methods to characterise heterogeneous porous media. For IRIS Instruments company (<http://www.iris-instruments.com/>), which is co-funding the project, the development of data fusion tools based on geophysical methods opens up new fields of application. Beyond infiltration areas, these initial results are important for the future use of geophysical methods, particularly in the environmental field.



©Fillaudeau L.(TBI), Rouis S. (CBS), Kallassy M (USJ)

# Innovative approach to Integrated Pest Management for Citrus (IPM-4-Citrus)



## Read more

Segura Monroy T. *et al.*

Dynamic model of d-endotoxin and spore productions by three *Bacillus thuringiensis ssp kurstaki* strains

Processes . 2021

<https://doi.org/10.3390/pr9122147>

## Partnerships

- Germany : JKI
- Italy : BIPCA (Private)
- Lebanon : USJ
- Turkey: BIYANS (Private)
- Tunisia: CTA, CBS, IPT, WIKI (Private), MEDIS (private)

## Contacts

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## Context

The IPM-4-Citrus project takes a multidisciplinary approach (biotechnology, bioactivity and transfer) in order to i) develop bioprocesses for a circular bioeconomy and ii) study and manage changes in scale in biotechnology. It aims to develop two new biopesticides (delta-endotoxins produced by the BLB1 and LIP strains of *Bacillus thuringiensis ssp. kurstaki*) that are active against citrus pests (*Phyllocnistis citrella*, *Prays citri*). Bt is an important industrial microorganism for the global biopesticide market. Modelling tools are crucial for evaluating and comparing the potential of endemic Tunisian (*Btk* BLB1) and Lebanese (*Btk* LIP) strains. Optimising bioproduction and obtaining exploitable formulated bioproducts pose scientific and technological challenges.

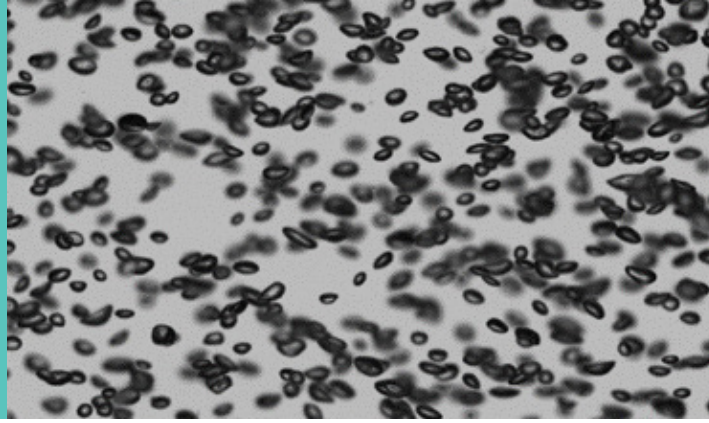
## Results

The dynamic optimisation of the production of proteins (including endotoxins), cells and spores during the different bioproduction phases requires the use of robust models coupled with control strategies. Two models have been proposed to describe protein and spore production by *Bacillus thuringiensis ssp. kurstaki* LIP. The calibration of the models allowed researchers to calculate the kinetic parameters and was well fitted with the experimental data set.

The results showed that optimisation based on a model control strategy (MCS) maximised protein and spore productivity. The simulations were performed with *Bacillus thuringiensis ssp. kurstaki* HD1, LIP and BLB1 under different experimental conditions (complex environment) in order to prove their robustness. Experimental validation of the control law was carried out to demonstrate the accuracy of the protein and spore productivities.

## Future outlook

By validating the efficacy of the formulations obtained through laboratory and field tests, the project has paved the way for potential commercial exploitation of these new biopesticides. Scaling up, integrating industrial production and formulating a cost-effective product are currently the most important challenges to tackle. The MEDIS laboratories are developing a plant (Nabeul, Tunisia) to produce formulations of *Btk*-based biological control agents for citrus following the results and expertise of the IPM-4-Citrus consortium. They are targeting the Middle East and North Africa (MENA) markets by 2023.



©Timo Larsson  
Bubbles within a bubble column.

## More responsible management of aerobic processes for wastewater treatment and reuse



### Read more

Larsson T. *et al.*

Development and validation of a comprehensive 1-D model to simulate gas hold-up and gas-liquid transfer in deep air-water bubble columns

Chemical Engineering Science . 2022  
<https://doi.org/10.1016/j.ces.2021.117210>

### Partnerships

- UR REVERSAAL
- UMR TBI
- SUEZ

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### Context

The energy consumption of water treatment and reclamation plants is strongly linked to the oxygen supply required for the aerobic biological processes of treating organic matter and nitrogen. As a result, optimising energy use at these facilities requires better management and knowledge of the transfer phenomena from the gas phase (generally air bubbles) to the liquid phase. The tools used to design aeration systems for these facilities are mostly empirical and extrapolation is sometimes difficult. There is a need to develop simple models that allow the integration of all the components of the transfer phenomena.

### Results

In partnership with the French utilities company SUEZ and INRAE's REVERSAAL and TBI units, PROSE researchers have developed a 1D model simulating both hydrodynamics and material transfer in a bubble column. This model integrates, for the first time, all the effects related to the water height (hydrostatic pressure variation, oxygen concentration in the gas) and the contamination of the gas-liquid interfaces. This model was compared with different databases from the literature on bubble columns with clear

water in order to study its robustness and relevance at different scales.

The integration of the effects of contamination of the air bubbles' interfaces allows researchers to faithfully reproduce the hydrodynamic behaviour and the oxygen transfer mechanisms observed experimentally. Additionally, air bubble depletion effects are particularly pronounced at high liquid heights and low gas flow rates. Taking them into account is essential to produce a relevant model of oxygen inputs.

### Future outlook

Additional research is being carried out to produce databases from experiments on complex fluids with regard to their rheological properties and interface contamination levels, representative of matrices from wastewater treatment and reuse processes.

Comparing the model with these databases will improve its robustness and reliability in order to transfer these tools to the operators over the long term.



©Magali Nuixé- Front view of a rhizotron containing three cocksfoot (*Dactylis glomerata*) plants separated from the ground by the cloth at the time of its installation.

## In-situ characterisation of the water status of grassland plant roots using a portable NMR sensor



### Read more

Nuixé M. *et al.*

Circadian variation of root water status in three herbaceous species assessed by portable NMR

Plants . 2021

<https://doi.org/10.3390/plants10040782>

### Partnerships

- UR QuaPA
- UREP (ECODIV division)

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### Context

Roots are vital components of the water dynamics in plants, but studying their structure and function requires destructive or indirect approaches. Nuclear magnetic resonance (NMR) is an interesting, non-invasive method to study the state (quantity and mobility) of water in biological tissues, such as plants. Portable low-field NMR would make it possible to perform these measurements in situ directly in the natural environment of plants. However, to our knowledge, no studies using this method have been conducted on roots. Our objective was therefore to demonstrate the feasibility of low-field NMR to characterise the evolution of the water status of grassland plant roots.

measurements. The NMR parameters measured are the total signal of the hydrogen nuclei, which are attributed to water, from the roots and the soil and the transverse relaxation time ( $T_2$ ) measured in the roots.  $T_2$ , a parameter that characterises the local mobility of water, is shorter the more the water is bound to tissues and/or large molecules.

For the first time, a circadian variation, according to a day/night cycle, was observed on the total NMR signal and on the value of the long  $T_2$  component. These results were consistent with the evolution of ecophysiological parameters and in particular the variation of water fluxes between day and night, measured at leaf and soil level.

### Results

The study was conducted in a climatic chamber on three species of herbaceous plants grown in a rhizotron, a parallelepipedal box closed at the front with a transparent plate allowing observation of root development and in which the roots and soil are physically separated by a piece of cloth. Concurrent ecophysiological measurements were performed to validate the NMR

### Future outlook

We plan to use this tool to estimate sap flow in plants or to characterise the water status of roots under different environmental conditions (e.g., water stress).





©Laurie Guilbert – H2020 GLOPACK Project

## Modelling biocomposite packaging by integrating data and expert knowledge



### Read more

Münch M. *et al.*

A process reverse engineering approach using process and observation ontology and probabilistic relational models: Application to processing of biocomposites for food packaging

Research Conference on Metadata and Semantics Research . MTSR 2021

[https://doi.org/10.1007/978-3-030-98876-0\\_1](https://doi.org/10.1007/978-3-030-98876-0_1)

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### Context

The massive amount of plastics used each year is causing waste to accumulate in our environment. Faced with the depletion of fossil fuels and the increase in organic residues (e.g., agricultural, urban, forestry), the development of new technologies to create biobased and biodegradable composite materials makes it possible to provide a recovery solution while producing replacements for plastic. Thus, a range of biocomposite materials formed by combining a biopolymer matrix (known as PHBV) and lignocellulosic fillers from different types of biomass are being developed to reduce the cost of materials and their environmental impact, while modulating their functional properties. Tailoring the design of biocomposite materials increases the need to understand and model the links between the structure of the materials and their final performance (e.g., water vapour permeability, thermal and mechanical characteristics).

### Results

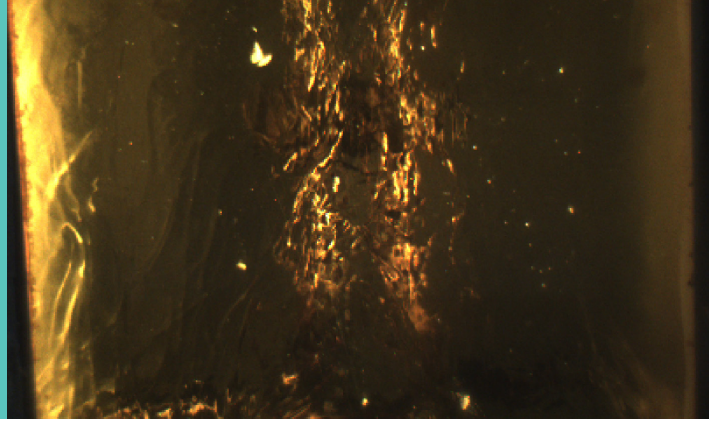
We designed and implemented a digital workflow for transformation processes, called POND (Process and observation ONtology Discovery), to support reasoning under uncertain conditions based on experimental data and expert knowledge. This workflow allows predictions to be made from a learning database.

POND is based on two artificial intelligence models: i) PO<sup>2</sup> (Process and Observation Ontology), an ontology dedicated to representing transformation processes; and ii) probabilistic relational models (PRMs). Combining them in POND allows users to perform a retroactive operation, where each learned model is submitted to the expert who can refine the integrated knowledge or add new knowledge. When applied to a dataset from four different projects, POND can be used to formulate optimal biomass types based on the desired characteristics of the final product. These results revealed a potentially interesting biomass not tested in the four projects (rice husk).

### Future outlook

The POND approach will be extended to: i) verify the quality of existing or future data inputs; ii) take into account environmental, economic and societal impact indicators of transformation processes in a sustainable design approach.

The POND approach will be developed within the framework of the CALIS (Consumer-Food-Health) national research infrastructure, which brings together certified collective systems for the production, processing and management of data relating to improving food quality, characterising the nutritional properties of foods, and the impact of foods on human health.



©Maxime Touffet

# Simulation of food processes at all scales: the case of oil oxidation during frying



## Read more

Touffet M. *et al.*

Coupling between oxidation kinetics and anisothermal oil flow during deep-fat frying

Physics of Fluids . 2021

<https://doi.org/10.1063/5.0055873>

## Value creation

Similar research is underway with the Cargill group (USA) on food deconstruction, taking into account food-mechanoreceptor interactions. Calculations are now carried out through Amazon's Cloud Computing Services and at Paris-Saclay University.

## Partnerships

- FUI Fryin'17 project (Seb, Lesieur, Adventys, Ethera, Université de Bourgogne)
- Cargill group (co-signatory).

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## Context

Frying is a cooking technique that has been used continuously since prehistoric times. Despite many innovations, domestic frying is still done in a cavity heated from below. This configuration creates significant natural convection, which is responsible for the dissolution of atmospheric oxygen and causes the oil to oxidate. This complex chemistry culminates in the appearance of volatile, odorous scission products, the production of waxes that cause equipment fouling, and the appearance of potentially toxic cyclic compounds. For the first time, these transfer and reaction phenomena were simulated in 3D at the scale of the components in a real fryer using supercomputers.

## Results

The history of the ageing of the oil was reconstructed. Unintuitively, volatile compounds are generated in the hottest regions far from the surface before desorbing on contact with air. The polymers are generated more homogeneously in anoxia before condensing on the cold walls. The detailed study of turbulent structures and anisothermal flows reveals an effect of the distribution of the heating elements and the shape of the vessel. Simulation at 'all' scales (from 100 nm to 0.3 m; from 0.1  $\mu$ s to 10 h) requires

numerous mathematical developments and considerable computing power, which has now become available. We coupled a very detailed Eulerian description of the turbulence (large eddy simulation) with a Lagrangian description of the chemical reactions. The detailed composition of the oil (by fatty acid) was analysed using a coarse-grained model.

The newly developed multiscale and multiphysics simulations allow the food processing operations to be explored in sufficient detail to virtually study the physical and chemical mechanisms while allowing the design, formulation (in this case of oil) and operational elements to be reviewed in relation to food safety and waste control.

## Future outlook

For food processing, this is a unique 'Sputnik moment' that opens up a new field of research: the direct computational study of food processing. This study highlights the creation of our young "Modelling and Engineering through Calculations" team in the SayFood Joint Research Unit.





©Nicolas Auvinet, OPAALE  
Illustration of an agricultural anaerobic digestion unit.

# Prediction of carbon and nitrogen flows in anaerobic digestion systems



## Read more

Bareha Y. *et al.*

A simple mass balance tool to predict carbon and nitrogen fluxes in anaerobic digestion systems

Waste Management . 2021

<https://doi.org/10.1016/j.wasman.2021.08.020>

## Value creation

Sys-Méthà is hosted on Data INRAE under a free licence. It has also just been integrated into the Maelia Integrated Assessment and Modelling platform (managed by the Joint Research Unit for Agronomy and the Environment – UMR LAE).

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## Context

The anaerobic digestion sectors are an integral part of the energy and agroecological transitions. These sectors bring about changes in agricultural systems that impact the major biogeochemical carbon and nutrient cycles. These changes generate environmental effects that need to be accurately assessed. For such assessments, a modelling tool for the anaerobic digestion sector is needed to produce input data for agronomic models (STICS for cropping systems, Syst'N for nitrogen leaching, etc.).

## Results

Sys-Méthà is a simulation tool developed as part of the MéthàPolSol and Proterr projects (French Agency for Ecological Transition – ADEME). The Sys-Méthà model is original in that it is compatible with agronomic models, which makes it possible to carry out studies on the scale of the entire sector in the context of numerous applications. It integrates the stages of anaerobic digestion, phase separation and digestate storage. Depending on the digested substrates, their flows and the characteristics of the anaerobic digestion unit, Sys-Méthà predicts the digestate flows produced, the associated carbon and nitrogen flows and the characteristics of the digestate produced. When coupled

with agronomic models as part of the MéthàPolSol programme, users can analyse the effect of different anaerobic digestion scenarios on the flows of carbon stored in soils and leached nitrates (water quality).

## Future outlook

Flows of other nutrients of interest (P, K) are currently being integrated. Developed within the teams of the Transform division, Sys-Méthà is used with research projects dealing with the environmental effects of anaerobic digestion carried out by units from other divisions. Research includes evaluating the effect of intermediate energy crops on soil carbon storage (Joint Research Unit for Functional Ecology and Ecotoxicology of Agroecosystems – UMR ECOSYS), comparison of the effect of anaerobic digestion versus direct return of biomass to the soil on CO<sub>2</sub> emission mitigation (Joint Research Unit for Soil, Plant and Atmospheric Interactions – UMR ISPA), effect of anaerobic digestion scenarios on nitrate leaching (Joint Research Unit for Soil, Agro-hydro systems and Spatial Modelling – UMR SAS). Sys-Méthà is therefore both a collaboration tool between Transform and the AgroEnv and AQUA divisions, and a tool for transferring the knowledge developed by Transform on these sectors to these divisions.



©Éric Dumont IMT, Nantes

# Modelling biotrickling filters for the removal of gaseous ammonia emissions from livestock facilities



## Read more

Dumont E. *et al.*

Biotrickling filters for the removal of gaseous ammonia emissions from livestock facilities. Theoretical prediction of removal efficiency and experimental validation

Chemical Engineering Journal . 2020

<https://doi.org/10.1016/j.cej.2020.126188>

## Value creation

Thesis research and participation of TC'Innov, an SME in Nantes, France, in the project (PROTEGE) for technology transfer are currently under way.

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## Context

Ammonia ( $\text{NH}_3$ ) is a gaseous pollutant produced by management of livestock waste. It is produced by animal urine conversion, which occurs by the conversion of urea by the enzyme urease into ammonia. This ammonia is also transferred to the gas phase depending of pH. Since 2017, emission levels associated with best available techniques (BATs) have been adopted in order to limit ammonia emissions from livestock farms. Farmers have four years from BAT publication to regulate these emissions. These constrains concern 3,300 farms in France, more than half of which are in Brittany. Treatment of exhaust air from buildings is now a necessity to limit ammonia emission. Aerobic bioscrubbing may be an interesting technology; however, its management is still at the empirical stage.

## Results

The maximum removal efficiency of a biological bioscrubber treating ammonia emissions from a livestock building has been theoretically predicted for the first time. Modelling work and experimental validation have shown that ammonia treatment with aerobic bioscrubbers can be a good option, but only if the water

quality in terms of pH and ionic strength remains controlled. On the one hand, pH should be close to neutral to promote both the transfer of ammonia and the biological process of nitrification, and on the other hand, the conductivity of the washing liquid should be controlled by regular draining and replacement of the water. Prediction of maximum removal efficiency can be achieved by simply measuring the properties of the washing liquid, either online or from samples, and can be used as a diagnostic tool to identify possible system failure.

## Future outlook

Combining an aerobic bioscrubber to treat ammonia ( $\text{NH}_3$ ) with an anaerobic bioscrubber to treat hydrogen sulphide ( $\text{H}_2\text{S}$ ) in the biogas generated by the anaerobic digestion of livestock effluents could allow the elimination of these two major pollutants without external inputs to the farm. Coupling these technologies is currently on going.





©Nicolas Bertrand INRAE

## A FoodOn ontology-based solution to integrate nutritional composition data



### Read more

Buche P. *et al.*

How to manage incompleteness of nutritional food sources? : A solution using FoodOn as pivot ontology

International Journal of Agricultural and Environmental Information Systems . 2021

<http://doi.org/10.4018/IJAEIS.20211001.oa4>

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### Context

To assess the nutritional quality of a food product, the first step is to obtain the values of the nutritional components. Food composition databases (FCDBs) managed by individual countries provide such values. Unfortunately, the values of some components of interest may not be available in the FCDB of the country for which the nutritional quality is assessed. Finding values of components of interest for similar foods in other FCDBs is one way to address incomplete data. An additional problem arises because the vocabulary used to describe a food in one FCDB is usually different from that used in others. This research focused on LanguaL and FoodOn. LanguaL is a multilingual thesaurus, used in major national FCDBs to define a food by a set of controlled terms. FoodOn is an ontology that aims to be the open reference vocabulary for food science. An original aspect of this research is to reuse these resources in order to provide a unified access to national FCDBs.

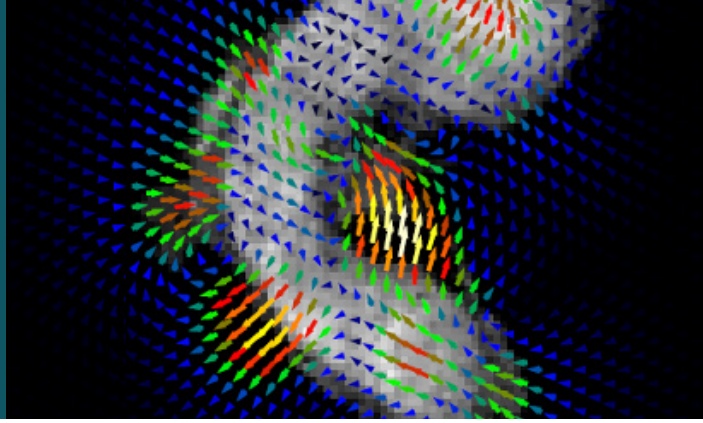
### Results

A new method was developed to automatically find similar foods referenced in different FCDBs. The method calculates a degree of similarity between foods using the FoodOn ontology, the LanguaL

faceted description and the English name of the compared foods. This approach has been implemented in a web application called MultiDB explorer, which integrates several FCDBs. MultiDB explorer was used to overcome the missing values in the ANSES-CIQUAL food composition table for three components of interest (iron, vitamin C, vitamin B<sub>12</sub>) in 76 foods. In all, 91 % of the missing values were determined and 96 % of the known values were completed with values from the US Department of Agriculture (USDA).

### Future outlook

Two extensions of the method are envisaged: i) to use the European FOODEX2 repository instead of LanguaL and ii) to take advantage of the validation of alignments made by experts through an iterative learning process. This work will be developed within the framework of the French national research infrastructure Consumer-Food-Health (CALIS), which brings together certified collective systems for the production, processing and management of data relating to improving food quality, characterising the nutritional properties of foods, and the impact of foods on human health.



©Florent Grélard - Illustration of the deformations applied to a grain section observed in MALDI to superimpose it over its counterpart observed in MRI. The colour of the arrows reflects the intensity of the displacement (weak in blue, moderate in green, strong in yellow and red).

## Coupling several imaging methods improves agronomic product characterisation



### Read more

Grélard F. *et al.*  
Esmraldi: efficient methods for the fusion of mass spectrometry and magnetic resonance images  
BMC Bioinformatics . 2021  
<https://doi.org/10.1186/s12859-020-03954-z>

### Value creation

The set of methods developed is distributed in the form of a library in Python language and is available from the GitHub development platform. It has been integrated into a 'compute capsule' to facilitate analysis reproducibility.

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### Context

Water and polysaccharides in cell walls are two components that have a strong impact on the technological or usage properties of agronomic products, especially cereals. Different imaging methods are able to characterise these two components: mass spectrometry imaging (MSI) can map the chemical composition of samples without any preconceived notions of the molecules to be analysed, and magnetic resonance imaging (MRI), a non-destructive method, reveals the anatomy of the organs and the state of hydration (content/mobility). To get a complete picture of the sample, researchers must be able to compare and merge the information obtained from these two methods. The difficulties concern the difference in image resolution, and the deformations caused by MSI during sample preparation.

### Results

We developed a complete image processing workflow to merge the information obtained from both imaging methods. The workflow includes method-specific preprocessing steps, image registration to spatially match information, and dimension reduction to simplify the data. Spatialised information can be superimposed to match the chemical composition and mobility of the water and thus to

understand, without any preconceived ideas, the molecules whose location correlates best with that of the water.

When applied to wheat grains at different stages of development, the method can be used to correlate the nature of hydration to the degree of substitution and acetylation of non-cellulosic polysaccharides present in the grain walls. In particular, the co-location of the most substituted and/or highly acetylated xylans in the most hydrated regions suggests a higher porosity of the walls related to the xylan modification. These two structural elements have been described as having an impact on wall organisation and polysaccharide interactions in the walls; however, to our knowledge this is the first clear evidence of this correlation at the plant level.

### Future outlook

After having validated the approach on 2D sections, we are now studying the joint variations in composition and water content/mobility at the 3D grain scale, at different stages of development. We are also exploring multi-block methods for analysing data to take advantage of the abundant information.



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# Classification of oligosaccharides using molecular networks based on ion mobility



## Read more

Ollivier S. *et al.*

Molecular networking of high-resolution tandem ion mobility spectra: A structurally relevant way of organizing data in glycomics?

Analytical Chemistry . 2021

<https://doi.org/10.1021/acs.analchem.1c01244>

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## Context

Carbohydrates are one of the most important chemical classes in the living world. They also have applications in various fields, such as biofuels or the food, pharmaceutical and cosmetic industries. However, minor chemical variations can have a major impact on their properties. While there is currently no satisfactory approach to finely characterise polysaccharides, particularly in complex extracts, mass spectrometry (MS) has emerged as a powerful tool for the structural analysis of other types of biopolymers, notably proteins. However, the methods cannot be directly transposed to carbohydrates, which contain many structural elements that do not affect their mass (a phenomenon known as isomerism). As a result, these isomers are invisible to MS – which detects only mass. Today, technological advances are making it possible to start lifting this analytical barrier. More specifically, molecules can be separated according to their 3D conformations by combining ion mobility spectrometry with MS (IM-MS).

## Results

We developed a method exploiting the latest advances in IM-MS to identify structural groups of oligosaccharides. This method is inspired by a strategy used in

metabolomics, called molecular networking. Classically, the strategy uses tandem mass spectrometry (MS/MS): the molecule of interest is isolated according to its mass and then broken down into fragments – akin to a structural signature of the molecule. A bioinformatics comparison allows similar spectra (and thus related structures) to be linked in the network. Unfortunately, this approach is not very informative for oligosaccharides because of the above-mentioned isomeric situations.

Our approach allows molecular networks to be constructed in which the mass of the fragments is replaced by their ion mobility. We tested this approach on several dozen oligosaccharides, representative of the carbohydrates found in plants. The constructed network proved to be superior in grouping oligosaccharides according to informative structural characteristics, chiefly related to their backbone.

## Future outlook

This new method should simplify the analysis of carbohydrates in biological media by grouping similar species and making the data less complex. Future research will improve the approach by making maximum use of the two dimensions available in the data, namely fragment mass and mobility.

PROBE





©INRAE FRISE

# How can machine learning help to detect and assess the impact of cold chain breaks?



## Read more

Loisel J. *et al.*  
Cold chain break detection and analysis: Can machine learning help?  
Trends in Food Science & Technology . 2021  
<https://doi.org/10.1016/j.tifs.2021.03.052>

## Partnerships

- UMR MIA (dpt NUM)
- Biotraq

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## Context

The temperature of every link in the cold chain must be controlled to ensure food product quality. However, field studies carried out by FRISE with temperature sensors placed in products have shown cold chain breaks. Such breaks, even for a short time, can affect food product quality. Cold chain breaks can occur when products are left on a loading bay or when a refrigeration unit malfunctions. Developing tools based on machine learning to detect and evaluate in real time the conditions in which these breaks occur (temperature, duration, etc.) and their impact on quality is thus an important issue for reducing losses and waste.

## Results

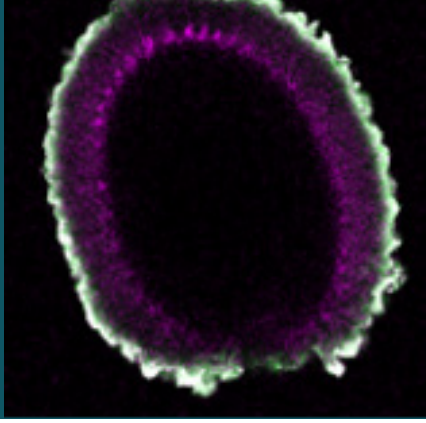
This research highlighted the variety of temperature data sources available in the cold chain that are needed for machine learning. Both experimental data (field, laboratory) and synthetic data generated from thermal models based on physical laws can be used. While machine learning methods have been used to predict temperature, they are not used to detect breaks in the cold chain. Methods for detecting anomalies in time series are already applied in other areas. Applying

these methods to the cold chain would be a step forward, on the one hand, to better understand the characteristics of these breaks, and on the other hand, to alert operators when it happens and thus reduce losses and waste.

## Future outlook

The impact of the different sources of temperature data used for training (experimental or synthetic) on the performance of the training models will be studied. Indeed, experimental data are more accurate but also expensive to generate while synthetic data are very quick to generate but have a degree of uncertainty due to the assumptions of the thermal models used. The objective is to evaluate and compare the performance of learning models trained either with synthetic data or experimental data. Finally, a case study of the use of anomaly detection methods applied to the cold chain will be carried out.





©Adeline Berger - Optical section through an *Arabidopsis* seed showing its mucilage halo composed of rhamnogalacturonan-I (green immunolabeling) and cellulose (Direct Red 23, magenta staining) obtained by confocal microscopy.

# Properties and diversity of *Arabidopsis thaliana* seed seminal mucilage



## Read more

Cambert M. *et al.*

Datasets of seed mucilage traits for *Arabidopsis thaliana* natural accessions with atypical outer mucilage

Scientific Data . 2021

<https://doi.org/10.1038/s41597-021-00857-3>

## Value creation

- **dataset 1. Portail Data INRAE** <https://doi.org/10.15454/1MZ1ZC> (2021).
- **dataset 2. Portail Data INRAE** <https://doi.org/10.15454/EYABB2> (2021).
- **dataset 3. Portail Data INRAE** <https://doi.org/10.15454/LBUN4X> (2021).

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## Context

Models predicting the effects of climate change on ecosystems require data on how adaptive plant traits are affected. To accelerate the dissemination of knowledge and facilitate innovation in this field, open science is a strategic tool. With this objective in mind, 187,490 data points from 20 natural variants of the model plant *Arabidopsis thaliana* were made available to the scientific community and described in a data paper summarising four years of work in the framework of the CEMMU project funded by the French National Research Agency (ANR). The aim of this project was to study the effect of temperature changes on an adaptive seed trait: the production of mucilage (a polysaccharide hydrogel) that forms upon imbibition of seeds in water. Various hypotheses have been put forward to explain the adaptive advantage of mucilage production, including its role in maintaining seed viability. In *Arabidopsis*, the mucilage consists of two structurally distinct layers, a water-extractable outer layer and an inner layer that adheres to the seed. These structural differences suggest that the two layers play different roles in seed physiology.

## Results

The CEMMU project used a transdisciplinary approach to produce three databases describing the

quantity, composition, structure and hydration rate of the inner mucilage layer for 20 natural *Arabidopsis* variants from various geographical locations in Europe and Central Asia. These variants were selected on the basis of atypical macromolecular characteristics of the outer mucilage. The data, which cover 33 traits measured on four biological replicates, have been described in a data paper and are now available to the scientific community on the Data INRAE data repository. These were derived from histological, biochemical and NMR relaxometry analyses. Being able to share and reuse the data should make it possible to explore different avenues that may explain the adaptive role of seed mucilage and the impact of variability on the ability of seeds to adapt to their natural environment.

## Future outlook

A first round of data exploitation (NMR and chemometrics) allowed us to make initial hypotheses on the impact of the natural variability of the internal mucilage on the hydration rates of the internal seed tissues. A statistical analysis of the potential relationships between the different characteristics measured is underway to confirm our hypotheses.

## Integrating knowledge and creating sustainable processes



To support climate and food transitions, researchers are optimising production and transformation processes to make them lean, efficient, flexible and robust. Expertise on exploiting natural biocatalysts such as enzymes is an undeniable asset for developing sustainable processes.

**Page 22:** Developing optimised and sustainable processes

**Page 28:** Using biocatalysis to transform resources into products



©Éric beaumont, INRAE - Pilot evaporator of the dairy platform at the Joint Research Centre for Science and Technology of Milk and Eggs (UMR STLO) in Rennes .

# A methodological framework to design eco-friendly food product processes



## Read more

Azzaro-Pantel C. *et al.*

Development of an ecodesign framework for food manufacturing including process flowsheeting and multiple-criteria decision-making: application to milk evaporation

Food and bioproducts processing 2021

<https://doi.org/10.1016/j.fbp.2021.10.003>

## Partnerships

- Chemical Engineering Laboratory (LGC) from Toulouse

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## Context

The food industry must make its processes more environmentally friendly. Systemic eco-friendly design approaches, developed for chemical and petrochemical processes, combine modelling and multi-objective optimisation, which makes it possible to obtain compromise solutions where several criteria are considered simultaneously. Applying these approaches to agrifood processes is hampered not only by a lack of knowledge of the impacts of the process on food product characteristics (and vice versa) but also by the lack of process modelling. In collaboration with the Chemical Engineering Laboratory in Toulouse, we developed a methodological framework for the multi-objective eco-friendly design of food processes.

## Results

The framework combines process description (using a commercial simulation tool) and multi-objective optimisation with life cycle analysis and cost assessment tools, all integrated in a multi-criteria decision support tool. The framework developed is illustrated by the example of milk evaporation, one of the most energy-intensive processes in the dairy industry, which has enormous potential for optimisation.

The process simulator was first adapted to model skim milk evaporators.

The economic and environmental criteria were then calculated from the inventories of the production and cleaning phases. Finally, we applied multi-objective optimisation (with a genetic algorithm) and multi-criteria decision making, separately or combined.

The engineering process of combining several tools to deal with a complex problem is in and of itself an outcome of this work. The framework's potential was first demonstrated through an analysis of three evaporator design solutions, and then as a support for the strategic choice of a fuel for the purpose of on-site energy production. Different fuel sources (oil, gas, wood chips) were compared, and on the basis of potentially conflicting objectives, rational choices were made using the tools and methods implemented in the methodological framework.

## Future outlook

This research paves the way for the development of eco-friendly designed approaches for agrifood processes and the construction of a digital platform to meet such demands. There are many possible avenues: integration in upstream and downstream operations to assess the drying process in its entirety, integration of the treatment of rejected products and waste, consideration of the quality criteria of the products obtained, among others.



© Eric Beaumont INRAE Rennes  
Membrane céramique de microfiltration pour la séparation des protéines du lait, protéines du lactosérum à gauche et micelles de caséines à droite.

## Integrating expert knowledge in multi-objective optimisation of processes



### Read more

Belna M. *et al.*

Formulating multiobjective optimization of 0.1  $\mu\text{m}$  microfiltration of skim milk

Food and bioproducts processing . 2020

<https://doi.org/10.1016/j.fbp.2020.09.002>

### Partnerships

This study was carried out as part of the OPTIMAL project (2017–2020), supported by a grant from the Brittany Region (contract no. 16006734, INRAE grant agreement 30001292) and the European Regional Development Fund (ERDF contract no. EU000171, INRAE grant agreement 30001293).

This research is the result of a collaboration between:

- Boccard
- USC I2M
- UMR STLO

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### Context

The optimisation of food processes is a complex task made difficult by i) the lack of knowledge about the mechanisms that limit process performance; ii) the many heterogeneous variables involved in prediction models, and iii) the inherent complexity of the food products themselves. A typical example is the microfiltration of skim milk, which uses a membrane with a pore size of 0.1  $\mu\text{m}$ . Microfiltration is commonly employed as a unit operation to separate the two main milk protein groups into fractions useful for cheesemaking and food formulation.

However, despite its importance in the dairy industry, this process has never been optimised due to conflicting stakeholder objectives: maximising the quality of the output products (retentate and permeate) while minimising input costs and considering environmental impacts.

### Results

To overcome these difficulties, the original approach of this work involved combining multi-objective optimisation with knowledge integration. Scientific and expert knowledge was collected and structured to model the multi-objective problem of skim milk microfiltration optimisation.

The microfiltration optimisation took into account five conflicting objectives specified by the stakeholders and integrated expert and scientific knowledge on output quality, roles of operational variables, process design and costs in formulating the multi-objective problem. The relationships of influence between the process variables and the five conflicting objectives were determined, the mathematical functions representing the objectives were defined, and the system was optimised using a metaheuristic.

More than a thousand Pareto optimal solutions were found, including solutions similar to current industrial practices but also new and innovative solutions.

### Future outlook

This approach opens up new possibilities for optimising microfiltration and, more generally, food processes for which the lack of scientific knowledge and data is an obstacle to modelling the mechanisms involved. The approach could be expanded to better account for design rules, environmental impacts (based on the results of life cycle assessments) or to integrate operations upstream and downstream of the operation studied.





©Christelle Labruyère - pilote de méthanation biologique au sein de l'équipe SYMBIOSE de TBI

# Modelling the interaction between hydrodynamics: hydrogen transfer and bioreaction in a biological methanation bubble column



## Read more

Rafrafi Y. *et al.*

Biological methanation of H<sub>2</sub> and CO<sub>2</sub> with mixed cultures: current advances, hurdles and challenges

Waste and Biomass Valorization . 2021

<https://doi.org/10.1007/s12649-020-01283-z>

## Value creation

Patent: METHABIO FR1903721

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## Context

Among the various methods of reducing CO<sub>2</sub> emissions, its direct capture by microorganisms is one that shows promise. Many types of microorganisms (bacteria and archaea) have the capacity to develop by transforming carbon dioxide into the organic molecules they need to grow or for their energy needs. These microbial metabolisms enabled CO<sub>2</sub> fixation when life began and could potentially be reused in what is now known as the Anthropocene epoch.

One of the first challenges that autotrophs can help us address is the replacement of fossil methane (also known as 'natural' gas) with gas produced from renewable resources. The methanation of CO<sub>2</sub> is, therefore, a promising reaction that is the subject of various research studies.

## Results

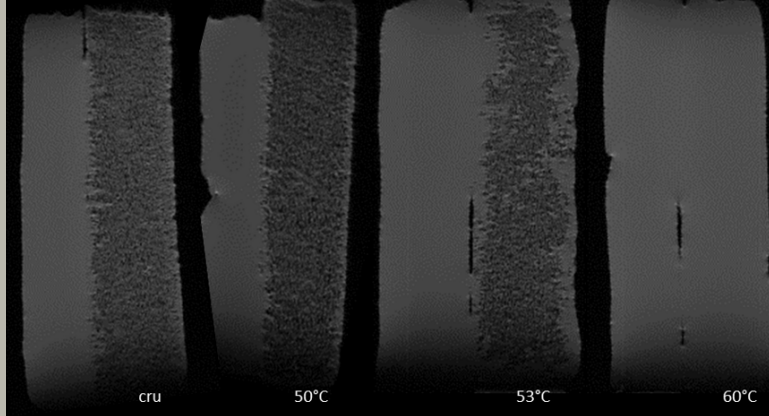
The SYMBIOSE team is working on the biological reduction of CO<sub>2</sub> to CH<sub>4</sub> using renewable hydrogen. An ex-situ methanation reactor (a 20 L bubble column) was developed and showed that biogas could be enriched to 97 % CH<sub>4</sub> content. The development of an innovative biological reactor allowed certain limitations to be overcome and to generate high percentages of CH<sub>4</sub> and noteworthy productivity levels. The development was the subject of a joint

INSA-ENOSIS patent. In collaboration with TBI's Transfer-Interface-Mix (TIM) team, we described, at microscopic scale, gas-liquid and liquid-biological transfer phenomena with multi-physics simulation tools and formalised the results in terms of efficiency, such as for heterogeneous catalysis. A reduced flow-based model describing bioreactions specific to methanation was developed. Experimental data were compared and the model was validated and the parameters of the multi-scale model were calibrated. This analysis between scales and model/experiment allowed us to use these biological models with multi-species transfers for larger scale applications, for example for optimising bioreactor design and predicting the performance of industrial bioreactors.

## Future outlook

These advances are crucial, not least in the development of the INSA GRDF chair and the SOLIDIA platform, which will be hosting projects developing industrial biological and catalytic methanation pilot units. Discussions are under way with manufacturers on modelling the change of scale that would be needed to develop pilot units on a semi-industrial scale.





©Sylvie Clerjon - High-resolution MRI images (0.1 x 0.1 x 0.5 mm<sup>3</sup>) of parenchyma of golden apple, raw and cooked for 10 minutes at 50 °C and 53 °C and 14 minutes at 60 °C

## Better understanding the structural changes in apples during cooking



### Read more

Leca A. *et al.*

Multiscale NMR analysis of the degradation of apple structure due to thermal treatment

Journal of Food Engineering . 2021

<https://doi.org/10.1016/j.jfoodeng.2020.110413>

### Partnerships

- UR QuaPA

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### Context

Heating fruit alters the texture perceived by consumers and the bioavailability of micronutrients. Apples are the most processed fruit in the world. They mainly undergo thermal processing, which is associated with three major issues: i) changes in texture perceived by consumers, ii) changes in bioavailability of nutrients and iii) high energy consumption. To control the final organoleptic and nutritional qualities of apples and limit energy consumption caused by processing, it is important to fully understand the underlying mechanisms in the parenchyma during cooking.

Texture analysis, the standard technique for determining the mechanical properties of fruit tissues following heat treatment, provides information at the tissue level. Nuclear magnetic resonance (NMR) is known for its ability to probe the interactions between water and macromolecules in tissues. These interactions indirectly inform us about the state of the structure at the cellular level.

### Results

Both methods converge at the pivotal temperature of 53 °C at which the cell structure of the apple

parenchyma breaks down and changes from a 'raw' to a 'cooked' state. The mechanism of texture loss is thus illustrated simultaneously at two levels. At the tissue level, the firmness of the parenchyma decreases sharply from 53 °C. At the molecular scale, NMR spectroscopy shows a unification of the T2 relaxation time, illustrating homogenisation of the interactions between the vacuolar water and the macromolecules of the structure, which is explained by the thermal degradation of the membranes. This is confirmed by NMR imaging with the filling of the pores with vacuolar fluid from 53 °C.

### Future outlook

The aim is to use these data to build deterministic models of apple processing and to optimise the heat treatment in order to control the texture of the finished product.

To achieve more accuracy in structural change determination, a longitudinal follow-up study with real-time cooking in the NMR spectrometer is planned. The same sample will be cooked and analysed by NMR throughout the process.





Dircom Cnam ©Sandrine Villain  
- Determination of the oxygen transfer coefficient in a bioreactor

## New economical and low-pollution method for the production of pure hydroperoxides



### Read more

Crouvisier Urion K. *et al.*

Optimization of pure linoleic acid 13-HPX production by enzymatic reaction pathway: Unravelling oxygen transfer role

Chemical Engineering Journal . 2022

<https://doi.org/10.1016/j.cej.2021.132978>

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### Context

Polyunsaturated fatty acid hydroperoxides are the first products of the cascade of fatty-acid oxidative degradation reactions. As such, they are frequently used in studies that seek to gain an understanding of the mechanisms and kinetics of lipid oxidation in general and in studies of the cytotoxicity of lipid oxidation products. Their production by chemical synthesis (an existing industrial process) does not allow pure isomers to be obtained and requires expensive purification steps at a later stage. Enzymatic production does not have these drawbacks because of the specificity of the enzyme. It does, however, involve controlling the transfer of oxygen, an essential substrate in the oxidation reaction, from the gas phase to the liquid phase throughout the catalysis step.

### Results

The use of soybean lipoxygenase-1 guides the reaction to the production of a pure isomer, linoleic acid 13-hydroperoxide (13-HPX). One condition that is often overlooked involves ensuring a gas-liquid oxygen transfer that is sufficient to prevent the reaction from moving towards an anaerobic pathway leading to the formation of by-products. By taking into account the oxygen transfer, we were able to adjust the reaction kinetics and the transfer kinetics between them and

to ensure a yield of nearly 100 % when converting linoleic acid to 13-HPX. In comparison with the isomers available on the market, this process results in a product of higher purity and thus a product that is more stable during storage. Furthermore, by taking into account the oxygen transfer we were able to correct the reaction kinetics and acquire a more accurate estimate of the enzyme kinetic rate constants.

The obtaining of this pure hydroperoxide has already had one significant impact: it is no longer necessary to obtain commercially available hydroperoxides, which are either not pure enough or very expensive. This makes it easier to conduct studies on lipid oxidation.

### Future outlook

Oxygen transfer can be further enhanced by designing microstructured reactors where the interfacial exchange area between gas and liquid can be significantly improved, which would allow the process to be intensified. The use of other polyunsaturated fatty acids and/or other enzymes could also expand the range of useful molecules for production.



©Nada Chami - Appearance of a mixed CP/CO<sub>2</sub> hydrate slurry

# Thermodynamic characterisation of cyclopentane slurry for secondary refrigeration



## Read more

Chami N. *et al.*

Thermodynamic characterization of mixed gas hydrates in the presence of cyclopentane as guest molecule for an application in secondary refrigeration  
Chemical Engineering Science . 2021

<https://doi.org/10.1016/j.ces.2021.116790>

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## Context

High-power refrigeration systems use large quantities of refrigerants, which are regarded as greenhouse gases. Secondary refrigeration can restrict the use of these fluids and confine them to cold production, as it involves using 'secondary refrigerant' fluids with a low environmental impact, such as water, to transport cold where it is needed. To increase the efficiency of these systems, two-phase refrigerant fluids called slurries and containing crystals suspended in a transport liquid can be used. These are high-energy density fluids due to the latent heat of the crystals. These slurries are capable of storing and transporting large quantities of cold. Ice slurries have already been developed for applications requiring temperatures below 5 °C. To extend the range of application to higher temperatures, a new slurry has been studied: a mixed cyclopentane CP/CO<sub>2</sub> hydrate slurry. In order to implement this mixed hydrate, its thermodynamic properties must be characterised and controlled.

## Results

Thanks to the study, the thermodynamic equilibrium of mixed CP/CO<sub>2</sub> hydrates was defined. The

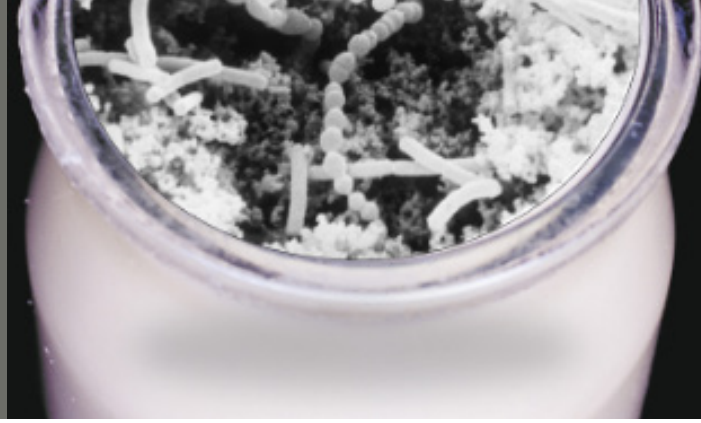
equilibrium points were measured using a calorimetry technique called Micro-DSC. They measured the dissociation enthalpy of mixed hydrates between 0 and 4 MPa. The enthalpy of the mixed CP/CO<sub>2</sub> hydrate is superior to that of water (e.g.,  $\Delta H = 462.5 \pm 1.5 \text{ kJ} \cdot \text{kg}^{-1}$  of water at an equilibrium pressure of 0.25 MPa). The specific heat capacity of the mixed CP/CO<sub>2</sub> hydrates was also determined from the results obtained by MicroDSC and from a heat balance calculation of all system components. The value obtained in the temperature range [277.9–281.2] K is ( $C_{p_H} = 1479.09 \pm 137.43 \text{ J} \cdot \text{K}^{-1} \cdot \text{kg}^{-1}$  of hydrate at 0.25 MPa).

## Future outlook

Rheological characterisation was undertaken to obtain a rheological model of mixed CP/CO<sub>2</sub> hydrates and simple cyclopentane hydrates. A particle-size characterization of mixed CP/CO<sub>2</sub> hydrates was also programmed using two particle-size analysis techniques: FBRM and PVM.

Thermal characterisation will be conducted both experimentally and digitally to predict the thermal behaviour of the slurry when it flows.





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Micheline

# Promoting positive interactions between lactic acid bacteria: towards new mixed dairy and plant-based food applications



## Read more

Canon F. *et al.*

Positive interactions between lactic acid bacteria promoted by nitrogen-based nutritional dependencies

Applied and Environmental Microbiology  
. 2021

<https://doi.org/10.1128/AEM.01055-21>

## Partnerships

• UMR CSGA

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## Context

The transition towards more sustainable food systems poses a societal, economic and technological challenge that involves changing our food habits by diversifying protein resources. New mixed foods, such as dairy and plant-based combinations, have their place in this transition. To improve the taste, nutrition and health benefits of these mixes, lactic fermentation can act as a driver, through well-thought-out lactic acid bacteria (LAB) co-cultures.

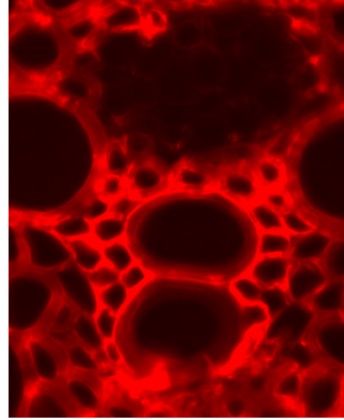
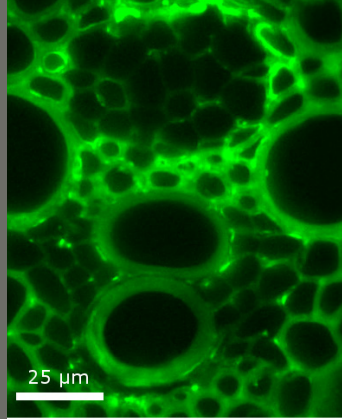
## Results

We developed a strategy for creating LAB co-cultures capable of fermenting hybrids based on milk and lupin proteins. The strategy involves three stages: i) *in silico* selection of LAB species capable of hydrolysing targeted sugars; ii) among these species, *in vitro* screening of 97 strains for their ability to ferment these sugars and to hydrolyse milk and lupin proteins; iii) combine some of these strains on the basis of the complementarity of their phenotypes. This strategy has proven to be successful in terms of sugar consumption, protein hydrolysis and aroma compound profile. It has also revealed the existence of positive interactions between strains. These interactions could potentially be

boosted by nutritional dependencies linked in particular to the nitrogen metabolism of LAB. We exploited the complementarity of LAB strains in terms of their nitrogen nutrition. We combined proteolytic strains (prot<sup>+</sup>) capable of providing peptides and free amino acids with non-proteolytic strains (prot<sup>-</sup>) in a chemically defined medium containing milk and lupin proteins. Prot<sup>+</sup> strains were shown to stimulate prot<sup>-</sup> strains in different ways, depending on the concentration and nature of the peptides and amino acids produced, particularly branched-chain ones, which are essential for the growth of lactic acid bacteria. Strong, medium or no interactions were thus observed, leading to improved functionality in the case of strong interactions.

## Future outlook

This research is continuing, the aim being to better understand the interactions between LAB by identifying the peptides produced by prot<sup>+</sup> strains and those used by prot<sup>-</sup> strains and to determine if the same interactions occur in yogurt-type foods. This study opens up ways of creating innovative fermented foods.



©INRAE - Cédric Montanier  
Activity on wheat straw

## Modulation of the spatial conformation of multi-domain glycoside hydrolases and the effect on catalysis



### Read more

Badruna L. *et al.*

The Jo-In protein welding system is a relevant tool to create CBM-containing plant cell wall degrading enzymes

New Biotechnology . 2021

<https://doi.org/10.1016/j.nbt.2021.07.004>

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### Context

Lignocellulolytic bacteria have developed an entire arsenal of enzymes that allow them to break down plant biomass and metabolise the carbon contained in plant cell wall polysaccharides. These enzymes are often organised into different domains, including catalytic domains and domains linked specifically to the target motif. While the individual domains of these enzymes are generally well characterised in vitro on synthetic soluble substrates, they are rarely characterised in their natural, dense and complex environment, and even less so in their entirety. The spatial conformation that these various domains can adopt during catalysis is not therefore taken into account. In response, we developed an approach enabling us to do this.

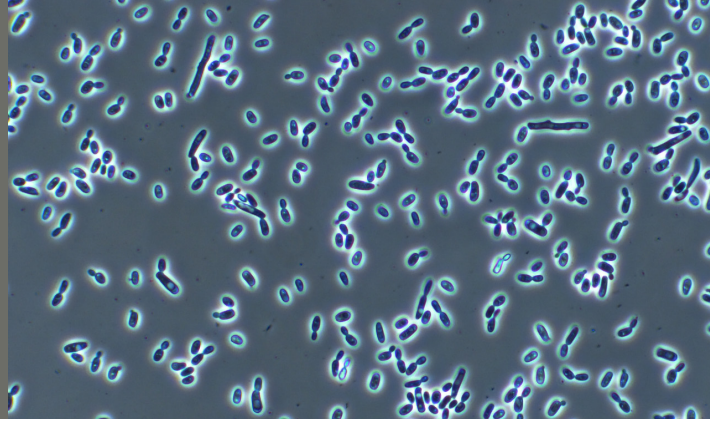
### Results

Our original approach involved using Jo and In, two proteins with a covalent and spontaneous association. The Jo-In complex allowed us to restrict the spatial geometry between two domains: a xylanase and a carbohydrate-binding module (CBM). A number of assemblies were created. We dissolved the models of these multi-domain proteins in solution through small angle X-ray scattering (SAXS), which allowed us to characterise

the position and distance between the two domains. We then studied the biological activity of the various domains on simple and soluble substrates or natural and insoluble ones, such as straw or wheat bran. The results show that our multi-domain enzymes do exhibit a spatial geometry dependent on the way in which the domains are assembled. The rigidity of the assembly caused by the Jo-In complex prevents any physical interference between the domains. Furthermore, the torsion angle formed between the two domains can be played with, depending on the position of Jo and In. We showed that this difference in topology between xylanase and CBM had an impact on enzyme activity in plant cell walls.

### Future outlook

The know-how we have developed now allows us to create enzyme assemblies tailored to the need. This tool also allows us to address the question of the impact of the organisation and spatial proximity of these assemblies in biological processes. We would like to implement these systems in metabolic pathways and, in broader terms, apply them to the synthesis of molecules of interest and the development of new polymers.



©INSA - *Yarrowia lipolytica*

## Control of the filament formation of *Yarrowia lipolytica* by managing the fermentation process



### Read more

Lesage J. *et al.*

Accelerostat study in conventional and microfluidic bioreactors to assess the key role of residual glucose in the dimorphic transition of *Yarrowia lipolytica* in response to environmental stimuli

New Biotechnology . 2021

<https://doi.org/10.1016/j.nbt.2021.05.004>

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### Context

Interest in the yeast *Yarrowia lipolytica* is growing due to its ability to use diverse carbon sources and produce a wide range of useful metabolites (lipids, proteins, organic acids, aromas). However, in response to fluctuations in the environment, *Y. lipolytica* triggers dimorphic transitions that can hamper bioprocesses (loss of performance, limitation of transfers, impact on the rheology of media). Until now, the onset of filament formation has been described as being induced by stress conditions and dependent on the strain in use. Our previous research has shown that the response of *Y. lipolytica* to stress conditions (pH, dissolved oxygen) was dependent on the culture mode and has allowed us to hypothesise a relationship between the concentration of residual sugar (i.e., an excess in batch mode and a shortfall in continuous mode) and the induction of filament formation in the presence of these stresses. The possibility of controlling filament formation by managing the bioprocess would aid the development of biotechnological processes with the yeast *Y. lipolytica*.

### Results

*Y. lipolytica* cells were cultured in a bioreactor using specific culture techniques (accelerostat) to enable a progressive and controlled increase in residual glucose concentration. The results showed that even under stress

conditions *Y. lipolytica* maintained an ovoid morphology when residual glucose concentration was below a threshold value of around 0.35 mg L<sup>-1</sup>. Transitions towards more elongated forms were triggered at this threshold and progressively intensified as residual glucose levels increased. We explored the mechanism for regulating the dimorphic transition in conjunction with the residual sugar concentration and cyclic AMP (cAMP) concentration – the signal molecule – involved in yeast stress response cascades. The effect of cAMP on dimorphic transition was assessed by the addition of cAMP and the quantification of its intracellular levels during the progressive increase in residual glucose concentration. We demonstrated that the exogenous addition of cAMP inhibited the mycelial morphology of *Y. lipolytica* even with glucose concentrations exceeding the threshold level of 0.35 mg L<sup>-1</sup>. The results suggest that dimorphic responses in *Y. lipolytica* are regulated by sugar-signalling pathways, most likely via the cAMP-PKA dependent pathway, for which cAMP has been described as an activator.

### Future outlook

Transcriptome studies should provide us with a better understanding of the regulation mechanisms involved.



©INRAE - Loïc Foucat et Estelle Bonnin - Spectromètre RMN bas champ

# The behaviour of pectin-degrading enzymes in dense media



## Read more

LI F. *et al.*

Effect of solid loading on the behaviour of pectin-degrading enzymes

Biotechnology for Biofuels . 2021

<https://doi.org/10.1186/s13068-021-01957-3>

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## Context

Pectin impacts plant biomass recalcitrance by affecting the ability of other cell wall components to access enzyme degradation. Their removal thus has a positive effect on the saccharification of pectin-rich biomass. Furthermore, the development of environmentally-friendly processes involves reducing water consumption and using high-solids loadings. The aim of this research is to thus study the behaviour of different pectin-degrading enzymes in the presence of a low (5 %) to high (35 %) solid citrus-peel loading. Two enzyme conditions were studied, with a pectin lyase being compared with a mixture of endopolygalacturonase and pectin methylesterase.

## Results

A high solid citrus-peel loading affects the solubilisation of pectin differently, depending on the enzyme used. The pectin lyase causes increased breakdown of the solid substrate and is less sensitive to a reduction in water content than the mixture of endopolygalacturonase and pectin methylesterase, with or without a processive mode of action. In conjunction with enzyme degradation, low-field NMR experiments showed that the solid loading clearly affects

water mobility and that these changes in mobility vary according to the enzyme used. Pectin lyase caused less significant changes than the mixture of endopolygalacturonase and pectin methylesterase.

At similar concentrations of pectin, pectin solutions inhibit the diffusion of polygalacturonase more than the solid substrate. This could be attributed to the high viscosity of highly concentrated pectin solutions, while the pores present in the solid substrate provide continuous diffusion pathways.

## Future outlook

Although pectin structure varies according to the plant source, the results obtained here can be extrapolated to other co-products, as the pectin motifs recognised by the enzymes are always present. This new information is useful in the biorefinery of pectin-rich plant materials when enzymes are used in the treatment.

PROBE





©Cyril Marchand

## Exploration of the diversity of fungal enzymes in mangrove soils



### Read more

Ben Ayed A. *et al.*

EE Exploring the diversity of fungal DyPs in mangrove soils to produce and characterize novel biocatalysts

Journal of Fungi . 2021

<https://doi.org/10.3390/jof7050321>

### Partnerships

- Ecosphère Continentale
- Côtière- MicrobieN)
- Universities of New Caledonia, Clermont Auvergne, Lyon, Aix-Marseille, Sfax (Tunisia) and Turin (Italy)
- INRAE
- IRD
- CIB (Spain)

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### Context

Coastal mangroves are fascinating ecosystems, composed mainly of woody plants that grow in extreme environmental conditions such as high salinity and high temperatures. They are considered the largest carbon sink in coastal ecosystems, while mangrove fungi play a vital role in recycling organic matter, especially lignocellulose-rich biomass. Very few studies have been conducted on fungal diversity at a global level, on the enzyme mechanisms used by mangrove fungi to decompose plant biomass, and on how the fungi adapt to marine conditions, especially to high salt concentrations.

### Results

In taking part in a multi-partner project (Ecosphère Continentale et Côtière- MicrobieN), we screened functional diversity (expression of genes encoding fungal dye-decolourising peroxidases [DyPs]) in surface and deep sediments collected beneath *Avicennia marina* or *Rhizophora stylosa* trees during the wet and dry seasons.

The greatest diversity of DyPs was observed during the wet season in surface sediments beneath the area occupied by *R. stylosa* compared to an area planted with *A. marina*. The most commonly identified enzyme in these

soils, Dyp1 (in 70 % of samples), was produced by a genetic engineering technique and was biochemically characterised to provide a better understanding of its origin. We showed that this enzyme is functional and possesses properties comparable to those of its terrestrial counterparts. It also has very interesting industrial dye-decolourising characteristics that are more versatility than two terrestrial DyP counterparts. Nevertheless, its enzyme activity in the presence of sea salt is more similar to that of a terrestrial enzyme, leading us to posit that the organism producing this enzyme came from a terrestrial environment next to the mangrove and ended up colonising the mangrove itself, which provides a buffer between sea and land.

### Future outlook

We want to study the taxonomic and functional diversity of mangroves in further detail by comparing natural mangroves and those affected by anthropogenic factors, by including bacterial and fungal communities and by diversifying the enzyme targets (e.g., ligninases and hemicellulases). In broader terms, the results of our study will contribute to other areas of research, such as the study of carbon recycling in the marine environment and the understanding of microbial communities in a wider sense.

## ✓ Analysing how food is made and perceived



Designing new foods involves acquiring new knowledge about the determining factors of the nutritional and sensory qualities of raw materials and foods. This two-pronged approach is all the more necessary to design foods from plant matter, and thus meet the current challenge of sustainable food. Researchers are also studying how these new foods are perceived by consumers in order to maximise their acceptability.

**Page 34:** Making food production more sustainable

**Page 37:** Developing the nutritional and sensory quality of food

**Page 43:** Food perception and digestion



© INRAE/Guyomarc'h Fanny - Dairy and plant-based protein co-cultures: the potential for pleasant-tasting and textured food that is nutritionally balanced and environmentally sustainable.

# Innovating with assemblies of animal- and plant-based proteins



## Read more

Hinderink E. *et al.*

Combining plant and dairy proteins in food colloid design

Current Opinion in Colloid & Interface Science . 2021

<https://doi.org/10.1016/j.cocis.2021.101507>

Guyomarc'h F. *et al.*

Mixing milk, egg and plant resources to obtain safe and tasty foods with environmental and health benefits

Trends in Food Science & Technology . 2021

<https://doi.org/10.1016/j.tifs.2020.12.010>

## Partnerships

- Wageningen University & Research (WUR)

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## Context

The increasing consumption of animal proteins by populations in developed countries places unsustainable pressure on resources and increases the prevalence of chronic metabolic diseases such as inflammatory bowel disease and cancer. The challenge of replacing animal proteins with plant-based proteins in Western diets involves successfully transitioning towards healthier and more sustainable food. For many consumers, this transition will only be acceptable if they have a choice of food products that are appealing, convenient and affordable. One possible solution is to offer innovative mixed products combining dairy, egg and/or plant-based proteins to consumers who find vegan products unappealing or reject them altogether.

## Results

To make this happen, the animal and plant sectors need information to drive their development and innovation activities forward. Recent research undertaken by the TRANSFORM teams describes interaction mechanisms between animal- and plant-based proteins. Dispersing proteins in the form of coacervates or heat-induced aggregates thus offers an effective technological solution to the instability that plant-based proteins are commonly known for. An intimate

understanding of interactions can also help diversify the texture of food gels, foams and emulsions to enable the use of plant-based proteins in foods that the public is more inclined to accept than purely plant-based foods. These 'mixed' or 'hybrid' foods provide essential amino acids and vitamins that are sometimes lacking in vegan diets. Fermentation further enhances this performance by degrading indigestible or anti-nutritional compounds arising from the plant fraction and by producing pleasant aromas. Hedonic testing has shown that these mixed foods are a lever for improving the extent to which Western consumers accept plant proteins.

## Future outlook

This global investigation of innovative mixed foods, from the molecular scale and manufacturing and functional properties to consumer preferences, seeks to provide a multi-criteria assessment of sensory, economic, health and environmental performance aspects. A benefit-risk analysis would also be useful in better identifying the effects of the various components of animal and plant fractions. This knowledge could open up areas in which the plant and animal sectors could complement rather than replace each other.





© Siddharth Sharan

## Fava beans: a useful legume to add to our diet



### Read more

Sharan S. *et al.*

Fava bean (*Vicia faba L.*) for food applications: From seed to ingredient processing and its effect on functional properties, antinutritional factors, flavor, and color

Comprehensive reviews in Food Science and food safety . 2020

<https://doi.org/10.1111/1541-4337.12687>

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### Context

Consumption of plant-based protein foods is booming. Not only are they beneficial in terms of human health, but they can also support a more sustainable diet. The fava bean (*Vicia faba L.*) is a legume that would appear to be a very promising protein source. It has useful functions to offer in food formulation, particularly foaming, emulsifying and gelling properties. There are, however, several limitations to its use, not least the presence of anti-nutritional factors and the existence of sensory drawbacks. The search for technological pathways for developing ingredients (flour, concentrates or isolates) based on this legume is essential in promoting its functional and nutritional properties, while also controlling its odour, taste and colour and reducing the presence of anti-nutritional factors.

### Results

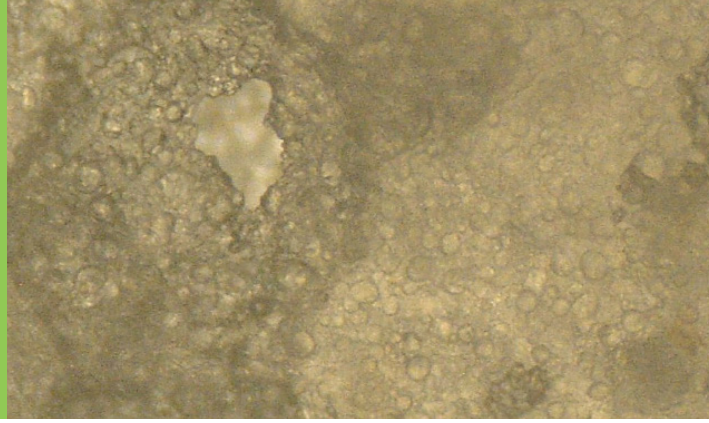
Realistic processing conditions (temperature, pH and treatment duration) were employed on a fava bean concentrate and their impact on functional and olfactory properties were assessed. The results of this research showed that foaming and emulsifying properties are mainly governed by the pH modification, and

the use of these ingredients and can be explained by the properties of proteins. Furthermore, processing conditions can have a significant impact on olfactory perceptions, which range from green, cooked notes to rancid notes, and are due to the presence of combinations of specific volatile compounds. Finally, the physicochemical and sensory properties of the compounds responsible for antioxidant potential, flavours (bitterness and astringency), colour, and anti-nutritional effects were also studied, confirming the significant effect of pH on these ingredients.

### Future outlook

This project has opened the way to new research on the best technology pathways to pursue in proposing low-processed ingredients in a realistic context for industrial development. A multi-faceted approach will need to be adopted to find a satisfactory compromise between useful functions that can be exploited, the limiting of anti-nutritional factors and the presence of olfactory notes or colours that consumers find undesirable.





©Kossigan Bernard Dedej - Continuous mesoscopic observation of a film of dough as it swells into a bubble in a laboratory-designed cell that controls temperature and gas composition; shown here after the film has broken.

# Can controlled functionalisation of flour mixtures with fewer additives be achieved?



## Read more

Grenier D. *et al.*

Gas cell opening in bread dough during baking

Trends in Food Science and Technology 2021

<https://doi.org/10.1016/j.tifs.2021.01.032>

## Partnerships

- UMR IATE, Montpellier for the analysis accompanying the bibliographic review in TIFS and for the mechanical characterisation of gluten during baking (post-doc, Heliciane Clément 2021–2023)
- UR BIA, Nantes and UMR PANTher, Nantes for the multi-scale characterisation of starch grain softening in bread dough (Marie Skłodowska-Curie Postdoctoral Fellowship, Nanci Castanha da Silva 2021–2023)
- The European PRIMA-FBM project (2021–2025) coordinated by INRAE (UR BIA, Nantes).

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## Context

Bread is a staple food in many cultures. Its honeycomb structure and soft and crisp textures are all valued qualities (contributing to 20 % of the overall appreciation). Controlling expansion in baking has relied mainly on the viscoelastic properties of the gluten network (8–12 % of wheat bread flour), while the variability between flour batches is smoothed with technological aids or additives. The approach is to better understand the role of flour constituents other than gluten, such as starch, in order to attempt a functionalisation using flour blends to achieve the desired expansion. The baking stage can no longer be neglected as previously, as it determines fundamental changes in the properties of the starch.

## Results

Analytical work revisited the stabilising/destabilising functions of the dough walls by the different components in a flour, in particular the starch grains, during rising and baking. Two candidate mechanisms for dough wall stabilisation by starch were investigated: the release of material into the extragranular phase and the softening of starch grains. None of these have been studied under the conditions of moderate hydration of the bread dough. The approach

developed for this research combines micromechanical modelling, which is still underdeveloped in the food industry, and original measurement methods, which make it possible to follow the migration of matter at the scale of starch grains (nuclear magnetic resonance – NMR) or to observe the deformation of a film of dough forming a bubble under a microscope. We drew inspiration from the Alveolab®, which many laboratories in the bread- and pastry-making industry are equipped with, but this time with an atmosphere that recreates the rise in temperature and the gaseous composition of the dough.

## Future outlook

The researchers want to play with the successive arrangement of the stabilising actions of the different ingredients of the dough (glutinous network, then liquid lamella, then granular phase consisting of starch). The objective will be to find combinations of flour blends that allow this series of actions to be obtained for a given gluten quality. This could make it possible to work with flours with a lower gluten content than those currently used and to better control the baking process by adjusting the pressure level in the oven (European PRIMA FLAT-BREAD-MINE project).



©Pixabay - Wernerdetjen

## Innovative processes for to improve meat quality: an international concern



### Read more

Supaphon P. *et al.*  
Structural changes in local Thai beef during sous-vide cooking  
Meat Science . 2021  
<https://doi.org/10.1016/j.meatsci.2021.108442>

### Partnerships

- King Mongkut's Institute of Technology (Thailand),
- Riddet Institute (New Zealand), the German Institute for Food Technology (DIL, Germany),
- University of Melbourne and Charles Sturt University Wagga Wagga (Australia).

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### Context

Currently, 30 % of the beef consumed in Europe comes from cull cows. On other continents, hardy breeds are favoured for their resistance to diseases and hostile environments (climate, parasites, resources). Although these meats have a high nutritional potential, they are quite tough. We have forged partnerships to apply to these fresh meats innovative technological processes (hydrodynamic shock waves, pulsed electric fields, proteolytic action) that are likely to reduce their mechanical strength while preserving their nutritional potential. "Sous vide cooking", known to improve texture, was favoured and structural changes were characterised to understand the mechanisms associated with meat tenderisation.

### Results

The application of hydrodynamic shock waves prior to cooking promotes the solubilisation of intramuscular collagen, whereas pulsed electric fields do not significantly modify the structure but favour the in-vitro digestibility of vacuum-cooked meat. The use of ginger extract, rich in protease, significantly tenderises the meat. The meat is more tender and

there is less juice lost when vacuum cooked at 60 °C (rather than 70 °C or 80 °C). It appears that slow-twitch muscle fibres retract on cooking, causing adjacent, probably fast-twitch, fibres to ripple. The palatable texture of vacuum-cooked meats is thought to be the result of a strong solubilisation of collagen and the gelling of myofibrillar and sarcoplasmic proteins.

### Future outlook

The goal is to make better use of meat from culled or hardy animals and to offer consumers pre-cooked meat with good sensory, sanitary and nutritional quality. We want to better understand the variability of muscle fibre behaviour when heated and the role of processes in meat tenderisation.



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## A new concept to study the nutritional quality of a food



### Read more

Peyron M-A. *et al.*

Deciphering the protein digestion of meat products for the elderly by *in vitro* food oral processing and gastric dynamic digestion, peptidome analysis and modeling

Food & Function . 2021

<https://doi.org/10.1039/d1fo00969a>

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### Context

Ensuring good nutrition in the elderly is a real challenge. The development of new, nutritionally sound foods that are suitable for this specific population is particularly complex. Addressing this challenge requires studies that are more mechanistic, and which include the entire digestive process, under well-controlled conditions, made possible by the design of *in-vitro* devices such as the AM2 masticator and the DIDGI digester. Developing food products that are both nutritious and functional remains a crucial objective. Elderly people need foods with specific textures adapted to their oral and digestive capacities.

To answer this question, we considered all the data from the oral phase, the digestive physiology of the elderly, and the modelling of the fate of protein in the stomach. Objectively assessing the bioavailability of nutrients at all stages of digestion and identifying protein profiles will ultimately make it possible to design foods that meet nutritional needs.

### Results

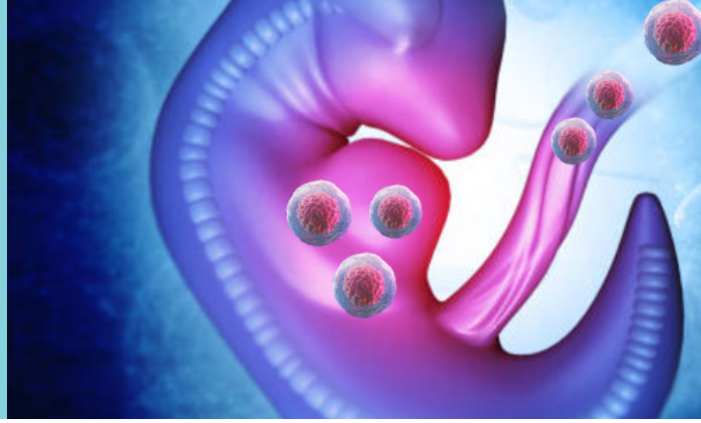
A pork sausage model was developed and subjected to normal and deficient chewing in order to mimic the oral deficiencies commonly

seen in the elderly, followed by *in-vitro* gastric digestion, mimicking the digestive tract of adult and elderly foods. Food boluses were characterised for their particle size and rheological properties. The kinetics of nutrient release and peptidomic analysis in the digesta were studied to establish a nutrient bioavailability index to help assess the effects of malnutrition on health status on older people. After impaired chewing, the food bolus was harder, with more coarse particles, lower free iron release and higher protein oxidation. The amount of peptides released in the stomach gradually increased, but to a lesser extent under the digestive conditions of the elderly. One novel finding was that the overall average amount of protein in the stomach after complete digestion was significantly lower in the case of impaired chewing in both adults and the elderly.

Two models of the kinetics of protein hydrolysis were proposed with specific parameters for digestion in adults and the elderly.

### Future outlook

Our research broadens the concept of food oral processing (FOP) by extending oral activity to digestion in a nutritional context. This new concept is called food oral and digestive processing (FODP).



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# Prebiotic supplementation during pregnancy alters the gut microbiota



## Read more

Brosseau C. *et al.*

Prebiotic supplementation during pregnancy modifies the gut microbiota and increases metabolites in amniotic fluid, driving a tolerogenic environment *in utero*

Frontiers in Immunology . 2019

<https://doi.org/10.3389/fimmu.2021.712614>

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## Context

When a woman is pregnant, her diet alters the intestinal microbiota. Diet can thus impact the immune system of the foetus *in utero* by modifying the transfer of immune factors, microbial factors and bacterial metabolites mediated by the umbilical cord, placenta and amniotic fluid. Prebiotics are fibres that act as fermentable substrates for specific bacteria, which either leads to the release of metabolites or exerts direct effects on immune cells. We hypothesised that prebiotic supplementation during pregnancy could shift the maternal microbiota towards higher production of metabolites and promote a healthy immune system in the foetus.

## Results

We demonstrated in mice that prebiotic (galacto-oligosaccharide/inulin) supplementation during gestation modifies the gut microbiota with an increase in the abundance of Bacteroidota and a decrease in Firmicutes associated with an increase in metabolite production. Of these metabolites, the concentration of acetate increases in the faeces as well as in the amniotic fluid. Prebiotic supplementation also increases the

frequency of immunoregulatory B and T lymphocyte cells in gestational tissues (uterus and placenta) and in the foetus (in the marrow and intestine). These cells are then found in the mouse pups at six weeks of life. We thus demonstrated that prebiotic supplementation during pregnancy leads to the transmission of specific microbial and immune factors from the mother to the offspring, allowing the establishment of a tolerogenic immune response in the foetus, which could protect the offspring from future conditions such as food allergies.

## Future outlook

We would like to determine in mice whether the tolerogenic environment induced *in utero* by prebiotic supplementation during pregnancy could prevent the occurrence of food allergy in the offspring. This nutritional strategy is being investigated in our PREGRALL cohort of pregnant mothers with allergies.

PROBE





©SQPOV - Purees used during this study

# From apple to plate: understanding and controlling texturing mechanisms



## Read more

Buergy A. *et al.*

Pectin degradation explains tissue fragmentation of fruits during thermomechanical processes for puree production

Food Hydrocolloids . 2021

<https://doi.org/10.1016/j.foodhyd.2021.106885>

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## Context

The texture of fruit purees is a key quality attribute that food manufacturers must get right if they want consumers to purchase such products. Texture is determined by structural factors such as the proportion and size of the particles and the viscosity of the liquid phase. These factors depend to a large extent on the tissue structure and cell wall properties of fresh fruit cell as well as the chosen processing technique. Pectins – parietal polysaccharides that are sensitive to heat treatment – seem to play a key role, as they ensure cell adhesion. However, the relationships between these structural factors and processing technique are not yet understood. Given the heterogeneous nature and variability of raw materials and the fact that they are further accentuated by climate change, how can apple purees with a uniform, controlled texture be obtained without the use of additives? Answering this question requires a better understanding of the complex mechanisms that affect the rheological properties of purees, in particular interactions between the microstructure and processing technique.

## Results

Purees with contrasting structures and textures were produced by modulating processing conditions (temperatures between 50 °C and 95 °C and grinding between 100 and 3,000

rpm), using apples whose structure was modulated by the choice of variety, cultural practices and post-harvest maturation. These purees were then characterised from the macroscopic to the molecular scale. Rheological measurements and sensory analysis revealed that particle size was the key determining factor in the texture of apple purees. The main factor affecting tissue fragmentation during the process was grinding. This fragmentation was further facilitated by apple maturation and heat treatment. This was correlated with pectin solubilisation and degradation, in particular side-chain hydrolysis, resulting in reduced cell adhesion. Processing conducted at 95°C, or six months of maturation, and gentle shearing provide more viscous purees. These results have aided understanding of tissue fragmentation and changes in texture during processing.

## Future outlook

In addition to offering the industry guidance on how to better manage fruit diversity and heterogeneity via processing technique modulation, this research will make it easier to create innovative, additive-free food products and bring about more sustainable processing.



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## Understanding interactions in aroma blends



### Read more

Ma Y. *et al.*

Perceptual interactions among food odors : Major influences on odor intensity evidenced with a set of 222 binary mixtures of key odorants

Food Chemistry . 2021

<https://doi.org/10.1016/j.foodchem.2021.129483>

### Partnerships

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### Context

About 80 % of our ability to identify and perceive food sensory characteristics is determined by the olfactory component. It results from the detection and integration by the olfactory system of chemically complex mixtures of volatile compounds. Our sense of smell is very efficient in coding this chemical complexity, which allows us to immediately recognise and differentiate between the smell of the various foods we eat. This coding is partly based on the interactions that can occur at different stages in the processing of olfactory information and that lead to mixture-induced effects such as masking or synergy, the mechanisms and determinants of which are still unknown today. In order to better understand these perceptual phenomena, the Flavour, Food Oral Processing and Perception (FFOPP) team at the Centre for Taste, Smell, and Feeding behaviour (CSGA) embarked on, within the framework of an international partnership with the Jiangnan University (China), a very systematic study of mixtures of odorants commonly found in foods.

### Results

We chose to study the simplest mixtures, i.e., those containing only two compounds. A total of 222 binary

mixtures were assessed by a panel of 30 people, who measured the intensity of the mixtures' smell, their quality, and their pleasantness. The raw data from this study were made accessible in a data paper linked to an open access dataset. The results showed that in most cases the smell of the components was perceived in the mixture and that their intensity stayed the same as it would in an out-of-mixture state. Masking was the second most frequent effect (44.8 %), while synergy was very rarely observed (less than 1 % of cases).

### Future outlook

Overall, this highly systematic and unique work provides general rules of thumb on what to expect when mixing the key odorants of food aromas. The data obtained are an important source for initiating research into the predictive modelling of the effect of mixing aromas. The study also identified some key associations that could be explored in more detail to identify the physiological and neurobiological mechanisms that underlie the perceptual effects in odour mixtures (masking, synergy, matching).



©Francis Canon

# The effect of tannins on the flavour of red wine before and after oxidation



## Read more

Pittari E. *et al.*

Effects of oenological tannins on aroma release and perception of oxidized and non-oxidized red wine: A dynamic real-time *in-vivo* study coupling sensory evaluation and analytical chemistry

Food Chemistry . 2022

<https://doi.org/10.1016/j.foodchem.2021.131229>

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## Context

Oxidation is one of the main chemical mechanisms that alters the organoleptic properties of wine as it ages. Oxidation modifies the structure of wine molecules, altering its flavour. The fruitiness of red wines tends to decrease over time as a result, with notes of maderisation or prunes appearing in its place. Thanks to their antioxidant properties, wine tannins are described as having a protective effect on the flavour of wines and combat the effects of oxidation. The addition of these tannins also alters the wine's aromatic persistence. It should be pointed out, however, that these effects are currently the subject of some controversy and that more needs to be understood about them.

Furthermore, the perception of flavour is a dynamic process, influenced as it is by a variety of factors, such as the kinetics involved in the release of flavour compounds. This study combined a dynamic sensory analysis method developed by the ChemoSens platform, the temporal dominance of sensations, and a measurement of the composition of volatile molecules in the judges' nasal cavities. This method made it possible to track both the dominance of sensations over time and the aroma compounds released when tasting a pinot noir before and after oxidation and the addition of the wine tannins proanthocyanidin and ellagitannin.

## Results

Results show that the presence of ellagitannins can protect the fruity character of red wines from oxidation. The findings of this study thus suggest that maturing wines in oak barrels has a positive impact on the preservation of fruit aromas during the ageing process, as it provides ellagitannins. This effect may be due to a higher release of ethyl decanoate in the first few seconds of tasting than is the case in oxidised wine with no tannins. The lack of effect following the addition of proanthocyanidins may be due to their already high concentration in the wine. The use of ellagitannins to preserve fruit aromas could help counterbalance the aromas extracted from the wood and mask the appearance of oxidation notes with a positive effect on the shelf life of wines.

## Future outlook

Further studies will provide a better understanding as to why a wine's fruity character can be preserved and help explain the increase in aromatic persistence in the presence of ellagitannins. This study benefited from the expertise of the ChemoSens and Polyphenols platforms, which both form part of the PROBE research infrastructure and highlight its added value.



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## Survival of *E. coli* in the digestive tract: a meal-scale study



### Read more

de La Pomelie D. *et al.*

Investigation of *Escherichia coli* O157:H7 survival and interaction with meal components during gastrointestinal digestion

Foods . 2021

<https://doi.org/10.3390/foods10102415>

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### Context

Shigatoxin-producing *Escherichia coli* (STEC) are zoonotic agents that are the third most frequently occurring and dangerous foodborne pathogens in the European Union. They are responsible for serious food poisoning, including haemolytic uraemic syndrome. Food products of animal origin, especially minced meat, as well as vegetables and beverages can be contaminated. The survival of STEC after ingestion of contaminated meat in the human digestive tract depends on many factors and is not yet fully understood. This study looked at the survival of STEC in a complex physicochemical environment from the stomach to the intestine, focusing on the chemical reactivity of nitrite (an antibacterial agent) at the meal level.

### Results

At the end of gastric digestion, 5 % of the initial *E. coli* O157:H7 CM454 bacterial population inoculated into the minced meat survived despite an acidic pH. This ability to withstand an acidic environment is attributed to the presence of five acid resistance systems in *E. coli* O157:H7. The combined addition of nitrite and ascorbate, here mimicking the contribution of vegetables to the digestive environment, resulted in a drastic reduction in the population of *E. coli* O157:H7 CM454,

at the end of both gastric digestion (0.30 % of the initial population) and intestinal digestion (0.04 % of the initial population).

In the presence of *E. coli*, more free iron is released during digestion of meat (2.6 times more) and meals (4 times more). This release of free iron is known to occur during the digestion of meat, which is rich in iron. Iron is an essential micronutrient for bacteria and for maintaining iron homeostasis. *E. coli* has developed several mechanisms involving siderophores such as citrate, low-molecular-weight thiols and ferritins. The release of iron is explained by cell lysis. Finally, the presence of *E. coli* altered the redox potential of the stomach towards a more reducing environment, perhaps due to the consumption of oxygen by the bacteria but also thanks to the ascorbate as an electron donor.

### Future outlook

Our research highlights the importance of considering meal complexity. A transcriptome analysis will be performed using RNA sequencing to gain insight into how genes are modulated in the digestive environment.





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## Assessing food/saliva interactions according to the rheological properties of artificial boluses



### Read more

Gibouin F. *et al.*

Rheological properties of artificial boluses of cereal foods enriched with legume proteins

Food Hydrocolloids . 2022

<https://doi.org/10.1016/j.foodhyd.2021.107096>

### Partnerships

- RFI Food for Tomorrow project (Pays de Loire Region)

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### Context

The breakdown of food during chewing determines its sensory and nutritional properties. With salivation, it forms a food bolus, which is then swallowed. For cereal-based foods, mechanistic models have shown that interactions with saliva determine the variation in bolus viscosity. These interactions are essential for the palatability of food products enriched with plant proteins.

The aim of this research is therefore to determine an interaction coefficient between saliva and cereal-based foods with different structures and compositions. Four foods, enriched and unenriched with pea flour, belonging to two families of cereal-based products, a sponge cake (soft) and an extruded flat bread (crisp), were studied. To overcome inter-individual variability, artificial boluses were obtained after hydration and grinding.

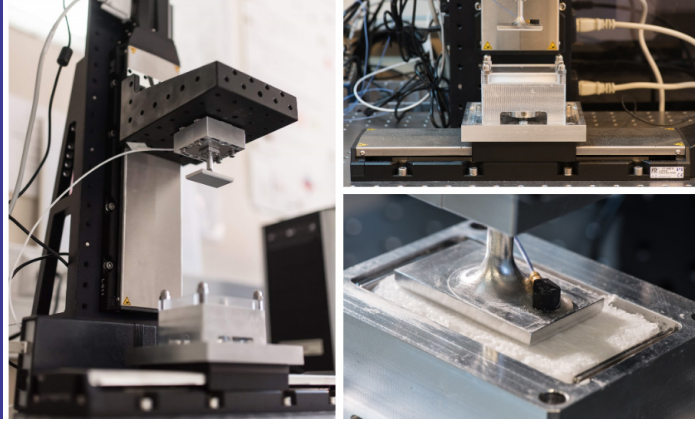
### Results

The variations of rheological properties of the boluses with their water content,  $X$  in the range of hydration by saliva, were determined by two types of rheometry, oscillatory and capillary rheometry. The results showed that at low strain the boluses

behave like a gel and at high strain like a yield stress fluid. Boluses can thus be characterised by two essential rheological properties: the characteristic stress at transition to flow and flow consistency. The decrease of these properties with increasing water content, adjusted with an exponential function, is used to determine a coefficient of interaction of the food with water  $\alpha$  ( $5 \leq \alpha \leq 30$ ). These  $\alpha$  values, thus calculated, are higher for pea-based extrusions ( $\alpha \geq 15$ ), than for sponge cake ( $\alpha \leq 15$ ). When compared to those found for real boluses, findings suggest that  $\alpha$  allows characterisation of the interaction of the food with saliva.

### Future outlook

These rheological methods can be applied to other foods, and by determining  $\alpha$ , their interaction with saliva can be taken into account to help design specific diets.



©Vincent Mathieu - Differing views of the biomimetic system for the study of biomechanical interactions between food, tongue and palate.

## Biomimetic tool for understanding the tactile perceptions between the tongue and the palate



### Read more

Srivastava R. *et al.*

A new biomimetic set-up to understand the role of the kinematic, mechanical, and surface characteristics of the tongue in food oral tribological studies

Food Hydrocolloids . 2021

<https://doi.org/10.1016/j.foodhyd.2021.106602>

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### Context

As the recent award of the Nobel Prize in Medicine 2021 showed, understanding the mechanisms behind tactile perception is a major scientific challenge. When food enters the mouth, these perceptions contribute to sensory pleasure, while also indicating when the food can be swallowed, in conditions that must be both comfortable and safe. Understanding the mechanisms of tactile perception involves having a grasp of both the properties of food and the complex physiological characteristics of the organs in the oral cavity, which are known to vary significantly from one person to another.

### Results

A biomimetic tool was designed to simulate, in laboratory conditions, the various factors involved in the manipulation of food between the tongue and the palate. The system is primarily based on the design of artificial tongues, the rigidity, roughness and lubrication of which are controlled. These tongues are then mounted on a tool in which sequences of movements can be programmed to study compression and shearing between the tongue and a flat rigid surface recreating the hard palate. The tool is equipped to perform series of physical measurements to study the forces

applied, the deformations endured by the tongue and the food, and the vibrations generated during this oral simulation. A proof of concept, focusing on friction phenomena during shearing between the tongue and the palate, confirmed the potential of the tool and showed the considerable impact of the rigidity and roughness of the tongue, until now largely overlooked, on the friction forces in play.

### Future outlook

The tool has been designed in such a way to allow a great degree of flexibility in its development. It is now possible to mimic properties that can vary markedly from one person to another (age, pathologies, etc.), to study and understand the extent to which these properties can alter the sensory experience. This tool can be used to develop innovative physical approaches (such as ultrasound methods) in order to characterise and gain insight into biomechanical interactions. It could also be used as a means of introducing personalised diets and adapting the structural properties of foods to the specific physiological condition of individuals.



©Teensy-Weensy Pouchou on Foter.com

# Very hot beverages could harm our ability to detect tastes



## Read more

Martin C. *et al.*

Impact of very hot drink consumption habits, age, and sex, on taste sensitivity

Foods . 2021

<https://doi.org/10.3390/foods10051139>

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## Context

Some foods are occasionally served and consumed at very high temperatures. This is the case in particular with hot beverages such as tea and coffee. When the tissues of the oral cavity (and especially the tongue) come in contact with these beverages, they undergo a very significant rise in temperature, which can cause cell damage. However, relatively little is known about the consequences that regular exposure to high temperatures can have on the ability of taste buds to function and finally, on taste perception.

The study we conducted was an initial approach designed to assess the impact of the regular consumption of very hot beverages on taste sensitivity.

## Results

We surveyed 82 consumers who regularly consume hot beverages. They each completed a questionnaire on their consumption habits and their taste sensitivity to the five basic tastes (sweet, salty, sour, bitter and umami) were evaluated. We then compared the taste sensitivity levels of consumers who said they drank their favourite beverage 'very

hot' (n=36) with those who replied 'moderately hot' (n=46). The age and gender of participants were taken into account in all analyses, as these are two factors known to influence taste sensitivity. Our results showed that consumers accustomed to drinking 'very hot' beverages showed less sensitivity for sweet and salty tastes. The same trend was observed with the sour taste. The results also confirmed the decline in taste sensitivity as people age (from 50 onwards) and showed that women were more sensitive to certain tastes (sour, bitter and umami).

## Future outlook

These initial results show that consumption habits can have a significant impact on our sensory system and, as a result, on food perception. However, further research is needed to reveal all the mechanisms involved.

# Understanding the metabolism and perception of flavours in the mouth by exploring oral and saliva proteomes



## Read more

Schwartz M. *et al.*

Oral enzymatic detoxification system: insights obtained from proteome analysis to understand its potential impact on aroma metabolization

Comprehensive Reviews in Food Science and Food Safety . 2021

<https://doi.org/10.1111/1541-4337.12857>

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## Context

The flavour of a food is perceived in the mouth thanks to the integration of taste, aroma and trigeminal sensations. Aroma is provided by odorant molecules, which are released in the mouth during chewing. Recent studies have shown that these odour molecules can be metabolised in the oral cavity. The metabolites formed have different sensory properties compared to the original odorant molecules. As a result, and according to these studies, oral metabolism should modify the aromatic perception from one individual to another. The enzymes responsible for these reactions are unknown, although there is considerable evidence to suggest the involvement of xenobiotic metabolising enzymes (XMEs). Using a systematic mining approach of existing data on oral and saliva proteomes, we conducted a comprehensive inventory of oral XMEs potentially involved in the metabolisation of flavour molecules.

## Results

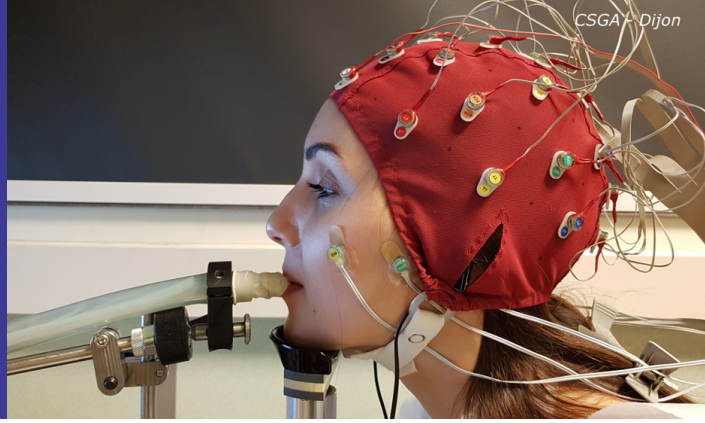
The exploration of four saliva proteomes and one oral epithelium proteome identified more than 90 XMEs present in the oral cavity. These XMEs comprise about 2 % of the total of different proteins present in saliva. The identified XMEs can be separated

into three groups according to their reaction mechanism: oxidoreductases, transferases and hydrolases. The greatest diversity was found in the group of oxidoreductases, with eight families of enzymes with 48 members. All of these enzyme families show similar reactions to those previously observed in *ex-vivo* saliva studies and *in-vivo* studies of flavour molecule metabolism in the mouth.

## Future outlook

More research is now needed to further explore the reactions involved. We plan to further characterise the molecular mechanisms involved by studying the enzymes of oral metabolism in more detail. XMEs from the aldehyde dehydrogenase family were selected on the basis of this study and are currently being explored. Additionally, the understanding of molecular mechanisms will also have to take into account the complexity of the oral cavity and involve *in-vivo* studies of real-time metabolism. This research is a first step towards understanding the metabolism of aromas in the mouth and their impact on flavour perception.





©Charlotte Sinding - Stimulation of participants with salty and/or flavoured soups during the recording of olfactory and gustatory evoked potentials by an electroencephalography technique (EATERS project, Centre for Taste, Food and Nutrition Sciences – CSGA).

## The human brain: the illusionist that makes smells taste good!



### Read more

Sinding C. *et al.*

Odor-induced saltiness enhancement: insight into the brain chronometry of flavor

Neuroscience . 2021

<https://doi.org/10.1016/j.neuroscience.2020.10.029>

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### Context

We often confuse taste and smell – we speak of the ‘taste’ of a food when we are actually referring to its smell, also known as ‘aroma’. This common confusion has a biological reason, nestled in our brain. To better understand the brain mechanisms involved in the processing of taste and smells, volunteer participants were fitted with electroencephalography headsets to measure the electrical activity of the brain while they tasted three pea soups in a random order. The soups contained either normal salt content, reduced salt content (-25 %), or reduced salt content plus beef broth aroma.

### Results

These soups all resulted in an early brain activation at 150 ms related to the processing of the salt concentration in the food, followed by a late activation (640 ms) related to the conscious processing of the salt intensity. While both soups without aroma produced an equivalent late activation peak (at 640 ms), the aromatized soup produced a later signal at 660 ms. These 20 ms are not trivial for our brain, whose neurons respond in a few milliseconds: they reflect a longer information processing pathway, probably linked to the processing of the odour as a

taste signal. This finding shows that the theory that positions the interactions between odours and taste at a very early level (in the primary olfactory and gustatory cortices) is unlikely. These interactions tend to take place in high-level brain areas related to cognitive and memory processing.

### Future outlook

The olfactory information from ‘beef broth’, or any other smell associated with saltiness, would reactivate a memory associating this smell with the taste of saltiness, which would stimulate the areas of the brain involved in processing the salty taste, without the smell activating our taste receptors. Thus, consuming a food with a salty smell would give us the ‘illusion’ that it tastes saltier than it actually is. We can therefore reduce the amount of salt and sugar by using particularly fragrant ingredients such as herbs, spices or vanilla. On a basic level, these temporal measurements still need to be associated with the brain areas involved in the perception of food. Another possible avenue of research is to delve into how these mechanisms are modified by the weight and diet of individuals, as well as by their culinary culture, which is the basis of smell-taste associations.



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# Oxidation of omega-6 fatty acids during digestion, a risk factor in vascular health, is limited by apple consumption



## Read more

Boléa G. *et al.*

Digestive n-6 lipid oxidation, a key trigger of vascular dysfunction and atherosclerosis in the Western diet: protective effects of apple polyphenols

Molecular Nutrition & Food Research . 2021

<https://doi.org/10.1002/mnfr.202000487>

## Partnerships

- LAPEC at the University of Avignon

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## Context

The Western diet, characterised by high consumption of polyunsaturated  $\omega$ -6 fatty acids, is blamed for the development of cardiovascular disease, the leading cause of death in our societies. Moreover, various epidemiological studies have clearly shown that fruits and vegetables, as well as certain classes of polyphenols, offer protection against coronary heart disease. Although the sites and mechanisms of lipid oxidation are still poorly understood in the development of atherosclerosis, the protective effects of antioxidant polyphenols against dietary lipids during digestion has been validated in our previous work in minipigs. Lipid oxidation is initiated during gastric digestion by a form of heme iron, myoglobin, that is predominant in red meat. However, the link between lipid oxidation during digestion and endothelial dysfunction had not yet been established, nor had the ability of polyphenols to limit the formation of lipid oxidation products leading to atherogenesis.

## Results

In partnership with the Laboratory of Cardiovascular Pharma-Ecology (LAPEC) at the University of Avignon, we conducted a 12-week study with ApoE<sup>-/-</sup> transgenic mice that show an increased susceptibility to the development of atherosclerosis. The addition of

sunflower oil ( $\omega$ -6) to a Western diet high in saturated fats and red meat leads to the formation of a cyto- and genotoxic aldehyde, 4-hydroxynonenal (4-HNE), in the gastrointestinal tract. 4-HNE also increases in plasma, as does the level of oxidation of plasma LDL. Nevertheless, co-ingestion of *Reinette de Flandre* apple puree or the corresponding phenolic extract brought the various markers (4-HNE, blood pressure, LDLox, *ex-vivo* aortic vasorelaxation, atheromatous plaque) back to the level observed in the absence of  $\omega$ -6 fatty acids or even to a level corresponding to a normolipidic diet.

Although the protective effect by polyphenols may also involve cellular action by colonic metabolites, these results support the epidemiological data and are useful in supporting public policies to encourage the consumption of five portions of fruits and vegetables per day.

## Future outlook

An *in-vitro* model of gastric digestion was developed and validated on apple and various polyphenols. It will enable investigation of the ability of other fruits and vegetables to inhibit heme-iron-induced lipid oxidation during digestion of a Western meal, as well as the bioavailability of phenolic compounds in a digestive environment.



# Designing biobased products and materials



Plant biomass is an abundant but recalcitrant raw material. Developing tools and advanced technological pathways to transform this bioresource into functional products are challenges for the division's researchers.

**Page 51:** Studying the biodegradation mechanisms of recalcitrant materials

**Page 57:** A closer look at the biomaterials studied at TRANSFORM





©INRAE - Marie berger

# A multiscale approach to understanding lignocellulosic biomass recalcitrance



## Read more

Leroy A. *et al.*

Evaluating polymer interplay after hot water pretreatment to investigate maize stem internode recalcitrance

Biotechnology for Biofuels . 2021

<https://doi.org/10.1186/s13068-021-02015-8>

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## Context

Biomass recalcitrance is a key issue for the development of low-cost and sustainable biorefineries. The recalcitrance conferred by the complex structure and composition of lignocellulose limits conversion by biochemical means (pretreatment and saccharification) of many lignocellulosic resources such as the co-products of maize cultivation (stalks, leaves). One of the most widely used strategies to overcome biomass recalcitrance is the application of pretreatment technologies. Optimising pretreatment technologies requires an understanding of how the structure and organisation of these parietal polymers influence recalcitrance and the nature of changes induced by pretreatment.

To this end, water mobility at the polymer structure level was characterised on native and hydrothermally pretreated maize stalk samples. For this purpose, complementary techniques were used, including compositional, spectral and nuclear magnetic resonance (NMR) analyses.

## Results

In addition to the removal of hemicelluloses (expected effect), pretreatment induces a loss of

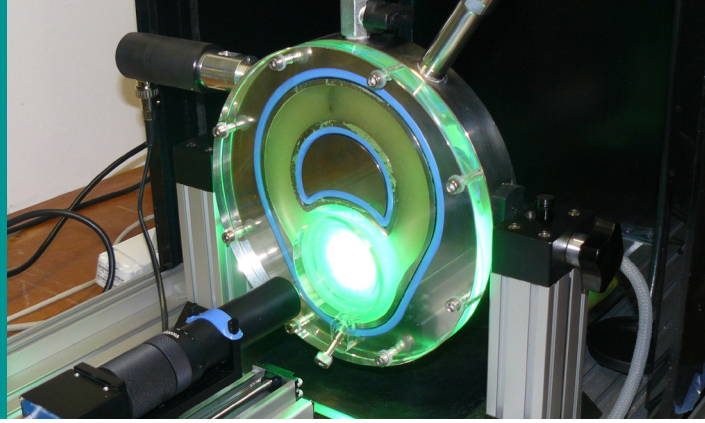
amorphous cellulose and structural changes in lignins. Our results also show that the environment and the organisation of the lignin are important factors to consider in addition to its composition to explain recalcitrance capacity. These changes at the molecular level induce modifications in cell wall organisation through increased porosity and accessibility to cellulose. This leads to a redistribution of water, which supports the diffusion and efficiency of hydrolytic enzymes. The combination of NMR analysis techniques has been shown to be of interest in studying the environment of cellulose to explain its accessibility.

## Future outlook

The recalcitrance capacity of lignocellulose is highly variable depending on the biomass considered. However, elucidation of the interactions between polymers is key to understanding the recalcitrance of lignocellulosic resources. Consequently, using the same multiscale approach on other types of lignocellulosic biomass will make it possible to optimise pretreatment and saccharification processes, and in the medium term, contribute to the development of new low-cost and sustainable biorefineries.







© Marie-Francoise Devaux - Torus reactor

# Kinetics of physical state changes accompanying chemical degradation of lignocellulosic biomass



## Read more

Barron C. *et al.*

Enzymatic degradation of maize shoots: monitoring of chemical and physical changes reveals different saccharification behaviors

Biotechnology for Biofuels . 2021

<https://doi.org/10.1186/s13068-020-01854-1>

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## Context

Overcoming the recalcitrance of biomass to enzymatic deconstruction is a key issue for biorefinery development. The factors involved in the phenomenon of recalcitrance are still under debate. Taking into account the heterogeneity of the biomass by looking at the changes in the physical state that accompany saccharification could shed new light on the breakdown process.

## Results

Dry fractionation was used to separate maize straw into five fractions based on particle size and electrostatic properties: one 'coarse' fraction, two 'medium' fractions and two 'fine' fractions, composed of particles with median diameters of around 300  $\mu\text{m}$ , 200  $\mu\text{m}$ , and less than 100  $\mu\text{m}$ , respectively. The physical changes (particle size and number) and saccharification were monitored using a torus reactor to collect hydrolysates and acquire images of particles during degradation by a commercial enzymatic cocktail.

Saccharification and the decrease in particle number and size were significant and rapid in both fine fractions. In contrast, in the coarse fraction, the saccharification rate was low and the physical state changed

very little. The two middle fractions showed comparable saccharification rates but different physical-state changes. For one of the medium fractions, the reduction in the total number of particles was similar to that obtained for the fine fractions, but was slower. For the second medium fraction, the reduction in the number of particles was lower, similar to the coarse fraction and more gradual. The small decrease in particle size at relatively high hydrolysis rates for the fine and medium fractions suggests that the enzymes diffuse and act within the particles. The linking of the fractions with the physicochemical characteristics shows that saccharification is controlled by the volume of pores accessible to the enzymes within the particles, while the lignin content contributes to preserving the macroscopic structure of the particles.

## Future outlook

The next step will be to examine the degradation process at the particle level. The addition of a dry fractionation step in a biorefinery process could allow for better exploitation of biomass by diversifying the uses according to fraction reactivity.

## Observation of the impact of alkaline pretreatments of lignocellulosic biomass at the plant cell and tissue level



### Read more

Thomas H.L. *et al.*

Alkaline pretreatments for sorghum and miscanthus anaerobic digestion: impacts at cell wall and tissue scales

Bioenergy Research . 2021

<https://doi.org/10.1007/s12155-021-10342-9>

### Partnerships

Biomass for the Future (BFF) Stimulus Initiative project in collaboration with the AGAP-PHIV Joint Research Unit

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### Context

Given the major development of agricultural anaerobic digestion, new substrates such as lignocellulosic residues, multipurpose intermediate crops (e.g., sorghum) and biomass that can be grown on marginal land (e.g., miscanthus) are being considered for biogas production. However, the presence of lignin makes bioconversion of these types of biomass difficult, so pretreatments are applied to improve methane yields. Alkaline pretreatments have been shown to be effective; one hypothesis is that this is due to their action on lignin.

The objective of this study, carried out as part of the Biomass for the Future (BFF) Stimulus Initiative project in collaboration with the Joint Research Unit for the Genetic Improvement and Adaptation of Mediterranean and Tropical Plants (UMR AGAP), was to understand the impacts of alkaline pretreatments at the tissue and plant cell level.

### Results

We developed a protocol for the pretreatment of internodes on sorghum and miscanthus. The pretreatments were applied to sections of approximately 100  $\mu\text{m}$  thickness, which were then stained before being observed under a microscope. Staining was done with Congo red, which is specific for cellulose, and with phloroglucinol, specific for

lignin, and showed that the FASGA staining method makes it possible to differentiate lignin and holocellulose on the same slide. We have shown that pretreatments with soda or lime break down lignin depending on the location of the tissue. In particular, they affect the parenchyma and, to a lesser degree, the sclerenchyma of both biomasses. However, the epidermis is not degraded. Alkaline pretreatments led to both the unmasking of cellulose, which was then more accessible for anaerobic degradation, and the release of parenchyma cells. This explains the increase in the first-order rate constant of degradation of 162 % for sorghum and 546 % for miscanthus. A principal component analysis showed the correlation between this rate constant and the percentage of surface area of holocellulosic compounds in the inner part of the parenchyma.

### Future outlook

In addition to the improved knowledge obtained regarding the mechanisms of alkaline pretreatments of lignocellulosic compounds to improve the performance of anaerobic digestion and biorefinery processes, this research opens up avenues in the study and development of new pretreatments. The action of these pretreatments should be focused on the epidermis, which is not degraded by alkaline treatments and is recalcitrant to anaerobic digestion.



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## Exploring and exploiting fungal biodiversity to degrade recalcitrant polymers



### Read more

Navarro D. *et al.*

Large scale phenotyping of 1,000 fungal strains for the degradation of non-natural, industrial compounds

Communications Biology . 2021

<https://doi.org/10.1038/s42003-021-02401-w>

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### Context

The fungal kingdom contains an estimated diversity of between 2.2 and 3.8 million species. The diversity of fungal nutrient acquisition strategies, as well as the diverse properties of their hosts, are the main drivers for the evolution of their extraordinary biocatalytic potential. In particular, plant-decomposing filamentous fungi have the unique ability to efficiently degrade lignocellulose, a notoriously stubborn biopolymer. The underlying biochemical strategies have inspired many industrial processes to exploit biomass as fermentable sugars and other biomolecules for second generation biofuels, bioproducts and biomaterials. Given the similarity of bioconversion challenges (e.g., crystallinity or toxicity) shared by these biopolymers and some human-made compounds (e.g., plastics and dyes), fungi and their enzymes represent an underexplored reservoir of biological tools for discovery and exploitation.

### Results

We performed the first large-scale phenotyping study to assess the degradation potential of the fungi collection at the CIRM-CF biological resource centre ([www.cirm-fungi.fr](http://www.cirm-fungi.fr)) on five non-natural compounds: two dyes from the textile industry (Reactive Blue 5 and Basic Blue 21), lignosulphonates from the pulp and paper industry, a soluble polyurethane from the plastic

industry and microcrystalline cellulose used in the food, pharmaceutical and cosmetic industries. We described the functional phenotyping (growth and degradation) of >1000 strains belonging to 26 different orders, 78 families and 231 genera.

Our study i) mapped the biotechnological potential of filamentous fungi, ii) demonstrated that functional diversity can be observed down to the intra-specific level (i.e., between strains of the same species), and iii) provided guidelines for the selection of fungal families/species for selected applications.

### Future outlook

We showed that the search for new fungal biocatalysts for novel biotechnological applications requires an unbiased screening covering a large diversity, both at different taxonomic ranks and between strains of the same species. In the coming years, we will continue the phenotyping campaign of the CIRM-CF collection, which continues to grow (it currently has over 3000 strains).

We believe that the exploration of the phenotypic biodiversity preserved by the biological resource centres to identify the most relevant natural catalysts, combined with the molecular engineering of these already naturally evolved tools, is key to meeting the various challenges of the twenty-first century circular bioeconomy.





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## Exploiting agricultural waste to obtain molecules of interest



### Read more

Vojvodić Cebin A. *et al.*

Valorisation of walnut shell and pea pods as novel sources for the production of xylooligosaccharides

Carbohydrate Polymers . 2021

<https://doi.org/10.1016/j.carbpol.2021.117932>

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### Context

Recovering agricultural waste is an essential part of sustainable crop and food production. The aim is to reduce the negative environmental impact of waste and generate economic benefits by creating value-added products. The non-consumable parts of plants such as peels, skins, hulls, husks, pods, fruit stones, stems, leaves and pomace represent readily available secondary raw materials that can be used to produce energy, materials and chemicals, or as new and unconventional sources of functional ingredients.

The objective of this research was to evaluate walnut shells and pea pods as sources for producing xylooligosaccharides (XOS). XOS are known to have prebiotic activity. They are non-digestible oligosaccharides that are selectively metabolised by part of the human microbiota, with health benefits arising from the production of short-chain fatty acids and the stimulation of probiotic strains, such as bifidobacteria.

### Results

Hemicellulose from walnut shells and pea pods was obtained by combining different delignification and alkaline extraction parameters.

Under optimal conditions, the soluble fractions contained up to 45 % xylose, and the insoluble fractions more than 75 %. Structural analysis of these polysaccharides shows a xylan, which was not highly branched, with very few glucuronic acid residues in the insoluble fractions and more complex structures in the soluble fractions. Different conditions of degradation by GH11 xylanase from *Neocallimastix patriciarum* were applied and resulted in a maximum xylan conversion rate of 70 % on walnut shells and 90 % on pea pods. Analysis of the hydrolysis products shows the presence of xylobiose and xylotriose with low enzyme concentration, and xylose and xylobiose with higher enzyme concentration.

### Future outlook

Given that the prebiotic activity of XOS requires further research, the choice of optimal hydrolysis conditions will depend on the contribution of each XOS to the prebiotic effect, their potential synergistic effects and the cost-effectiveness of hydrolysis in relation to time, the amount of xylanase added and the xylan conversion rate.





© David Pot - Three forage sorghum ideotypes potentially suitable for biogas production

## Identifying a sorghum ideotype for biomethane production



### Read more

Thomas H.L. *et al.*

Mobilizing sorghum genetic diversity: Biochemical and histological-assisted design of a stem ideotype for biomethane production

GCB-Bioenergy . 2021

<https://doi.org/10.1111/gcbb.12886>

### Partnerships

Biomass for the Future (BFF) Stimulus Initiative project, and in collaboration with the AGAP and SELMET joint research units and various seed companies.

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### Context

Sorghum has been identified as a suitable resource for energy production, particularly biogas or biomethane. Its biomass composition shows a high genetic diversity, which is a vector for adaptation to various value chains. While commercial genotypes proposed for biogas production aim for high per-hectare biomass yields, a better understanding of the impacts of stem composition and structure on methane potential could guide the identification of an ideotype that maximises biomethane yield.

As part of the Biomass for the Future (BFF) Stimulus Initiative project and in collaboration with the Joint Research Unit for the Genetic Improvement and Adaptation of Mediterranean and Tropical Plants (AGAP) and the Joint Research Unit for Mediterranean and Tropical Livestock Systems (SELMET) as well as different seed companies, a panel of 57 genotypes representing a large part of the compositional variability available in *Sorghum bicolor ssp bicolor* was characterised at five cultivation sites. The development of histological phenotyping of stems has allowed researchers to describe the genetic diversity of the internodal anatomy and the distribution of key cell wall compounds.

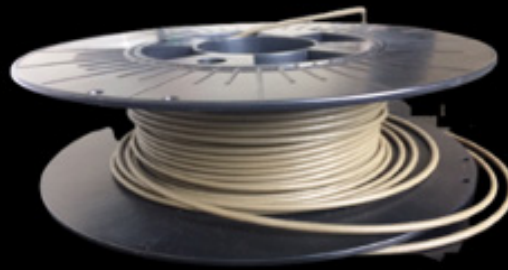
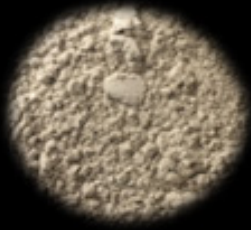
### Results

Moderate to high heritability was observed for stem biochemical composition and high heritability associated with moderate genetic variability for biomethane potential. These findings pave the way for significant genetic gains in the future. Genetic correlation and hierarchical clustering identified soluble sugar content as the primary driver of genetic variability in biomethane potential. Breeding programmes should therefore target high levels of soluble sugars but should also consider cell wall components and their location. In particular, low lignin and high cellulose content should be targeted while maintaining good lodging resistance.

### Future outlook

This ideotype identification should lead to genetic gains in the medium and long terms with regard to the biomethane potential of sorghum. These future improvements, combined with sorghum's strengths in terms of adaptation to environmental stress, point to increased use in agricultural anaerobic digestion systems. Moreover, this species is likely to play a key role in optimising the economic and environmental sustainability of agricultural systems currently facing the effects of climate change.





©Claire Mayer

## Smart materials with fluorescent properties designed using 3D printing



### Read more

Mayer-Laigle C. *et al.*

Flax shives-PBAT processing into 3D printed fluorescent materials with potential sensor functionalities

Industrial Crops and Products . 2021

<https://doi.org/10.1016/j.indcrop.2021.113482>

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### Context

Ensuring efficient use of natural resources and reducing post-harvest losses and plastic materials are ways to lower the environmental impact of human activities and support the transition towards a sustainable bioeconomy. Additive manufacturing of biobased materials such as composites reinforced with plant fibres is a relevant alternative to conventional processes. These techniques allow for the development of new functional designs (such as sensors) that are difficult to achieve through traditional approaches (moulding or subtractive) and help significantly reduce plastic waste. Since these approaches are still new, many technological challenges remain to control the functionality and durability of products obtained from biobased materials.

### Results

Flax shives are fragments of straw recovered after scutching and are still not widely used. After grinding, shives were grafted with a pH-sensitive fluorophore using a simple and inexpensive chemical reaction. The grafted shives were then formulated with a polybutylene terephthalate matrix and extruded to form a filament used for 3D printing. Extensive microstructural characterisation

demonstrated that the particles were homogeneously distributed throughout the 3D printed material. Despite the relatively low content of fluorescent flax shives in the final material (1 % by mass) and the successive heating steps (during extrusion and 3D printing), strong fluorescence emission was measured. Material immersion in buffer solutions of varying pH showed a sensitivity of the fluorescence to pH although these variations remained small in intensity, most likely related to the hydrophobic properties of the polymeric matrix and low open porosity. Indeed, the low open porosity of the material containing the fluorescent flax shives may limit the penetration of liquids by imbibition.

### Future outlook

Fluorescent molecules can respond to many stimuli (mechanical, thermal, chemical) and 3D printing allows the incorporation of such sensors into complexly shaped materials. The design of the object, the printing process and the choice of polymer are key elements to increase the reactivity of the materials. This research is continuing as part of the SMARTPOP project (EU-MSCA-Grant Agreement Number 893040) in collaboration with the SCION institute in New Zealand.





© Jessica Vanier, FARE - Enzymatic glycosylation reaction from wheat bran

## Using bran as surfactants



### Read more

Jocquel C. *et al.*

An integrated enzymatic approach to produce pentyl xylosides and glucose/xylose laurate esters from wheat bran

Frontiers in Bioengineering and Biotechnology . 2021

<https://doi.org/10.3389/fbioe.2021.647442>

### Partnerships

This research was carried out within the framework of the Interreg Fr-W-FI ValBran project led by FARE :

- FARE-AFERE Chair
- Reims Institute of Molecular Chemistry
- University of Picardy Jules Verne
- University of Liège

VITO

- ValBiom
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### Context

Biobased surfactants are very popular as they are biodegradable, non-toxic to humans and the environment and produced from renewable carbon. Alkyl glycosides and sugar esters are non-ionic biobased surfactants of interest for food and cosmetics. Currently, these surfactants are produced industrially by chemical synthesis, which consumes energy and can lead to undesirable by-products. In this context, the AFERE Chair and the Joint Research Unit for the Fractionation of Agricultural Resources and the Environment (UMR FARE) have developed an innovative biocatalytic process to convert wheat bran into alkyl glycosides and sugar esters.

### Results

An integrated approach combining biocatalytic reactions was developed using commercial enzymes (cellulase, xylanase, lipase) to fractionate wheat bran polysaccharides and produce pentyl xylosides as well as D-glucose and D-xylose laurate esters. Under optimal conditions, the total production yield of both families of surfactants was estimated at 150 kg per tonne of wheat bran. The process of synthesising these surfactants is innovative at several levels. The first innovation was to exploit both the xylan and

cellulose from a single batch of wheat bran that had not been pretreated to produce the various surfactants. The second level of innovation is based on the development of a biocatalytic process carried out under mild reaction conditions compared to the chemical synthesis processes classically used in the industrial synthesis of these surfactants.

The process has been validated at a technology readiness level (TRL) of 3–4, demonstrating the technological feasibility of converting several kilos of bran into biobased surfactants.

### Future outlook

The prospects for this study aim to optimise the developed process, with the recycling of enzymes and solvents in particular to limit the environmental impact and reduce the cost of the process. The technology will also have to be scaled up to test the technical and economic viability of the biocatalytic process for the production of the targeted surfactants.





© Philippe Evon - UCAI

# Life cycle inventory of a technical textile made of flax fibres



## Read more

Gomez-Campos A. *et al.*

Flax fiber for technical textile: A life cycle inventory

Journal of Cleaner Production . 2021

<https://doi.org/10.1016/j.jclepro.2020.125177>

## Partnerships

French Agency for Ecological Transition (ADEME) 'BOPA' project, in partnership with:

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## Context

Flax fibre appears to be an interesting raw material for developing a sustainable bioeconomy. The environmental performance of composites reinforced with flax fibres has been evaluated in previous life cycle assessment (LCA) studies. However, these studies do not focus on the production of the technical linen textile itself and therefore lack detailed information on the life cycle inventory. In addition, co-products are handled using economic allocation techniques and there is little discussion or clarification of their fate.

This research aims to fill these gaps and provide data for future LCAs on flax fibre production and processing. The study focuses on the impacts of the production of a biobased reinforcement material (technical textile) from a system expansion perspective.

## Results

A detailed life cycle inventory of flax fibre production and processing fibre was established, and the environmental performance of a technical linen textile was assessed by a cradle-to-gate LCA. The fate of co-products was documented, and correctly defining co-products and their fates was shown to be as crucial as having a complete

and accurate life cycle inventory.

This research improved existing LCAs on flax fibre production by including emissions from the retting process, exploiting co-products through system expansion, and analysing a larger number of impact categories.

The results show that agricultural activities and electricity generation (from flax cultivation and spinning and weaving processes) are the main contributors to the negative environmental performance of a technical linen textile; the contribution of the impact generated by land use change is small in comparison. For this specific case study, the sensitivity analysis proved that an entirely French production was preferable from an environmental point of view.

## Future outlook

To promote the use of biobased materials in the development of a bioeconomy, future research should focus on optimising the production processes of these materials to increase their market competitiveness.







© UR BIA-INRAE

## From studying fruit skins to developing new elastomeric materials ...



### Read more

Marc M. *et al.*

Bioinspired co-polyesters of hydroxy-fatty acids extracted from tomato peel agro-wastes and glycerol with tunable mechanical, thermal and barrier properties

Industrial Crops and Products . 2021

<https://doi.org/10.1016/j.indcrop.2021.113718>

### Partnerships

INRAE (TRANSFORM BIA, IATE, UCAI),  
GEPEA, ITQB Lisbonne

### Valorisation

Patent

Bakan, B., Marc, M., Lourdin, D., Leroy, E., Lopez, C., Valentin, R., Mouloungui, Z., Marion, D. Method for preparing an elastomer from a hydroxylated fatty acid and elastomer obtained by such a method. Ref. FR1906915

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### Context

All plants are covered by a cuticle that provides crucial biological functions for the plant (e.g., resistance to dehydration, adaptation to climatic and biological stresses). This cuticle is also a source of original substances that are currently undervalued, and so we developed a biorefinery process to exploit those substances.

Glycerol is another agro-industrial co-product from the oil and biodiesel production process. The study of the fine structure of cutin polyester in crack-resistant plant cuticles led us to mimic this polyester using a solvent- and catalyst-free polycondensation process.

Inspired by the natural polymer of the plant cuticle, our aim is to produce hydrophobic elastomers from synthons extracted from agro-industrial waste (i.e., cuticle monomers and glycerol).

### Results

By modifying the ratio of these two synthons, it is possible to modulate the degree of cross-linking of the polyesters and their functional properties, such as their thermomechanical and barrier properties (e.g., oxygen permeability or adhesion of pathogenic bacteria). In the co-polyesters formed, increasing the amount of esterified glycerol (up to 6 %)

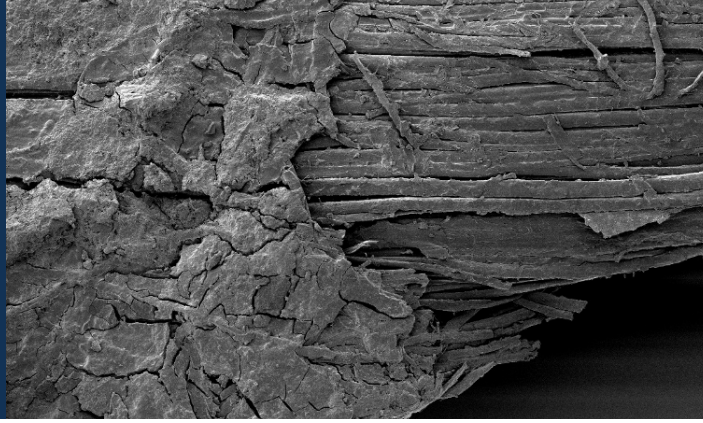
induced a decrease in the cross-linking rate of the polyesters and the formation of crystalline domains. These structural changes are directly correlated with:

- i) an increase in rubber elasticity (to over 200 % extensibility),
- ii) a reduction in oxygen permeability and
- iii) a reduction in bacterial adhesion.

### Future outlook

This research opens up opportunities where several thematic fields overlap, such as being able to:

- i) determine biorefinery processes for the exploitation of agro-industry co-products (biomass use, bioeconomy)
- ii) design new biobased and bioinspired materials
- iii) develop new hypotheses on how plants adapt to changes in their environment.



© Anthony MAGUERESSE and Alessia MELELLI - Flax threads from a painting covered with layers of different materials (glue, plaster, paint, etc.) viewed under a scanning electron microscope.

## Old fibres from art objects can be used to design sustainable ecomaterials for the future



### Read more

Melelli A. *et al.*

Lessons on textile history and fibre durability from a 4,000-year-old Egyptian flax yarn

Nature Plants . 2021

<https://doi.org/10.1038/s41477-021-00998-8>

### Partnerships

- Henri Dupuy de Lôme Research Institute (IRDL, CNRS/ENSTA Bretagne/Université Bretagne Occidentale/Université Bretagne-Sud)
- School of Science and Technology (SST) of the University of Camerino (Italy)
- 'Capitani-Segre' magnetic resonance laboratory of the Italian National Research Council (CNR, Italy)
- Mechanical and Civil Engineering Laboratory (LMGC, CNRS/University of Montpellier)
- Synchrotron facility SOLEIL
- UR BIA

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### Context

Organic materials such as flax fibres, composed of renewable biogenic carbon, are often considered to have a short lifespan, but they can last for centuries or millennia by accommodating structural and biochemical changes. Flax can be sustainable, both in terms of the environment and as a material. To better understand the secrets of its durability and its limits, a study was conducted on linen threads extracted from four paintings dated between the 17<sup>th</sup> and 18<sup>th</sup> centuries. To further complete our study of these hundreds-year-old samples, we also looked at mortuary linen fibres from Egypt, dated to 4,000 years ago. These two complementary studies pinpoint how the environment, in addition to the extraction process of natural fibres, creates defects in the structures and plays a key role in their preservation and performance. This research brought to light ways to create the most sustainable ecomaterials possible to meet today's challenges.

### Results

Our results show – counter-intuitively – that the fibres extracted from the paintings dated a few hundred years ago have undergone more marked changes than the thousands-year-old fibres from Egypt. A general stiffening was found in almost all of the ancient fibres compared to a modern reference linen thread,

but the threads in the paintings also showed structural changes not only in the structural defects already identified as weak points by the study of Egyptian threads but also due to other issues. These include oxidation and hydrolysis, which break up the fibres into small pieces, or fungal attacks, which more than any other factor contributes to degradation, through the formation of 'tunnels' and fractures in the fibres.

Despite the effects of ageing, two-photon microscopy showed good stability in the organisation of cellulose (flax is 80 % cellulose). Solid-state NMR and infrared spectroscopy provided additional information on changes in the biochemistry and structure of the fibres. The interdisciplinary team identified the major role of water penetrating and damaging these materials through their structural defects.

### Future outlook

These findings provide insights on the ageing of modern flax fibres, which are used, for example, in the design of composite materials for the automotive, nautical and aeronautical industries. Based on the strength of these results that could impact various disciplines, a new ANUBIS project has been launched, financed by the French National Research Agency (ANR, topic Bioeconomy).

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