

### THE DREAM Project Book of Results

Design and development of

REAlistic food

Models with well-characterised micro- and macro-structure and composition





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# Chapters



### Foreword

DREAM for "Design and development of REAlistic food Models with well-characterized micro- and macrostructure and composition" was an EC-funded FP7 large-scale integrating collaborative project who started in 2009 for almost five years. DREAM embarked 18 partners from nine countries on a very stimulating scientific and technological adventure around the question: how could the consumer demand for safe, high quality food be supported by developing modeling approaches?

The DREAM ambition was to develop food models realistic enough to be used by the industry and sufficiently versatile to be used as predictive tools to understand the impact of changing composition and/or processing conditions on food quality. The ultimate goal is to save time in the development of an innovation and to provide decision support for food professionals.

In this book we present the results obtained by the different teams on the four generic categories of food. These categories were chosen according to their generic structures: filled cellular solid (fruit and vegetables), proteinous cellular network (meat), combined gelled/dispersed systems (dairy products and cheeses) and open solid foam (cereal products such as bread and biscuits). In practice, on each product, the DREAM project has developed three types of models. First, generic models foods for which the relationships between the structure, chemical composition and functional properties are known. For these realistic physical models, several parameters can be varied (fiber content in cereal products, type of fat in dairy matrices...), leading to a series of samples representative of each type of food studied. Second, in-silico food models which allow simulating for example the role played by the temperature, pressure, chemical composition, etc. on the food structure and resulting material properties. Third, integrated knowledge models to bring together the technical expertise gained by professionals and the data obtained from research. This modeling uses the most advanced recent cognitive science and complex systems science to extend the methods developed in other food products. Such models also allow assessing the impact of processing conditions on the nutritional food properties and safety issues.

The applicability of the model foods and food models was assessed before transferring the protocols and disseminating the gained knowledge to industry and other stakeholders through demonstration training. To support the practical application of realistic food models a Practical Guideline on use of models was developed. The main objective of the guideline was to provide an overview on different food models and on modeling tools/software for the industrial users in order they benefit from models to optimize their existing processes or to come up with new ones to develop innovative products.

Dissemination to the general public, students, scientists, industry, food authorities was made through DREAM website, E-news releases, peer reviewed publications, workshops, training and congresses and at the DREAM international conference. All results are available on the DREAM web site: http://dream.aaeuropae.org/

Finally, I would like to thank all colleagues and teams who have contributed to the DREAM project and congratulate them for their achievements. It was also a stimulating collaborative public-private research challenge and an incredible human adventure.

#### Monique Axelos

INRA - Head of the division for science and process engineering of agricultural products DREAM project Coordinator



numeric simulation mathematical knowledge

#### Task 1.1

Generic structure for the modelling approach: The objective of the task was to propose a structure of the way to integrate knowledge that could be available for all the cases studied in WP2 to 5.

#### Task 1.2

Construction of the numeric food model and uncertainty management: In deep interaction with the researchers of WP3 to 6, mathematical concepts and methods have been established and applied to food models of DREAM. The main objective in this task was to integrate the knowledge (generated by the experimental trials in WP3 to 6), the technical expert knowledge extract from WP1T1.1 and the uncertainty on the knowledge as to reconstruct the dynamic network at each time linking the scales of the system under process conditions.

#### Task 1.3

**Reverse engineering using the numeric food models:** In this task, the challenge was to develop methods able to help to determine the sequences of actions driving the food process along admissible evolutions and requirements. The problem was turn into a theoretical adaptation of a viability approach initially developed Saint-Pierre (1994) for dimension lower than 6.

#### Main outcomes

Numeric IKM's able to answer to the problematic fixed under the WPs have been developed. Manage the uncertainty and lack of knowledge on some phenomenon was a prerequisite. Approaches of modeling, coupling stochastic and mechanistic approaches in the form of equations or graphical models has thus been developed and applied to food models. For example an IKM has been developed and validated as to contribute to the understanding of the structure and texture dynamics of a dairy oil-in-water emulsion. It has been applied to a dairy dessert (Foucquier et al., Descamps et al.) and generalized to a cream cheese model (Jelinko, D.) in collaboration with INRA Nantes and IFR Norwich. An IKM mimicking the thermal treatment of a broccoli including guality degradation and microbial Inactivation has also been developed to be used for decision support purposes in collaboration with the WUR Wageningen (Gaucel, et al) and the ADRIA Quimper. A graphical model of biscuit baking was also in test in INRA, GMPA in collaboration with UB. Theoritical developments at the interface of applied mathematics and computing have in parallel been proposed (1) to help and simplified the modeling task (Tonda, Lutton et al., 2013). (2) to help the reverse engineering task by a coupling between viability theory and methods of clustering (Alvarez et al., 2013) and otpimisation.

### Introduction of WP1

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#### Aims and background

The challenge of the WP1 was to develop or adapt new applied mathematical tools able to predict the emerging organization of a food model at different scales and the functions associated to it, so called IKM's (Integrated Knowledge Model). To do that, several authors had shown the necessity to resolve the enormous challenge of unifying complex and dissimilar data, knowledge and models, specifically to understand the dynamics of such a complex food system (Perrot et al., 2011). It is particularly true when it is applied to real systems. Food structure prediction and structure-function relationships are only well established for simple - or "simplistic" - models of foods (gels, emulsions, dry foams...), which are far from the foods they are aimed to represent, at least for their composition and function, and thus useless with respect to industry's needs. Nevertheless the improvements of food processes by optimization methods are restricted to the few applications where the mathematical modeling is complete (Banga et al., 2003). In this context the development of mathematical approaches having the ability to take into account the heterogeneous knowledge and simultaneously the uncertainty on the system were promising and has been investigated in DREAM.

#### Prospects

Promising results have been reached to facilitate the task of numerical modeling in food science. Such models can be of a precious help in a context of sustainability, either through an help for a better understanding of the emerging organization of the food model during its elaboration or by optimization of the process based on numerical simulators.

# In silico comprehension and prediction of the structure and texture of a dairy dessert

Dairy products have been experimentally shown to behave like complex systems: Their resulting textures depend on various factors, including their composition and their processing conditions. Out of these processing conditions, the most influential are: the nature of heat treatment, processing parameters applied during acidification and during the homogenization process. Nevertheless being able to predict the texture upon process conditions is an interesting challenge for the industry. The work developed under the WP1 was to answer to this challenge by developing an in silico model able to integrate the available knowledge and the uncertainty on the domain. The originality of the approach is about the integration of recent theoretical developments crossing over applied mathematics and computing science. The predictions of the model have been validated on experimental data reached under the WP4 applied to different neutral dairy emulsions and generalized to a cream cheese model.

#### Research aims and background

Dairy products have been experimentally shown to behave like complex systems: Their resulting textures depend on various factors, including their composition and their processing conditions. Out of these processing conditions, the most influential are: the nature of heat treatment, processing parameters applied during acidification and during the homogenization process (Foucquier, 2011). From an industrial point of view texture of dairy products have crucial importance.

Consumer appreciation of dairy desserts such as gels, yogurts or cream cheese variants is influenced by the texture of the product. Due to difficulties of thorough experimental product characterization along the production chain, mathematical simulation and modeling approaches are well suited tools to gain deeper understanding of how the composition and some of the processing parameters can be related to the final structure of the product. Nevertheless the task is not easy because of the complex interactions that can occur between key variables at different scales (Perrot, 2011) if we want to mimic a real system. The model proposed is based on knowledge originated from

different domains (physical chemistry, microbiology, computer science, applied mathematics, etc.). dairy dessert, the story starts at a lower scale : the nanoscale level (Descamps, 2013) (Figure 1). A dairy dessert is an oil in water emulsion stabilized by milk proteins. From the behavior of each type of the particles, whey denaturated proteins or native whey proteins, agregates of proteins and casein micels, depend the organization of the macrostructure of the gel. Particles are indeed in competition to colonize the interface of a fat globule. From this dynamic system is reached a more or less regular and stabilized fat globule interface. From this individual interface and the connexion between all of them, is going to emerge a connected organization at an upper level. We propose an integrated model that mimic in silico this competition. Knowledge of the laboratories working in the WP4 has been integrated in the structure and some parameters of the model. Last developments crossing over applied mathematics and computing science have been included in the mathematical functions allowing to couple deterministic and stochastic algorithms.

### Significance and benefits

The model is a coupling between a first order differential kinetic model of protein denaturation (M1) and a stochastic model of simulation of the fat droplets interface colonisation (M2a) and bridge creation between fat droplets (M2b) (Figure 2). Coupling M1 and M2 allows to simulate the emergence of a network at a mesoscale level from local droplet considerations. The inputs needed are the initial relative concentration of each particle in the solution, the droplet size and volume distribution and the thermal denaturation rate. The fat droplet local interface organization is predicted through two dimensions: the interface composition: % of the different particles fixed at the interface and interfacial concentration. A good prediction is observed for data reached during experiments led by the WP4 partners (Descamps, DREAM congress). The macroscopic structure is explorated upon the number and organization of the links between the particles in the space. For example for the three experiments represented figure 3, the percentage of caseins has been evaluated by the experts to have a strong influence on the perception of the texture of the product which has been shown in the literature to be in link with the number of fat droplet connections. The prediction is in good accordance with this knowledge. Thus for a ratio 80/20 of casein/whey proteins, a weak viscosity is measured (40 Pa.s at 0.001s-1) and few local interfaces are predicted to be connected. In the contrary for a ratio 5/95, the gel is structured with a viscosity of 2600 Pa.s at 0.001s-1) with a prediction of a highly connected structure.

This approach has been generalized and applied in collaboration with data coming from the industry to a cream cheese model (Jelinko, 2013). The experimental data and model simulations show similar tendencies, also demonstrated in previous studies, where increasing homogenization pressure in cream cheese production resulted in increased storage modulus ('G) in the interval of (0 – 50 Mpa) and increased texture and number of links.



Figure 1: Interaction of casein micelles (only interacting parts are shown), casein from heated milk with attached whey protein/ $\kappa$ -casein complexes. These complexes prevent coagulation even if the  $\kappa$ -casein is removed and provide points of attachment between micelles during acid gelation (Dalgleish & Corredig, 2012).



Figure 2: an IKM in silico model of a dairy dessert including heat treatment and homogenization



Figure 3: Example of the in silico IKM prediction at the macroscopic level

#### Prospects and challenges

This work is a first contribution able to predict real dairy dessert structures crossing over the scales. The fertilization of the different disciplines: food, computing and applied mathematics has led to a model of understanding and comprehension that can be used by the industry for optimization or sustainability purposes.



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#### Results and applications

A generalisation of this approach is needed to be sufficiently generic to cover the wide range of dairy desserts produced in the industry. For that a deeper understanding of what it takes place at the interface is necessary coupled to complex system tools developed specifically to handle this understanding and to propose way of process optimization.



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# Reduction of complexity of an in-silico milk-gel model, using visualization and optimization

# Dairy products, complex system, in-silico model, reduction of complexity and visualization

Replicating in-silico the structuring dynamics of food models is a relevant challenge for a better understanding of these systems. It is then important to simplify as much as possible the structure and the number of parameters of in-silico food models: given their intricate structure, however, it becomes hard for experts to thoroughly explore the behaviour of the system and search for meaningful correlations between parameters. In this work, developed under the WP1, we combine visualisation with model exploration to search for correlations in an established computer model of a milk gel, following the subsequent steps: (a) data are collected during the computations of a learning algorithm, (b) data are made available via a multidimensional visualisation tool, (c) subset selection tools and navigation in the multidimensional parameters space help the expert to evaluate the behaviour of the model. Through this approach, we found a correlation between two parameters of the model, that we were able to support with a formal analysis.

#### Research aims and background

If the structural characteristics of pure protein aggregates submitted to heat treatment are widely studied (Rabe 2011), research on aggregates of casein coupled to whey proteins (denatured or not) is still in the initial stages (Morand 2012). Models that are built become more and more complex, and necessitate the use of robust and efficient algorithmic techniques. This work is a contribution to the design of such complex models: it addresses the question of parameter learning using robust optimization techniques and visualisation. The issue here is to show that the observation of the behaviour of an optimization algorithm yield important informations about the optimization problem itself, and as a consequence on the model under study. The experiments conduced in this work were based on an evolutionary algorithm (EA), a stochastic optimisation technique that relies on the computer simulation of natural evolution mechanisms. EAs are specially well suited for the resolution of difficult optimisation problems, and particularly for learning the optimal

parameters of complex models (Baeck 1993). Classical uses of EAs only consider the best individual of the last population as an estimation of the optimum, but recent works points out the potential benefit of visualizing data collected during the execution of an EA (Lutton 2011), and shows how a multidimensional visualisation tool, GraphDice (Bezerianos 2010), can help to efficiently navigate inside the data set collected during the execution of an EA.

#### Significance and benefits

Even for skilled scientists, it is often extremely hard to validate the behaviour of an in-silico model: due to complexity an extensive exploration of the search space is often impossible. The proposed methodology generates a limited amount of data that is likely to be of interest for the user.



Figure 1: In-silico model

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#### Results and applications

Data has been collected during two emulsification experiences (respectively used as training and validation sets), where the continuous phase of the emulsion is formed by dissolving milk proteins in permeate. The analysis is based on a model previously developed (Foucquier 2011), that predicts the structure characterised by the percentages of adsorbed caseins and native whey proteins, and the interfacial concentration. This model depends on 5 unknown parameters, that can be learned from experimental points (learning set), using an EA that searches a 5 dimensional space. A visual exploration (using GraphDice, see figure) of the set of points visited during the optimisation process shows a convergence toward a rather large area of values for a couple of parameters (see red points of Figure below), highligthing some evidence about a possible dependence between these parameters. This evidence has then been confirmed by a mathematical rewriting of the differential equations of the model, allowing to consider 4 unknown parameters instead of 5. An optimisation run within this reduced search space yield a good fitting of the 4 parameters model. These results have been confirmed on the validation set.

#### Prospects and challenges

This work can be extended to a general methodology for model exploration. Such a technique could be invaluable to assist experts in assessing the validity and the weak points of their in-silico models, both in the agri-food and other domains.

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#### Viability theory, kd-trees, controlled dynamical system

The mathematical viability theory is very useful when trying to confine controlled dynamical systems into a set of desirable states. It defines states area (the viability kernel) where the system can evolve safely.

Unfortunately the computation of the exact kernel is a complex and computationally intensive task. We propose a method coupling viability algorithm and active learning with kd-tree in order to provide a compact representation of the viability kernel and to limit the call to the model, which, in case of food models, are generally time consuming. Viability theory is a set of mathematical and algorithmic methods proposed for maintaining the evolutions of controlled dynamical systems inside a set of admissible states, called the viability constraint set. This framework has proved in the recent past to be useful for food control in domain where the knowledge of what exactly is a good candidate objective function for optimization is not so clear [Sicard et al, 2012]. Unfortunately the algorithms that are available presently have a complexity which is exponential with the dimension of the state space. This is a severe limitation to the use of the method in real applications. The work we present aims at providing a compact storage of the set being computed, in order to limit the number of calls to the dynamical model. It aims also at being reusable.

#### Results and applications

An algorithm based on kd-tree has been developed in order to learn the boundary of an hyper-volume [Rouquier et al, submitted], considering the viability kernel as a classification function [Alvarez et al, 2010]. The objective was to limit the number of call of the underlying model. A second algorithm has been developed in order to compute the viability kernel of a viability problem (a dynamical system defined as a black box and a set of desirable states), using the previous kd-tree storage algorithm. This new algorithm has been proved to converge to the true viability kernel [Alvarez et al, 2013].

#### Research aims and background

This work is a crucial step in order to provide a complete set of tools to compute the viability kernel and capture basin of viability problems. The code of the algorithm is open source and will be freely available at the ISC-PIF forge. Sustainability problems (such as resilience study) and control problems which can be defined as viability problems could then be studied with the algorithm we provide.

### Prospects and challenges

This method focuses on the boundary of the viability sets rather than on the sets themselves, so it allows to consider state space with one additional dimension. But it still suffers the curse of dimensionality. Further work is in progress in order to parallelize the refining part of the algorithm.

Real application of the algorithm will also be implemented in order to validate the platform.



Figure 1: Level sets of the capture basin of a viability kernel (bilinguism), to define the resilience of the system.

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# An integrative modelling of quality and safety of vegetable during thermal treatment: application to the cooking of Broccoli

Mathematical modelling can be very useful in the food industry for control and design of processes. Thermal treatment is a widespread process and literature provides several models, from mechanistic to purely based on data. Unfortunately, they often focus on the process itself or on a single macroscopic indicator of the food product. As a consequence, model coupling is a required step to provide an overview of the food macroscopic characteristics. Process control so implies to find a tradeoff between complexity of the model and accuracy of the prediction, allowing to use the tools of control theory. In this work, we propose a model taking into account food quality, including consumer acceptability, and food safety, applied to the cooking of Broccoli. The results show a satisfactory prediction, albeit improvable, of the experimental data. Furthermore, the low complexity of the model make it a good candidate for control application.

#### Research aims and background

Glucosinolates (GSs) are beneficial components present in Brassica vegetables, which shown an ability to reduce the risk of several cancers. The concentrations of these compounds are strongly affected by the processing of vegetables, especially the heat treatment. A model has been developed to describe the fate of GSs during thermal processing (Sarvan et al. 2012). However, this model alone is not suitable for process control as maximisation of GSs concentration corresponds to raw cabbage. Indeed, optimisation of the control temperature only makes sense for models requiring antagonistic constraints on temperature , e.g. low temperature to preserve the GSs content and high temperature to ensure food safety. Our aim is to extend the previous model to other macroscopic features of vegetable during heat processes, related to the consumer acceptability (colour, texture) and the food safety. Literature provides simple models for this purpose, dealing with texture (Rizvi and Tong 1997) and colour (Tijskens et al. 2001). Inactivation of foodborne pathogens is extensively studied but the recent works mainly focus on the Weibull frequency distribution model (Mafart et al. 2002).

In addition, efficiency of the tools from control theory is directly related to the dimension of the model. The issue of this work is to show the feasibility of well-predicting the dynamical behaviour of several macroscopic characteristics in a low complexity model.

#### Results and successful application

A model has been developed in order to reproduce the dynamics of the concentration of a single (or mean) GS in vegetable and cooking water, colour and texture of the vegetable and concentration of the biological pathogen. The single control variable is the temperature which is assumed to be homogeneous in both vegetable and cooking water and equal to the temperature of the heating device. As there is no available data on thermal inactivation of a specific biological pathogen for this study, a "virtual" biological pathogen is considered, allowing to test different thermal resistances. Finally, the enzymatic degradation of GSs was neglected so that all sub-models (GSs, texture, colour and biological pathogen) are independent, the only link being the temperature dependency. The corresponding mathematical system (not shown) is a set of 7 ordinary differential equations with 16 parameters. Parameter estimation has been performed on data from the cooking of Broccoli. The fitting between experimental and simulated results, for some of the state variables, is shown in the Figure 1. Due to the low complexity of the mathematical system, analytical solving has been performed, leading to expression of direct function of time for all the state variables (result not shown).



Figure 1: Fitting between experimental and simulated results for some of the state variables for istothermal heat treatments. Hatched areas correspond to targets at the end of the process.

#### Prospects and challenges

While the presented model shows an acceptable fitting of the experimental data, it must be extended to a larger range of control temperatures, including kinetics. Further work is in progress to apply viability analysis tools in order to find control temperature kinetics leading to the identified targets.

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# Semi-supervised learning of a biscuit baking model, using symbolic regression and Bayesian networks

Machine learning methodologies can be an important aid to modeling experts in the food industry, allowing them to obtain reliable models with more efficiency. While human expertise cannot be replaced, it is possible to exploit automated techniques to obtain several candidate models from which an expert could later choose the best, or draw general conclusions on recurring patterns. In this work, developed under the WP1, we perform a feasibility study to learn the model of an industrial biscuit baking process, using two different machine learning paradigms. Symbolic regression is employed to obtain a set of equations, starting from raw data; and an interactive approach is used to learn a Bayesian network model, after discretizing the original dataset. The results show a good prediction capability, albeit further improvements must be studied in order to produce physically meaningful models.

#### Research aims and background

Machine learning techniques have gained increased popularity in recent years, mainly due to the increasing complexity of problems faced in industry. Symbolic regression, an evolutionary technique based on Genetic Programming (Koza 1992), is able to automatically reconstruct free-form equations from data, uncovering hidden relationships between variables in a dataset. Commercial software using symbolic regression is already available to the public domain (Schmidt 2009). While symbolic regression can work on the original data with no modification, models produced can be very complex and not adherent to the physical reality of a process, thus being hard to understand for a human expert. Bayesian networks are graphical probabilistic models that work with discretized variables. They represent a set of variables and their conditional dependencies via a directed acyclic graph, and are widely used to represent knowledge in many different domains, ranging from computational biology to decision support systems. While the discretization of variables might introduce further sources of error, Bayesian network representations are intuitive for the end user; they can be validated by experts of a specific process with little to no knowledge of their inner working;

and they can be even manually modified. Several research lines work on the automatic and interactive reconstruction of Batesian network starting from data (Tonda 2012) and several libraries have been developed for the purpose (Druzdzel 1999). Our aim is to perform a feasibility study on the application of these techniques to the food domain, verifying whether it's possible to produce reliable models for a specific industrial process.

#### Results and successful application

Data has been collected during 16 runs of an industrial baking process by the company United Biscuits: 12 runs are used for training, 4 for validation. The variables measured are top and bottom flux of heat in the oven, color, height, and weight loss of the biscuits. Additional features include the temperature in each zone of the oven, which might vary between different experiences. Both machine learning techniques are trying to find models that predict the considered variable at instant t+1, having access only to observable values (such as top flux, bottom flux, heat in the different zones of the oven at time t, and initial value of the considered variable at time t=0). Symbolic regression is able to find several good equations to predict color, height and weight loss, performing well also on the validation set.





The Bayesian network obtained through an interactive learning approach shows several relationships between variables that also appear among the best models produced by symbolic regression, thus supporting the findings of the previous step.

#### Significance and benefits

Modeling experts usually need a long phase of trial and error before finding a satisfying model of a complex process, such as is often the case in the food industry. Semi-supervised learning techniques can generate several candidate solutions with good fitting in a small amount of time, from which the expert can then choose the most promising or physically sound. An added benefit is the possibility of examining the candidate models for recurring patterns, which might unveil some unknown relationships between variables of the process.

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#### Prospects and challenges

While the obtained models show a good performance even on unseen data, the machine learning algorithms completely ignore the physical meaning of the models themselves. Further studies on semisupervised approaches are needed, in order to get high-quality results coherent with the physical phenomena underlying the processes.

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fruits & vegetables fruits & vegetables describing observed changes thermal impact on fru mpact on mathematical model mechanical

#### Task 2.1

Selection of three plant food models taking into account works done in FP6 projects, especially EU-SOL, ISAFRUIT, FLORA and FLAVO: (INRA, WUR) In this task the specific varieties and cultivation conditions to obtain the biological material on brassica vegetables, apple and tomato for the further studies have been defined.

#### Task 2.2

Processing of the three plant food models to represent mechanical and thermal treatments of fruits and vegetables by industry: (INRA, WUR) For brassica vegetables the effects of thermal processing on the content of glucosinolates, texture and colour have been investigated. For tomato the effect of hot/cold break on the content and accessibility of lycopene has been investigated. For apple the texture and the characteristics of polyphenols in cell wall polysaccharides were studied.

#### Task 2.3

Characterisation of the macro- and microstructure in the fruit and vegetable GMFs before and after processing in terms of different kinetic, mechanical, chemical, and enzyme events leading to changes in the bioavailability of selected phytonutrients: (IFR) The changes in cell wall structures and composition during processing have been investigated by imaging techniques.

#### Task 2.4

Mathematical modelling (in connection with WP1) to describe the main mechanisms of changes in product structure, phytonutrient (content and availability) and sensory properties during processing: (WUR) A mathematical model has been developed that describes the content of glucosinolates in brassica vegetables based on the various mechanism that occur during thermal processing: cell lysis, leaching, enzymatic degradation, enzyme inactivation and thermal degradation in different compartments. Kinetic texture and colour models have been developed.

#### Prospects

The developed approaches to study fruit and vegetables, as illustrated on the selected varieties and quality attributes in this workpackage, can serve as guidelines to study nutritional and sensory related product properties in fruits and vegetables in a much broader perspective. With limited additional experimental efforts, the parameters of the developed thermal process model can estimated. With these specific parameters the model can be applied to simulate and optimise the content of many phytochemicals within fruits and vegetable varieties.

### Introduction of WP2

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#### Aims and background

To develop well-characterised, realistic, food models for plant foods able to serve as tools for integrating and harmonising food and nutrition research on plant food products. Fruit and vegetables are physiological active products that, even for the same variety, show variation in many characteristics depending on the cultivation conditions (location, soil, weather, light,...) and post-harvest conditions. Therefore even with standardised protocols variation will remain. Therefore there is a need to link processing behaviour with measurable characteristics that are determining kinetics of nutritional changes. Mechanistic mathematical models are needed that are robust to deal with the natural variation and still give meaningful results to be used for product and process optimisation with respect to nutritional, sensory and safety quality attributes.

#### Main outcomes

Selected varieties of brassica vegetables, tomatoes and apples have been studied for their chemical, physical and nutritional properties. For brassica vegetables the main focus has been on developing a mathematical model for thermal processes that can describe the content of phytochemicals (glucosinolates) in the products as a function of processing conditions. In addition kinetic models have been developed for the texture and colour of the products. For tomato the focus has been on relating the processing conditions with the content and accessibility of lycopene. For apples the focus was on the distribution and absorption of polyphenols in the cell walls and on texture development.

# Using mathematical modelling to optimise processing of fruits and vegetables with respect to nutritional and sensory quality

#### Mathematical modelling for improved food quality

The phytochemical content of processed fruit and vegetable products is highly variable and unpredictable. By studying the underlying mechanisms of changes in the contents during processing, mathematical models have been developed to simulate and optimise the thermal processing conditions with respect to the phytochemical content in the final product. Combining these models with models describing texture and colour changes depending on the conditions allows multi-criteria optimisation of fruit and vegetable overall quality. The modelling approach was illustrated with brassica vegetables and the phytochemicals glucosinolates. This case study can serve as a blueprint for the application of mathematical modelling to enhance the nutritional properties of plant foods, while respecting the sensory quality, in a much broader sense.

### Research aims and background

Fruit and vegetables are an important part of our diets. The intake of fruit and vegetables is associated with reduced risk for many diseases like cancers, cardio vascular diseases, diabetes type 2, etc. Phytochemicals in fruit and vegetables show important biological activities related to their health promoting effects. The content of these phytochemicals in current fruit and vegetable products is highly variable. Next to breeding and cultivation, processing and preparation have been shown as main sources of this observed variation.

Understanding the changes in the phytochemical content during processing and preparation allows the development of mathematical models to describe the effects of processing conditions on the final level.

With these models product and process optimisation can be done in order to enhance the phytochemical composition of the final product, while in the meantime the sensory quality attributes of the products are respected.

The results of this research in terms of developed models and the approach to broaden the applicabil-

ity to a wide range of phytochemicals in fruits and vegetables can be used by the food industry to efficiently improve the quality of plant foods with respect to their health promoting and sensory qualities.

### Results and applications

As a case study the thermal processing of brassica vegetables (broccoli, cabbages and Brussels sprouts) was studied in detail. By investigating the mechanisms that are responsible for the changes in the phytochemical (glucosinolates in the case of brassica vegetables) composition during thermal processing models were developed to describe:

- Cell lysis kinetics
- Glucosinolate and enzyme leaching kinetics
- Enzymatic conversion kinetics
- Enzyme inactivation kinetic
- Thermal degradation kinetics of glucosinolates in vegetable tissue
- Thermal degradation kinetics of glucosinolates in

processing water.

By specific experimental set-ups the parameters in these models can be efficiently estimated. In addition to the phytochemical model, also semi-mechanistic models te describe the kinetics of texture and colour changes during processing have been developed and their parameters have been estimated. With the developed set of models process conditions can be optimised with respect to product quality (health promoting effects as well as sensory quality). The developed approach was illustrated on a specific group of phytochemicals in specific vegetables. However the same approach and often even the same models can be used on a much broader range of plant foods and their specific phytochemicals. What is needed to apply this is to estimate the specific parameters in the models for each case. The experimental procedures developed in this project can serve as a guideline for that.

#### Significance and benefits

With the developed models and experimental approaches to estimate model parameters it is now possible to efficiently describe and optimise the phytochemical content of plant food products, while respecting their sensory quality. The results can be used by the food industry to efficiently improve products, but also by nutritionists that want to have an overview of the phytochemical contents of plant foods after processing and/or preparation. Most nutritional data bases only mention the contents in raw materials and perhaps one standard processed or prepared product. With the developed model the effects of many process and preparation conditions can be estimated and used to improve the quality of intake data in e.g. epidemiological studies.



Figure 1: schematic representation of mechanisms that effect the content of phytochemicals during thermal processing of vegetable tissue. (green triangles: phytochemicals, red squares: active/inactive enzymes, beige circles: thermal breakdown products, green squares: enzymatic breakdown products).



Figure 2: simulation results of the effect of a sterilisation process on the content of phytochemicals (GS) and breakdown products (BDP).

#### Prospects and challenges

The developed approaches to study fruit and vegetables, as illustrated on the selected varieties and quality attributes in this workpackage, can serve as guidelines to study nutritional and sensory related product properties in fruits and vegetables in a much broader perspective.

With limited additional experimental efforts, the parameters of the developed thermal process model can estimated. With these specific parameters the model can be applied to simulate and optimise the content of many phytochemicals within fruits and vegetable varieties.

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# Optimisation of mathematical model describing transfer of polyphenol from fruit to juice by understanding non-covalent interaction between procyanidins and pectins

#### Non-covalent interaction between procyanidins and pectins

Procyanidins are the main phenolics in cocoa and many Rosaceae fruit, and have a major role in their bitterness and astringency, as well as potential health benefits. Disruption of the natural matrix during processing and interaction between procyanidins, and cell walls may have a strong influence on the release, on the bioavailability, and on the biological activity of procyanidins. A mathematical model has been developed, which is already applicable to predict retention of procyanidins by cell walls, e.g. in juice extraction processes. However, it needs to be improved by adding the influence of pectin characteristics that are degree of methylation and neutral side chain composition, in order to be used by the food industry to enhance food quality.

#### Research aims and background

Polyphenolic compounds, including procyanidins, are commonly perceived to be found mainly in the vacuoles of plants where they are separated from other cellular components. However, many may also be associated with cellular components, such as the cell wall, especially after cell injury when vacuoles rupture during processing. This results in the release of phenolic compounds which may then associate with cell wall polysaccharides through hydrogen bonding and hydrophobic interactions. These interactions have a strong influence in the release but also in the bioavailability, and in the biological activity of procyanidins.

The cell wall capacity to bind procyanidins depends upon compositional and structural parameters, such as contents and structure of the various cell wall polymers, stereochemistry, conformational flexibility and molecular weight of procyanidins, and cell wall and procyanidin concentrations. It also depends on surrounding conditions, such as temperature, ionic strength or ethanol content. A mathematical model has been developed to describe the transfer of procyanidin from fruit to juice. Among the different polysaccharides classes (cellulose, hemicellulose, pectins), pectins are those that have the greatest affinity for procyanidins. However, the mechanism by which procyanidins and pectins interact and the structural and compositional parameters that influence their association are not known.

Understanding the influence of structural and compositional parameters during procyanidin-pectin interactions may allow optimising the initial model to better describe the effect of mechanical processing on juice procyanidin concentration.

The model developed may be used by the food industry to identify influenced parameters and to simulate them in order to optimise their process and to enhance food quality.

#### Results and applications

The interactions between procyanidins and pectins were studied in details by varying both the composition of procyanidins and pectins. Experiments confronted B-type procyanidin from apple with various degrees of polymerization to the different pectin substructures, such as homogalacturonan presenting different degree of methylation, rhamnoglacturonan I with different neutral sugar side chains and rhamnoglacturonan II as monomer or dimer.



Figure 1: Neutral sugar side chains of pectins limit interactions with procyanidins

The affinity constants of procyanidins-homogalacturonans interactions in solution are the highest when both the procyanidins degree of polymerization and the homogalacturonan degree of polymerization are the highest. Procyanidins interacted with high methylated homogalacturonan mainly through hydrophobic interactions.

Associations between rhamnogalacturonan I fractions and procyanidins involved hydrophobic interactions and hydrogen bonds. No difference in association constants between rhamnoglacturonan I with different neutral sugar side chains and procyanidins

of degree of polymerization of 9 was found. Nevertheless, rhamnogalacturonan I rich in long arabinan chains showed lower association constants, and rhamnogalacturonan I without neutral sugar side chains showed higher association with procyanidin of degree of polymerization of 30. Only very low affinities were obtained with rhamnogalacturonans II. It seems that the ramification state of rhamnogalacturonan I limits their association with procyanidins. This might explain some of the variation in transfer of procyanidins from fruit to juice with maturation, as one of maturation features is loss in galactose and/or arabinose from the cell walls. The influence of degree of methylation of pectins could now be integrated in the mathematical model in order





#### Significance and benefits

Interactions between procyanidins and pectins have been studied in details and could be now integrated in the mathematical model describing the transfer of procyandins from fruit to juice after mechanical treatment. The result obtained could be used to the food industry to improve food quality.

Moreover, the process of fining for the removal of procyanidins for clarification and astringency reduction in wines traditionally uses protein extracts in its application. The wine manufacturer could use the result obtain here to optimise their fining treatment by using fibers as an alternative to fining with proteins in winemaking.

#### Successful applications

The impact of temperature and duration of pressing that were identified in the initial model have been validated by the Institut Français des Productions Cidricoles for use in cider apple pressing.



#### Prospects and challenges

The two main research prospects at this point are:

- Application to understanding the impact of maturation on procyanidin extraction, with a thesis starting on that topic (applied to pears due to their simple phenolic composition and existence of a large range of degrees of polymerization);

- Validation of the impact of the interactions on the colonic fermentation patterns for cell wall – procyanidin complexes.



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# Tomato processing method modify the bioaccessibility of its lycopene

#### Modulation of tomato carotenoid accessibility through appropriate processing

Tomato product is the main source of lycopene of our diet. Its accessibility, i.e. its liberation from the food matrix, and its bioavailability, i.e. the subsequent transfer to its cellular targets at the end of the digestive process, determine its true health benefit against several cancer or degenerative diseases. The accessibility is greatly influenced by the physical properties of the food matrix and especially, it is enhanced in cooked products. Hot break (HB) and Cold Break (CB) treatments used by industries to control the viscosity of tomatoes purees was shown to partially control the ability of the tomato lycopene to diffuse from puree to an oil phase. Food particle size and lycopene/matrix interactions were identified as the main factors affecting the diffusivity.

#### Research aims and background

Modelling the availability lycopene in response to processing was the objectives for the tomato model in the DREAM project. Lycopene has been already identified as a health benefit product. The initial step of the digestive process for such lipophilic micronutrients consists in diffusing from the plant matrix to reach the lipid phase of the emulsion of the bolus. For lycopene, the major tomato carotenoid, bioavailability is known to be enhanced in cooked products. Indeed, if most of industrial tomatoes processes do not much modify the overall lycopene content, its bioaccessibility can be greatly affected. The aims of the research were therefor to identify the factors limiting the lycopene diffusion and how these factors are modified by the processes applied to fruits. Controlling the carotenoid bioaccessibility by the process is a challenge for the fruit and vegetable industries in order to boost the nutritional value of their product.

#### Results and applications

The starting hypothesis to set up the model linked particle sizes to the ability of lycopene to diffuse to an oil phase. Tomato is a filled-cellular model, and then, 1- the smallest the particles generated by the process (grinding, cooking temperature), the quickest the diffusion of one nutrient, according to the second Fick law's and 2- depending of the numbers of intact barriers (i.e. intact cell-wall or membranes) that may remind in the matrix between the lycopene and the intestinal membranes. The case of study chosen for the project was two tomato purees obtained either using cold-break (CB) or hotbreak (HB) processing, which exhibited contrasted lycopene diffusion rate when they were mixed to oil in a standardized protocol. Our first results indicated that no clear difference of particle sizes could explain the contrast that we observed (Page et al. 2012). Wet sieving experiments indicated that most of the HB/CB difference was explained by the behaviour of the smallest particles (Figure 1). We also studied more into details, using one standard commercial tomato juice, the other physicochemical parameters affecting the lycopene diffusion, including pH effect, temperature and how much the diffusion including pH effect, temperature and how much the diffusion is modified when tomato purees where mixed to an emulsion instead of pure oil. For this last, the rate of the diffusion rose, but the partition factor of the lycopene between puree and oil was not much affected. Even more, it was reduced by some interactions with the emulsifier (Degrou et al. 2013). These last results indicated that molecular interactions between lycopene and other components of the matrix may affect the availability of the lycopene, making the model more complex than expected, and making necessary a set of new experiments to recover variables that should be implement into the tomato model.



Figure 1: Threshold effect on particle size. Most of the available lycopene is included into the smallest class of particle sizes (limit at 125µm, i.e. roughly the average fruit cell size).

#### Prospects and challenges

At the end of the DREAM project, the main factors affecting the lycopene diffusion from the matrix have been identified. Modelling needs now to be achieved in order to set up a mathematical model that could predict the availability of the carotenoid from the processing parameters. And, moreover, the identification of the smallest particles as the main factor of the contrast between HB and CB puree make it a further research challenge to understand the biochemical change of the fruit tissues that lead to lycopene release. Structural description of these small particles is a challenge.

#### Significance and benefits

From the Dream results, it is now clear that carotenoid bioaccessibility can be deeply modified by the processing methods, and particularly if the very first temperature ramping (i.e. during the first minutes of heating, which correspond to the main difference between HB and CB purees) is efficiently applied, leading to a quick rise of the initial temperature. In this case a significant quantity of carotenoid is made bioaccessible.

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#### Task 3.1

Rationalisation of meat tissue selection. Three meat categories were characterized in composition and structure and two distinct muscles were selected (Longissimus thoracis and Masseter). Main raw meat parameters associated with the nutritional target property (in vitro protein digestibility) were identified. Guidelines to select categories of raw meats based on in vitro digestion were developed.

#### Task 3.2

Prospective investigation to build artificial mimetic gel models representative of animal tissues. Meat tissue structure couldn't be represented using gel models. Thus, kinetic laws which govern changes in proteins' physicochemical state induced by heating were measured using an in vitro model: myofibrillar proteins in a buffer with the various compounds that can be found in different types of meat: Fe, ascorbate, oxidants and anti-oxidants. Heat treatments were applied at 45°C, 60°C, 75°C and 90°C up to 120 min.

#### Task 3.3

Microscopic image analyses of typical different meat tissues to assess their structural characteristics. Methods and algorithms to semi-quantify in situ the morphology and distribution of cell types in muscle tissue from histological images were developed and applied to the meat categories. It was shown from statistical analysis that connective tissue and fibre type distributions vary considerably with muscles and / or collection areas. The overall structure was also assessed from MRI images using a Diffusion Tensor Imaging technique.

#### Task 3.4

Use of artificial gel models to understand reaction routes promoted by heating of animal tissues. While thermal denaturation can be considered as a unique first order reaction oxidation is more complex since many interactive chemical reactions are involved. A mathematical model which accounts for this complexity was successfully developed to analyze and predict the effect of (i) iron content which differs depending on the meat type, and of (ii) the heating conditions through the time-temperature couples.

#### Main outcomes

The physical and mathematical models developed in WP3 proved to be useful tools to evaluate the impact of heating on in vitro protein digestion. These models could be used to evaluate the impact of other conditions or processes applied to proteinous foods on nutritional, sensorial or technological target properties of scientific or industrial relevance. Further research should focus on sensory properties using the defined meat categories and the mimetic and mathematical models will be progressively completed to include all the possible compounds in meat.

### Introduction of WP3

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#### Aims and background

Heating is one of the most important processes applied to animal tissues, since meat is usually consumed after cooking at industrial or domestic scale. This physical process induces structural and chemical changes that can affect meat nutritional value. The work developed in WP3 was focused on meat and the impact of heating on its protein physicochemical changes and digestibility. Homogeneous muscle models are needed to evaluate the effect of different processing parameters on the nutritional quality of meat. One objective was to define the best experimental models representative of meat products by selecting and assessing meat tissues or by creating artificial mimetic samples. Another objective was to evaluate the effect of compositional and structural properties of proteinous foods on the reactions promoted by heating.

#### Prospects

Two models were developed: three meat categories (pork muscles) that differ in their structure and composition, and a mimetic model (suspension of myofibrillar proteins). The pork muscles allowed to characterize their structural variability by imaging and histology, to set up a non-invasive noble method (MRI), and to identify heat induced protein changes linked with in vitro digestion. The mimetic model allowed to evaluate the effect of iron content and temperature on the kinetics of myofibrillar protein oxidation and denaturation. A mathematical 'stoichio-kinetic' model was developed to investigate from calculations the main chemical reaction routes involved in protein changes.



protein digestibility

#### Models to better analyse and control meat cooking

Meat cooking is an important process applied at industrial or domestic scale that has nutritional impact. Two experimental models were developed to investigate how meat characteristics and heating conditions, respectively, affect the nutritional quality of meat: (1) meat categories that differ in their structure, composition and metabolic type, and (2) a mimetic model composed by suspension of myofibrillar proteins which composition can be modified by adjusting the concentrations of major meat compounds: iron, oxidant and antioxidant enzymes. In parallel, a mathematical model called 'stoichio-kinetic' was developed to integrate knowledge. The model is composed of differential equations that represent all elementary reactions. Model predictions agree with the experimental measurements. This approach can be applied to other meat processes and sensorial properties.

#### Research aims and background

Apart from a few exceptions, animal tissues (meat and fish) are eaten after being cooked. Heating is therefore the most important process applied to this type of foods either at industrial or domestic scale. This physical process induces structural changes, at microscopic and macroscopic levels, and promotes protein changes that can have nutritional impacts.

A great number of works had been carried out on meat and fish products to assess the impact of production parameters (genetic, breeding, feeding, slaughter conditions, processing conditions...) on meat product qualities. This is usually done by comparing samples that undergo a specific treatment to a reference sample. However, in technological or laboratory tests it is rather difficult to well control and measure all the parameters that can have an impact on a specific output. This often prevents to generalize conclusions of a particular study and leads to contradictions between studies because of the interactions between sample properties and phenomena involved.

With the aim to improve the generalization of laboratory results, our work was focussed on the development of experimental and mathematical models. We worked in two main directions:

• To define the best way to prepare samples representative of meat products either by selecting and assessing meat tissues or by creating artificial mimetic samples;

• To evaluate, using the above meat models and mathematical modelling, the effect of meat tissue characteristics and cooking conditions on the reactions which are promoted by heating and can have nutritional consequences.

The tools that were set up during the DREAM project can help engineers to develop precooked or cooked industrial products with better nutritional properties. They can be also used by scientists to investigate a larger range of processing conditions: other meat tissue characteristics, other processes.

#### Results and applications

Parallel works have been carried out to develop two complementary experimental models: (1) extreme examples of meat tissues representing three "meat categories" that differ in their structure, composition and metabolic type (Realini et al., 2013 a & b), and (2) a mimetic model composed by a suspension of myofibrillar proteins (Promeyrat et al., 2013 a &b).

Changes in protein state and nutritional value were related by applying two extreme time-temperature heating couples (10 min, 75°C or 45 min, 90°C) to the two extreme meat categories. The nutritional value was evaluated from in vitro digestion tests using either gastric pepsin or trypsin and a-chymotrypsin. It is clear from Fig. 1 that the variables associated with protein changes in conformation and those linked with oxidation are positively and negatively correlated, respectively, with rates of in vitro protein digestion. The mimetic model was developed to avoid the confusing effect due to uncontrolled biological variability generally observed in animal tissue. Its composition can be modified by adjusting independently the concentrations of the major chemical compounds in meat (Fig. 2): iron, oxidant and antioxidant enzymes. This experimental model allows to easily determining kinetic laws that govern changes in protein state and to assess how much the kinetic parameters are affected by initial product characteristics and cooking conditions. Thermal denaturation can be modeled by a unique first order reaction. Oxidation is more complex (many interactive chemical reactions that are differently affected by pH and temperature). Thus, a mathematical stoichio-kinetic model which accounts for this complexity was successfully developed (Promeyrat et al., 2012). Simulation calculations allow to analyze and predict the effect of (i) iron content which differ from one meat to another and of (ii) the heating conditions through the time-temperature couples (Fig. 3). The predictions are accurate enough to be used for practical purpose.



Figure 1: Two meat categories were cooked either "10 min, 75°C" or "45 min, 90°C". A Principal Component Analysis showed that protein denaturation (hydrophobicity) and oxidation were correlated to digestion rates measured in vitro.



Figure 2: Kinetic laws of protein denaturation and oxidation were determined using a mimetic model: aqueous suspension of pure myofibrillar proteins, control of both physic-chemical environment (pH, ionic strength) and compound contents to mimic many kinds of meat.

#### Significance and benefits

Our collaborative work provides a sound basis to build a mathematical tool, or simulator, that can predict the effects of various processes such as chilled storage, modified atmosphere conditioning, curing, heat treatments, etc on the sensorial and nutritional qualities of processed meat products.

This approach needs that new scientific knowledge is progressively added to improve our simulator by assessing parameters associated with each individual phenomenon or chemical reaction. The strategy based on experiments with both realistic and mimetic models is certainly the best way to achieve this goal.



Figure 3: A mathematical model based on differential equations representing numbers of interactive chemical reactions can predict kinetics of protein changes; for example, carbonyl production according to Fe content and heating conditions.

#### Prospects and challenges

We took a big step forward in predicting the impact of practical cooking conditions on protein denaturation and oxidation through the use of both experimental and mathematical meat models. These phenomena affect protein digestibility but also various other technical and sensorial qualities. For example, the kinetic of protein denaturation is certainly linked to cooking losses and oxidation of myoglobin is known to determine colour. Thus, our strategy could be applied to many other targets. Using a mathematical model is essential to extrapolate laboratory results to industrial process conditions. For example, protein denaturation and oxidation can now be predicted for time variable cooking conditions, since all the reactions are represented by differential equations. Moreover, these calculations can be easily inserted in other mathematical models that predict the time evolution of the temperature distribution within meat pieces during cooking (Heat transfer mathematical model based on Finite Element technique).

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# Microstructure characterization of muscle tissue by quantitative imaging

# in situ high resolution imaging of meat tissue microstructure by MRI using diffusion tensor imaging (DTI)

Meat results from the post mortem transformation of animals skeletal muscles. These muscles are mainly composed of water (75%), muscle cells of 3 different types (I, IIA and IIB), connective tissue and fat (3 to 5%). The muscle is strongly organized in fiber bundles more or less aligned depending on its type and functionality. The muscle structural architecture can be roughly modeled as a square lattice array of fluid filled cylinders surrounded by fluids. With the MRI technique used here, we look at diffusion: the physical parameter related to the water local motion. Diffusion in tissues differs from diffusion in free solutions because compartments made by fibers hinder and restrict water motion. The structural meat architecture is hereby assessed, not directly and destructively as done by tomography microimaging (microscopy) but indirectly and non-destructively.

### Research aims and background

Even if diffusion quantitative magnetic resonance imaging is a generic method to assess Brownian random motion of molecules, it has been mostly applied to water which is abundant in tissues and visible by MRI. Both muscular cells size and muscular cells shape as well as interactions within the different compartments of muscular cell can influence the diffusion properties. If the structure is anisotropic, as in muscle, water diffusivity also displays anisotropic behavior. As muscle, and then meat, is highly organized, the diffusion of water is facilitated in the fiber direction, meaning that, in a group of fibers having the same direction, the apparent diffusion coefficient is: maximum in the fiber direction, minimum orthogonally to the fiber direction. We decided to make use of this anisotropy to indirectly and non-destructively characterize meat microstructure. The anisotropic water diffusion can be modeled voxelwise using ellipsoid by means of a first order tensor, according to Diffusion Tensor Imaging (DTI). Moreover, by varying the intensity of the magnetic gradient field, one can obtain the diffusion decay. This decay is then fitted by a bi-exponential curve, at each voxel, in order to obtain quantitative mapping at low and high gradient field values, exhibiting hindered and restricted diffusion, respectively. One first objective was to obtain high resolution imaging of meat tissue microstructure in situ by MRI using diffusion tensor imaging (DTI) at different b-values in order to assess by quantitative imaging the fiber structure of muscle tissue at the mesoscopic scale. Moreover, knowing that the muscle structure can be roughly modeled as a square lattice arrangement of fluid-filled cylinders surrounded by fluid, we can plot the theorical diffusion decay. This plot exhibits deep gaps in attenuation curves that have been directly correlated to the geometry of the cylinder lattice.

We have so attempted, in a second objective, to determine if such attenuation behavior can be found in the real meat structure made of aligned fibers. The tools that were set up in this part of the DREAM project can be used as input for creating artificial mimetic meat sample and to feed mathematical models.
# Results and applications

Regardless of the sample orientation relative to the magnet, diffusion weighted signals were obtained with gradient fields applied in 6 directions and then calculation (tensor diagonalization) was made in order to consider diffusion in the meat fiber oriented frame. The huge advantage of this approach is that the diffusion (closely linked to the spatial fiber organization) is then assessed free of the effects of local fibers orientations. After diagonalization, the first eigenvector corresponds to the main fiber axis direction. The second and third eigenvectors correspond to the 2 orthogonal directions of the fiber axis. The same process was applied to each 6-directions dataset at increasing b-values (ranging 100-20000 s/mm<sup>2</sup>).

#### Quantitative mapping

Parallel and perpendicular decays both deviate from the Gaussian diffusion, expressed by a mono-exponential decay since they follow a bi-exponential shape (Figure 1). This highlights hindered and/or restricted diffusion of water, out of and into muscle fibers, or exchanging between the two compartments. Diffusion maps reveal details due to spatial variations of structure at a resolution much below the acquisition resolution.



Figure 1: Diffusion-weighted intensity attenuation images from the trace of the diffusion tensor matrix. L image (left) is at low b value, H image (right) is at high b value, inhibiting respectively high diffusion corresponding to hindered diffusion and low diffusion corresponding to restricted diffusion.



Figure 2: Superimposition of histological and diffusion image.

# Identification of structural and morphologic components

After MRI experiment, samples were frozen at -180°C to avoid muscular cells degradation and microscopic histological observations were performed. After spatial registration, histological images were first superimposed high-resolution on images (susceptibility-weighted gradient-echo images, with 300 × 300 voxels) to identify morphologic components (fat/collagen network), and subsequently superimposed on quantitative diffusion maps (Figure 2).

#### Relationship between diffusion and muscle fibers types

To assess the architecture of the muscle, we investigated into the relationship between diffusion and muscle fibers types. Histological cuts (approximately 1mm x1mm) were observed after histoenzymological ATPase staining with pH 4.35 preincubation. At low b-values, diffusion parameters seem to correlate with metabolic characteristics of meat fibers, as highlighted by photomicrography of areas characterized by histoenzymological ATPase staining (Figure 3).



Figure 3: Superimposition of histoenzymological ATPase stained and diffusion images. On the histoenzymological images the three intensities are black (type I), white (IIA) and grey (IIX plus IIB). Diffusion is mapped with a blue/green to orange/red scale.

#### Variability of the dimensions of structural components

Less deep minima amplitudes on acquired data in homogeneous region are observed compared to the prediction of the mathematical models. The experimental data plot deviates from the theoretical plot mainly due to the heterogeneity in the fibers diameter, the no strictly parallelism of the fibers and also perhaps the no strictly impermeability of the fibers membranes.

# Significance and benefits

It is essential in the building of a mimetic model where structural components are involved to precisely access these components in situ on real material. The tools that were set up in this part of the DREAM project and results describing structural meat components can be used as input for creating artificial mimetic meat sample and could be applied to many other targets. We have showed that characterizing the behavior of water diffusion within a voxel provides a means for describing the inner microstructure at a cellular scale, taking into account apparent diffusion coefficients measured in muscle. Using the anisotropy of water diffusion due to the highly fibrillar structure of meat, we modeled this diffusion in three dimensions using tensors. We used Diffusion Tensor Imaging (DTI) with different b-values to obtain high resolution diffusion parameter mapping of tissue which were registered to highresolution susceptibility-weighted gradient-echo images and histological images to determine if there existed relationships between meat microstucture and diffusion observed at a meso-scale.

### Prospects and challenges

The results of this research are a step ahead towards the construction of realistic food models such as meat and will make possible to feed the models built in the other workpackages. Promising results have been obtained showing structural details correlated with metabolic characteristics. To the best of our knowledge, it is the first time that a scatter-like behavior was observed in a biological matrix which seems very promising for quantifying structural information from the resulting diffusion attenuation plots. Future efforts will be put on the differentiation within type of meat muscle and meat fibers types and on the variability of the structural components. Furthermore developed methods could be applied on other food product.

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#### Task 4.1

The objective was to produce and characterise a range of gelled / dispersed / aerated generic model foods. The primary model was a dairy dessert that could be used for risk assessment and was tested for use in following lipid oxidation. The changes in properties of the dessert were described as a function of the ratio of caseins to whey proteins.

#### Task 4.2

This task focused on producing a range of model cheese systems that could be used for assessing the effect of changes in formulation or processing on industrially relevant parameters. In particular the effect of salt reduction was assessed in the micro-cheese, the generic cheese and the standardised pilot-scale cheese milk models.

## Main outcomes

This workpackage was split into two tasks, each looking at a different food group, namely dairy dessert and cheese. For the main dairy dessert model, three kinds of rheological behaviour and four different interfacial structures were characterized.

In the first group, the emulsions were liquid, showing no connections between droplets.

In the second group the emulsions were structured, the droplets being weakly connected by WP aggregated.

In the third group the emulsions gelled, the droplets being connected by CM/WP complex or in other case by aggregated WP. In the same task the impact of enzymatic cross linking of the protein was demonstrated and in a slight variation to the basic model the impact of homogenisation pressure on the textural properties of cream cheese was also demonstrated.

The studies on cheese models yielded a generic cheese model for studying for studying the effect of composition of texture and bacterial growth. The use of a micro-cheese model for high throughput screening of bacterial growth was also demonstrated. Finally the primary cheese model yielded a robust protocol for the standardisation of the milk used for pilot scale production of experimental cheeses with very low variability. The usefulness of the approach was demonstrated for a wide range of cheese types including Camembert, Brie and Swiss cheese.

# Introduction of WP4

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# Prospects

At the start of the project there were no gelled or dispersed model systems that could be consistently reproduced and that could be made widely available for testing nutrient, allergen or toxicant release, microbiological safety etc.

Therefore we aimed to produce well characterised, realistic, food models for these types of food systems including cheeses and desserts that could serve as tools for harmonising food quality, safety and nutrition research on these types of food products.

We also aimed to produce mathematical models that could relate model composition such as fat content, protein ratio and processing to model functionality such as nutrient release and texture.

# Aims and background

The two main models produced from this WP both have great potential for the future but the development of the models also leaves a number of questions unanswered. The standardised cheese milk model is already being used by Actalia in the TeRiFiQ project and a simplified version has been developed by Soredab for use with SMEs who do not have the expertise or equipment to use the complex model. Additionally, results from the dairy dessert model are being combined with other data from the literature to build an integrated knowledge model of protein based emulsion systems that will be much more predictive in nature.

# The Dairy dessert: A model for designing tailored interfaces in food systems

#### Composition and structure of interface impacts texture of emulsions

The main objective of this work was to understand the impact of interfacial composition and organization on the connectivity between fat droplets and the microstructure of oil-in-water emulsions.

Four kinds of object were obtained by different processes applied to proteins solutions containing various protein ratios. Their different properties led to competition between these objects at the interface generating four types of structure with different connectivity leading to various rheological properties: liquid, structured or gel. In complex dairy desserts, only the presence of aggregated whey at the interface increased the firmness of these desserts. When emulsions and dairy desserts were enriched in PUFAs, the oxidation of lipids remained low after 2 months storage at 4°C.

## Research aims and background

Most processed foods contain gels and dispersions of some sort that consist of small particles such as fat or protein dispersed in another medium. There are currently no gelled/dispersed model systems that can be consistently reproduced and that can be made widely available for testing nutrient, allergen, toxicant release, microbiological safety etc. The work described here addresses this issue with a standardised dairy dessert model. The production of the dairy dessert is built on experience at IFR (UK), INRA and Soredab (France) in studying and understanding proteins/surfactant interactions in emulsions and foams, fat composition, and on the use of biopolymers such as starch or pectin as thickening/gelling agents. We hypothesized that changes in protein structure caused by heat treatment could induce different structures of the interfacial layer (difference of thickness and of homogeneity) with or without disulfide bridges.

These different morphologies would then have an impact on the structure and the texture of emulsion, that could moreover affect release and bioaccessibility of nutrients contained in fat (PolyUnsaturated Fatty Acid  $\omega$ -3, for example) or digestibility of

proteins. Consequently, the aim of this study was to evaluate the combined effect of heat treatment and CM/WP ratio change, on the structure of interfaces, in relation with the impact on the texture of O/W emulsions.

For that, our strategy was to generate emulsions with a range of well-defined different interfaces and combining different compositions (weight ratio of CM to WP: 80:20 to 12:88) and heat treatment of milk proteins (60°C or 80°C).

## Results and applications

The results of the work on the dairy dessert have been published in a series of papers from INRA Nantes and INRA Grignon (Foucquier et al. 2011; Surel et al. 2013). The way the dessert model is made is shown in Figure 1. Initial measurements determined the mean size of the casein micelles (CM) to be 140-160 nm regardless of processing temperature (60°C or 80°C) whereas the whey protein (WP) increased in size from 6 to 100 nm as the processing temperature increased. The emulsion droplet size was remarkably insensitive to either CM/WP ratio or temperature.

The primary controlling factor of the model was the interfacial composition as a function of CM/WP ratio and processing temperature. When the ratio was below 0.2, the interface was dominated by WP and at 0.2-0.3 there was a minimum in the amount of protein adsorbed to the interface. At CM/WP ratios above 0.3 the interface was dominated by the CM and the effect was more marked at the higher temperature. These differences in interfacial composition also had a marked effect on the interactions between the droplets and thus the rheological behaviour of the emulsions.

The overall effect is shown in Figure 2 in which the first group with a liquid texture consists of emulsions whose proteins were treated at 60°C regardless of the CM/WP ratio and those with a ratio of 0.8 or more treated at 80°C. The second group producing a structured liquid with higher viscosity includes emulsions with CM/WP ratios from 0.19-0.26 treated at. The final group of gelled systems are all treated at 80°C and have CM/WP ratios from below 0.15 or from 0.3 to 0.5. In these last systems the emulsion droplets are all interconnected by aggregated protein causing the system to gel. In parallel with the experimental work undertaken to develop the physical model, a mathematical model was developed that aimed at predicting the texture of the final model based on a number of inputs, including the amount of casein and whey, the size of the whey aggregates, the processing temperature and the homogenisation pressure. From this information a number of internal variables are generated that provide information on the amount of adsorbed CM and WP, the amount of aggregated WP adsorbed and the emulsion droplet connectivity. From these variables the structure of the resulting emulsion system can be predicted.

Equipment required in order to produce the model is relatively basic, comprising stirrers, heaters and an Ultra Turrax to make the pre emulsion that is then passed through high pressure homogeniser capable of delivering 50 bar.



Figure 1: Flow diagram for the production of the Dairy Dessert.



Figure 2: Schematic of the different structures induced by composition and processing.



Figure 3: Schematic of the mathematical modelling approached used to link formulation and processing to texture.

# Significance and benefits

This model offers the possibility of using a standardised dessert model with a range of textural properties. The texture of the dessert can be predicted from the amount of micellar casein and whey protein added and from the thermal treatment and the homogenisation pressure used to make the emulsion. The standardised and predictable nature of the model makes it ideal for use in risk assessment involving the growth of pathogenic or spoilage organisms or involving toxin contamination. In addition it is also suitable for use in studies on bioaccessibility or bioavalability using in vitro or in vivo methods respectively.

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#### Prospects and challenges

This model has successfully been used to determine lipid oxidation rates after the incorporation of kiwi seed oil containing high levels of  $\omega$ -3 polyunsaturated fatty acids into the formulation. No statistically significant differences were seen in the levels of oxidation after 2 months of storage at 4 °C regardless of formulation or processing temperature.

#### Successful applications

The development of mathematical models able to predict the formation of structure and thus the texture of dispersed systems represents an interesting prospect for the future. It also offers the possibility of broadening the range of dispersed food systems that can be included in the modelling.

In particular, coordinated approaches to producing healthier food systems with lower salt and/or fat and/or sugar are becoming increasingly important. The development of standardised models of significant food types offers the possibility of designing healthier foods with the desired textural properties and shelf-life.



# A pilot scale model for the reproducible production of soft cheese

#### A pilot scale model for the reproducible production of soft cheese

A realistic cheese model (Brie cheese manufactured with industrial technologies, Figure 1) was built as tools for experimental studies on cheese. A great attention has been paid to the authenticity and repeatability of these small scale models (around 1kg). Twenty replicates per cheese model were made to determine the repeatability of about a hundred state-variables and control-variables: from the milk composition to the cheese qualities. Their coefficient of variation was around 1%-1.5%. For each of these variables, statistical analyses were performed to characterize the dispersion of the data and the origin of the variability, in order to improve the models. Reliability of the models was confirmed in experiments dealing with the influence of salt content in cheese on Bifidobacteria in Brie-cheese.

### Research aims and background

Many problems in the field of dairy research and development require the implementation of cheese making trials. These trials include technological topics such as process modifications, yield, raw material, spoilage microorganisms, sensory studies (effect of starter or adjunct cultures), nutritional questions (reduction of salt or fat content, decrease of the proportion of saturated fatty acids in fat and the use of probiotics and/or prebiotics) and food safety issues (survival of pathogens, presence of harmful chemicals). However, cheese making experiments are expensive and time consuming, even on a pilot scale. They require specific equipment and required environmental conditions are difficult to control. Therefore, several alternatives have been suggested for experimental studies on cheese. The improvement of cheese models and the proposition of a strategy for the development of cheese models, as proposed in our study, are useful for the cheese industry and its suppliers (e. g. enzymes, lactic and ripening cultures, proteins, etc). Indeed, cheese models are too often considered as black boxes in some laboratories and so their improvement will increase the reliability of the results. Indeed, the dairy research contributes in a part of the progress made in the cheese industry by the improvement of the knowledge in the dairy science. More particularly, experimental results on food safety (e. g. growth and survival of pathogenic bacteria in cheese) or on the cheese milk quality are very important for the cheese makers and require suitable cheese models. The screening of the ingredients for cheese making must also be performed using viable models. Finally, the strategy of characterisation of the representativeness and the repeatability can be applied to the pilot plant in the cheese factories.

# Results and applications

Preparation of the cheese milk as outlined in the schematic diagram in Figure 2, starts with the heat treatment of raw milk (88°C/1min). This is to denature the whey proteins and to inactivate v egetative cells of bacteria present in the milk. This is followed by the preparation and addition of protein concentrate to increase of the casein content of cheese milk. The next stage is to adjust the fat and recoverable proteins content which in turn controls the fat-in-dry-matter of the final cheese.

# Results and applications

The last step in the process is the microfiltration of the skim milk (1.4  $\mu$ m) and heat treatment of the cream (120°C for 1 minute) in order to remove spores and thermo-resistant bacteria.

The cheese itself is prepared as follows: The milk is prepared with starters and acidifiers at 39°C for 30 min in order to standardize the pH at renneting. The milk is then coagulated with recombinant chymosin to gel the milk, which is then cut into 1.7x1.5x1.5 cm pieces to promote syneresis. The vat containing the milk gel is then drained and the cheese placed in moulds, which are in turn drained at 32°C for 3h then 18°C. After 1 day the cheese is placed in saturated brine at 12°C for 55 min and then ripened for 12 days (12°C, 96% RH) on grids before wrapping and storing. Mean value and standard deviation were measured for the composition of the cheese milk, day-1 cheeses and the ripened cheeses respectively. A good reproducibility was obtained for all state variables with coefficient of variation around 1-1.5%

The equipment needed for milk standardization is: Pilot scale microfiltration with 1.4 µm ceramic membranes, Pasteurizer, Tri-blender (or mixer) and a skimming centrifuge. Cheese manufacture requires a cheese vat, cutting blades or wires, cheese moulds and a thermostated cheese making room. Finally, cheese ripening requires wire grids and a ripening room (RH and temperature controlled).



Figure 1: Brie style soft cheese produced using the cheese milk model

# Significance and benefits

A detailed, realistic and reproducible cheese model was built and characterized. The model is currently used in our laboratory and could be used as a basis for model development in research institutions or industrial laboratories. A simplified version of the model has been developed in collaboration with Soredab.

#### Successful applications

This model has been successfully applied to study the influence of salt in moisture on the growth and survival of Bifidobacterium lactis BB12. The experimental data obtained from the model was in agreement with previous studies, showing an inhibitory effect of the increase of the salt in-moisture on butyric acid fermentation. Our results obtained in Brie cheese also confirm previous findings which showed little influence of salt content on the survival of B. lactis BB12 in cheese.



Figure 2: A flow chart for the standardisation of cheese milk in the soft cheese model

# Prospects and challenges

This model offers a way of producing pilot scale cheese with low variability. One of the main limitations of the model is its complexity. Therefore, one task for further development is the simplification of the model so that it may be used more widely in the cheese making industry.



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# Coupled impact of product process and composition in lipids as emulsifiers on the structure and the texture of a cream cheese

#### Impact of product process and composition on cream cheese texture and oxidation.

A cream cheese is a dairy emulsion where proteins act both as emulsifiers at the fat globule interface and as protein network components. The objective of this work is to understand the impact of the process and the addition of lipid emulsifiers on the interface composition then on the model texture. The most discriminating step of the process on product's rheological properties is the homogenization. Nevertheless, its influence is modulated by the heat treatment. The addition of lipid emulsifiers decreases the connectivity and hence decreases the product's firmness. These molecules compete with proteins and replace them at the interface. The cream cheese model is not sensitive to oxidation either after intense thermo-mechanical treatment, storage, or enrichment in polyunsaturated fatty acids.

#### Research aims and background

The problem was to have a cream cheese model easy to produce (Fig 1.), with well characterized composition to understand the impact of the process and its composition in lipid and protein emulsifying agents on its structure, texture and oxidation stability.

There are some previous works on the impact of product process on product texture (Sanchez et al., 1996). However, there is a lack of information regarding the influence of each process step on final product.

Moreover, the emulsifying capacity of phospholipids is well known but the influence of its addition on fat globule interface, product structure and texture stays little known.

#### Results and applications

Regarding the impact of the process (described in Fig 2) on the structure and the texture of the cream cheese, the project showed that the final homogenization pressure has a large impact on the cream cheese model texture. When the pressure increases, the fat globule size decreases. This leads to an increase in the specific surface of fat, number of interactions between particles and consequently in the cream cheese model firmness.

The pH at the end of acidification has a very limited impact on rheological characteristics of the model, but it has a significant one on its sensory properties: products acidified to pH 5.2 are considered more spreadable and brighter than products acidified to pH 4.9 (Coutouly et al., 2013).

Regarding the impact of enrichment of the cream cheese with lipid emulsifiers, the project showed that the phospholipids proves to have a larger affinity for fat globule interface than proteins. As low molecular weight surfactants (Mackie et al., 1999), they replace proteins at the interface (Fig 3.). Consequently, the number of interactions between particles decreases and the cream cheese model firmness decreases consequently. The presence of phospholipids results in partial coalescence of fat droplets which yields softer and smoother final products. Finally, regarding the stability of the cream cheese model, enriched or not with polyunsaturated fatty acids, and whatever the process to manufacture it, the cream cheese model proved to be very stable regarding the lipid oxidation, during 3 months of storage at 4°C.

This project allowed better understanding of how the interactions between particles and microstructure impact the final cream cheese texture.

# Significance and benefits

The project was performed with cream cheese models analogous to actual products.

The cream cheese model manufacture integrated a process and some ingredients commonly used in dairy industries.

The different parameters studied here (process parameters such as heat treatment temperature, final acidification pH and homogenization pressure; or ingredients such as proteins or phospholipids) can be tested straightforwardly in dairy industries.

The project outsides can give direct applications / solutions for industries.

Thorough characterization of the product was achieved at each stage in the process and at different scales.

The consequence of each variation of the process or of the formula has been characterized from nanoscale through electron microscopy to macroscale through rheology and sensory analysis.

The project allowed obtaining an overview of the possible parameters that can be used to modulate cream cheese texture. These parameters include both technological and formulation factors.

#### Successful applications

The cream cheese model could be used in other research projects.

The direct results provide information to link process, formula and product structure and texture.

Moreover, this work allowed the production of various data which are currently used for mathematical modeling. Two studies are in progress: modeling protein aggregates formation in tubular exchanger during heat treatment and the modeling colonization of the fat globule interface upon homogenization and induced connectivity.



Figure 1: cream cheese model



Figure 2: Cream cheese process





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# Prospects and challenges

This work raises various perspectives. Firstly, the analysis of the fat globule interface has to be more specific. In order to reach this goal, a method for the characterization of the fat globule interface in the final product should be developed.

Moreover, for further work, a source of purified phospholipids or other emulsifiers should be used. Indeed, in this work we used buttermilk isolates as source of polar lipids. Finally, it will be necessary to quantify the connectivity and link it practically with rheological properties and the texture of product.





#### Task 5.1

Definition of the working domain and relation with WP1 and WP6: Food models and process and recipe variables were selected. Processes required for manufacturing the food models were detailed according to the possibility of control and in-line measurement. They were transferred to WP6 for safety and nutritional tests. The available numerical models and the know-how for structure-properties and structureprocessing relationships (BKM) were collected.

#### Task 5.2

Cereal foam structure design and processing: Laboratory scale processes, and in biscuit model also pilot processes, were investigated to elucidate the most essential phenomena for cereal foam design. Samples were taken during and after processing for further analysis, or measured in-situ during processing. The phase/state changes were assessed at different structural levels, by determining starch digestibility, rheological properties, porous architecture by X-Ray tomography, electron and light microscopy, as well as water distribution by NIR and calibrated colour imaging.

#### Task 5.3

Improving (numerical) models and generating pathways for cereal model foams: Partners' know-how and expertise were combined for specific process operations (mixing, bubble formation, baking) and product specifications in order to complete BKMs, where differential equation (PDE) based models are too complex or not available; the existing models were extended to the computation of other variables (colour during baking, for instance), in WP1. Integrating BKMs will allow to design complete pathways for cereal food processing.

#### Main outcomes

Generic model foods (GMF) were developed for solid cereal foams: bread and biscuit, and accurate methods to characterize these were developed. A preliminary cake model was also developed. Structural changes during processing were ascertained for bread, with emphasis on the creation of the cellular structure and its heterogeneity. Dough liquid phase, including sugar and fat, was studied as a stabilizer of the air bubbles in dough and bread matrix. We have used and further specified the model for bread in the ring test, and protocols for the bread model were validated and disseminated for end users. Effects of oven heat flux on biscuit moisture distribution and colour were studied, and a model for bubble growth and biscuit thickness was made. The models were used to study effects of dietary fibre /bran addition in bread and biscuits, and to study effects of fat and sugar in bread.

# Introduction of WP5

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# Aims and background

Open solid foams represent the structure of baked cereal products, the properties of which are closely related to the intrinsic properties of the solid material, its density and cellular structure. The solid phase is a composite material blend of biopolymers and components of lower molecular weight. The size and distribution of pores is also important in the product behaviour. Fracture properties and dynamics of water in the matrix are important for the sensory quality of products based on solid foam structure. The micro and macro level structure is likely to have an important effect on digestibility and delivery of small molecular weight components in the human gastrointestinal tract.

The aim was to develop models for open cereal solid foam foods with special reference to effects of whole grain and fibre on structure.

# Prospects

The results showed the importance of models in understanding the role of food formulation and process variables at different structural levels of the product and further on nutritional properties, stability and safety. In bread, important nutritional benefits include high fibre content and glycemic response. In the former, maintenance of good sensory texture is critical, and in the latter, food structure has a decisive role. In biscuits, additionally fat quality and amount of sugar should be considered. Fundamental understanding and modelling of structure formation helps to develop solid foam foods with consumer benefits.



# Structural challenges in nutritionally improved biscuits

# How to produce whole grain and high-fibre biscuits with slow starch digestibility and high sensory quality?

Biscuits are an important cereal food category, made most often of white flour, fat and sugar and consequently typically have high calorie but low nutrient density. We wanted to understand effects of adding dietary fibre (whole grain flour and wheat bran) on biscuit microstructure, texture and in vitro starch digestibility. Fine and coarse wheat bran were used to make five different biscuits with various dietary fibre content (5-15 % ). The biscuits with small bran particle size had the best sensory texture. The generally low biscuit starch digestibility in vitro was increased slightly by wheat bran addition.

## Research aims and background

There is a large consumer and public health demand to increase the nutritional profile and expected health benefit of cereal based snacks. Biscuits, with white flour, fat and sugar as major ingredients, are popular snacks and also breakfast items. The production steps during biscuit manufacturing do not allow starch to gelatinize completely in spite of the baking process, and the glycaemic response of biscuits is typically lower than that of bread. On the other hand, the nutritional profile of biscuits is not optimal Making biscuits of whole grain flour or adding dietary fibre in the recipe would improve their nutritional profile, but poses challenges to the open solid foam structure determining the mechanical properties and sensory quality of biscuits.

We wanted to elucidate the role of dietary fibre in the form of cereal bran, as well as that of whole grain flour on biscuit structure and starch in vitro digestibility of biscuits. The aim was to be able to change the process conditions so as to maintain good sensory perception of texture and low starch digestibility while increasing the amount of cereal fibre and associated phytochemicals in the biscuit formulation. The results are important for the baking industry and biscuit manufacture in development of more nutritious products. The knowledge of interactions of dough components and bran particles benefits also producers of other baked snacks and dry products.



Figure 1: Biscuit assessment methods: Texture (2mm rod penetrometry)

## Results and applications

Five types of biscuit were produced containing 5-15 % dietary fibre, including a standard recipe and biscuits made with coarse (260-560  $\mu$ m) and fine (25-160  $\mu$ m) wheat bran. Textural measurements were made with a three point bending test. Further measurements were made by a penetrometry method for 40 types of biscuit with a wider range of fibre variations including bran and soluble inulin fibre. Starch crystallinity was analysed by differential scanning calorimetry, and in vitro starch digestibility rate was determined as hydrolysis index by an enzymatic method.

Biscuits became darker with increasing bran content. Bran particle size had little effect on average colour, but the particles were visible as specks.

The mechanical properties of the bran-containing biscuit matrix were affected by both by bran content and particle size. Addition of wheat bran increased the penetration energy ('firmness'), only slightly increased the elastic modulus, and reduced the number of penetrometry force peaks ('crunch'). Inulin also increased firmness but, unlike bran, resulted in a high initial penetration force. Bran particle size reduction had little effect on firmness, but increased the elastic modulus and hardness measured by three point bending. Bran supplementation level had a greater effect on penetrometry tests than particle size, but particle size had a greater effect in three point bending. The failure strain at maximum bran loading level was the lowest among the coarse bran supplemented biscuits. These samples had very low strain at failure which varied between 1.1-1.4%, which is unique to brittle materials.

Structural factors had more impact on in vitro starch digestibility rate compared to the status of starch which was measured by degree of gelatinization. Biscuits with finely ground bran had a visually more compact structure without any surface or internal defects and were harder than those with a coarse bran particle size. Increasing fibre content from 5 to 15 % increased the hydrolysis index by 20%. The effect of bran particle size reduction in biscuit formulations did not influence hydrolysis index.



Figure 2: Biscuit assessment methods: Colour



Figure 3: Biscuit assessment methods: Mass



Figure 4: Biscuit assessment methods: Dimensions

# Significance and benefits

It is a food engineering challenge to increase content of dietary fibre in biscuits while retaining structural and sensory characteristics, including texture, colour and taste. There are very few reports available about adding dietary fibre in biscuits, and effects on these characteristics or starch digestibility. The use of whole grain flour or bran seems a logical way of increasing fibre content of biscuits, but no reports of their use are available. Fundamental understanding about structure- function relationships in biscuit structure and fracture properties also is limited. Current milling techniques enable efficient particle size reduction of bran, delivering new types of ingredients for the baking industry. Their applications have hitherto been reported to a limited extent. The approach of this work provided thus many aspects on novelty.

The results, when applied, would encourage the use of finely milled bran and whole grain flour as biscuit raw materials, to produce biscuits with good sensory texture and colour, and retain the naturally low starch digestibility in biscuits. The benefit for biscuit manufacturers would be the production of new, healthier products, providing consumers with healthier options. The use of bran in food production would also increase sustainability, as a side stream could be utilized in a mainstream food product.

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#### Prospects and challenges

We hope that the results will encourage biscuit manufacturers in development of high-fibre products, thereby diluting the amount of refined ingredients in the product. The nutritional and health claim regulation by European Food Safety authority would allow a claim for fibre content of products with adequate nutritional profile. The idea of bran pre-treatment prior to incorporation in baking could also be developed further in collaboration with milling and baking industry.



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# Creation of bread cellular structure

## Designing bread texture thanks to the solid foam model

The growth of gas bubbles in viscous matrices is the main mechanism responsible for the structure of open solid foams, like bread.

The creation of the cellular structure in wheat flour dough and its heterogeneity was ascertained at different structural scales, focusing on proofing. Dough liquid phase, including sugar and fat, was studied as a stabilizer of the air bubbles in dough and bread matrix. The contribution of each scale was integrated by adapting a capillary number. We have used and further specified the model for bread in a ring test, in order to increase its fibre content, and protocols for the bread model were validated and disseminated for end users.

#### Research aims and background

The texture of bread is a fundamental element of its acceptability by the consumers, and it has a strong impact on its nutritional properties. For instance, increasing the fibre content leads to denser, and less acceptable breads. Like solid foam mechanical properties, texture depends on density and cellular structure of bread. So, there is a need to better understand the mechanisms of cellular structure creation during dough processing and provide models to better control process and final properties.

Wheat four dough contains about 45% water (tot. basis) and its cellular structure is created during fermentation, or proofing; during this stage, porosity increases from 0.1 to 0.7 and CO2 bubbles are connected together, although the dough does not collapse. This stability could be due to the formation of a liquid foam, constituted by a liquid phase, co-continuous to the starch/gluten visco-elastic matrix. To determine the role of this phase in the creation of the cellular structure, the aqueous phase, so-called dough liquor (LdP), has been considered as a good model for these interfacial films. In complement, elongational properties of dough can be determined by lubricated squeezing flow test (LSF),

which underlines the importance of minor components. Starting from the study of bubbles growth and coalescence in model dough systems, the aim of our work was to determine the role of the aqueous phase and the starch/gluten matrix on the mechanisms which govern the creation of cellular structure at microscopic scale, and at macroscopic scale on the loss of stability at the end of fermentation.

The results are important for the baking industry, first for manufacturing breads with increased fibres content, without loss of sensory quality. Secondly, it strengthens the knowledge on the breadmaking chain by providing engineers with basic knowledge models.

#### Results and applications

About twelve recipes of dough were processed with varying content of sugar (0-15%), fat (0-10%), fibres (0-15%), in a range for which a typical bread cellular structure was always obtained. The elongational properties of starch/gluten matrix, measured by lubricated squeezing flow, largely influenced dough proofing stability, the evolution of which was assessed by 2D image follow-up and adjusted by an exponential decay; this result could not be explained by the single bubble growth model. Porosity kinetics determined at macroscopic level were in good agreement with results determined at microscopic levels by X-ray microtomography (XRT) at ESRF (F38-Grenoble), and both followed a Gompertz model. Analysis of 3D-XRT images showed that most bubbles were connected, for highest porosity of dough ( $\geq 0.5$ ). The homogeneity of the cellular structure was defined from the size distributions of gas cells and walls; it was characterized by a critical thickness of walls ( $\approx 1\mu$ m), below which the cells were separated by liquid films.



Figure 1: Two dimensional images of dough from beginning (66 minutes) to the end (178 minutes) of proofing, diameter 5 mm. Images are issued from Turbin-Orger, A., Boller, E., Chaunier, L., Chiron, H., Della Valle, G., Reguerre, A-L. (2012a). Kinetics of bubbles growth in wheat flour dough during proofing studied by computed X-ray micro-tomography. J. Cereal Sci., 56, 676-683. Images have been obtained at the European Synchrotron Radiation Facility at Grenoble, France.



Figure 2: Three dimensional images of dough at the beginning (66 minutes) and at the end (178 minutes) of proofing. Images are issued from Turbin-Orger, A., Boller, E., Chaunier, L., Chiron, H., Della Valle, G., Reguerre, A-L. (2012a). Kinetics of bubbles growth in wheat flour dough during proofing studied by computed X-ray micro-tomography. J. Cereal Sci., 56, 676-683. Images have been obtained at the European Synchrotron Radiation Facility at Grenoble, France.

The fermented dough could thus be considered as a three-phases medium: visco-elastic matrix / gas cell / liquid phase.

Dough liquor was taken as a model of this liquid phase, and extracted from dough; it behaved like a macromolecular solution, and was characterized by the surface tension ( $\approx$  40mN/m), related to the presence of polysaccharides-proteins complexes at interfaces.

The contributions of the different levels of organization of the dough were then integrated by defining a (dimensionless) capillary number, that ruled the overall behavior of the dough. These results, including process specifications, were used in a ring test to study the effect of fibre addition, in different labs; although various texture properties were obtained, they could all be integrated in the same relation between texture and density, which finally validated the open solid foam model.

# Significance and benefits

Improving bread nutritional properties without decreasing its sensory properties and, mainly its texture, is a real challenge in baking industries. A better understanding of various operations is necessary to control the density and cellular structure of these products, which can, in turn be related to texture by available mechanical models. In this purpose, a common representation of these operations can be obtained by the definition of Basic Knowledge Models (BKMs), which captures the main physics of the phenomena involved. For instance, the relation between the capillary number and stability can be considered as a BKM; it suggests that the simple measurement of dough elongational viscosity and the knowledge of dough liquor surface tension can lead to the prediction of the cellular structure of the dough.

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# Prospects and challenges

The integration of such BKM requires the upgrading of computer tools. Their use for designing cereal food products requires (a) to integrate the available know-how and expertise for specific process operations where the use of models based on differential equations is still difficult, and (b) to extend the existing models to a domain of composition, in order to cope for the necessary increase in dietary fibre, that will modify greatly the rheological behaviour of dough. The use of such integrated models may be thought of to design products with target nutritional and sensory properties, provided their porosity and cellular structure are precisely characterized, and defined the pathways for reaching them, according to the so-called reverse engineering approach.



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#### Task 6.1

In WP6, model foods used for nutritional studies as well for microbiological food safety and quality assessment. It is important to ascertain that GMF are reproducible and well characterized. In this task, protocols for the realization of seven model foods have been standardized and cross-validated among the partners and tested for their practicability by industries

#### Task 6.2

Models were further characterized by using analytica methods and high level structural studies. Main physico-chemical features, e.g. firmness, color, bioactive molecule content have been assessed in WP2-models Protein concentration and oxidation surface hydrophobicity were assessed to characterize WP3-mimetic model. Deep investigation characterized WP4-models for viscosity, fat globule size distribution, proteir content/oxidation, while hardness, texture profile, and color have been monitored to describe WP5-models

#### Task 6.3

Through the development of appropriate methods and using the most recent analytical technologies the *in vitro* and *in vivo* studies have been focused a determine how different processes, modifying the molecular and physical structure of GMF, can influence the bioavailability of selected nutrients or bioactive compounds and their kinetics of appearance in blood of minipigs during digestion of a complete meal composed by GMF

#### Task 6.4

In T6.4, the impact of environmental conditions or microbial behavior or mycotoxin production have beer obtained by inoculating GMF with molecularly characterized spoilage, pathogenic or beneficial strains. The use of experimental kinetics performed during the challenge test has allowed the determination o growth/destruction parameters specific for each combination microorganism/GMF. Mathematical models have been then used to simulate growth for other conditions

#### Prospects

Administration of different GMF *in vivo* as a complete meal can highlight the effect of processes in changing the food structure and consequently nutrient/ bio-molecule bioavailability giving a realistic evaluation of possible interactions of foods within the digestive tract. A specific task includes the implementation and consolidation of microbiological data deriving from GMF exploitation in the Sym'Previus-decisionmaking-tool. The predictive module will assist scientific community and industrials to i) select food model formulations and optimize processes and storage; ii) determine the appropriate shelf-life according to microbial behaviour; iii) assess quantitative risk.

# Introduction of WP6

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#### Aims and background

Change in composition or process conditions may deeply impact the microbiological quality and safety of foods, as well as the bioaccessibility of toxic or bioactive compounds and therefore their absorption during digestion. To assess their reproducibility and industrial practicability, Generic Model Foods (GMF) were standardized and validated. Their physico-chemical characterization can provide information on bioaccessibility of nutrients and/or toxicants, fundamental for understanding the effect of different processes on the bioavailability - in vitro and in vivo studies - of selected nutrients and their impact on consumer health. Additionally, GMF can represent a powerful tool for microbiologists. According to microbial behaviour of beneficial, spoilage and pathogen populations, the use of GMF can help in optimizing formulations, processes and storage - finally improving risk assessment - via the implementation of available decision making tools.

## Main outcomes

Protocol standardization, cross-validation and physical-chemical characterization were achieved for seven generic model foods - WP2-Brassica and Tomato purée; WP3-Mimetic meat model; WP4-Dairy dessert and Soft cheese; WP5-Bread and Biscuit. The *in vitro* digestion and *in vivo* studies highlighted the effect of the different processes in changing the physical and molecular structure of food and consequently the bioaccessibility and bioavailability of nutrients or bioactive compounds. The quantification of the impact of process and storage on microbial food safety and quality was obtained after inoculating GMF, following the microbial behaviour and implementing the decision making tool to simulate their performance in food.

# Acting on micro- and macrostructure of meat, cooking conditions can modify the nutritional potential of meat proteins

## How to cook meat for obtaining the best health effects?

In addition to the composition in amino acids and the digestibility, new criteria are appearing to fully describe the nutritional potential of proteins: the kinetics of amino acid absorption, and the potential to release bioactive peptides during digestion. For meat, these parameters can be modified by cooking conditions, which act on the micro- and macrostructure of the product. Drastic cooking conditions lead to protein aggregation, which slows enzymatic digestion of proteins. However, this effect, related to the microstructure, is limited in comparison to the effect of the macrostructure, and the chewing efficiency of the consumer. Furthermore, a simple difference in cooking conditions significantly modified the postprandial plasma peptide profile of the consumer, and therefore the potential health effect of meat.

#### Research aims and background

The classic criteria for evaluating the quality of a protein source are based on amino acid composition, and protein digestibility in the digestive tract. It is now known that these basic criteria are not sufficient to fully describe the nutritional potential of a protein. For instance, it has been shown that the rate of protein digestion can regulate postprandial protein retention (Dangin et al., 2003). Thus, the ranking of protein sources according to their rate of digestion is necessary. Additionally, total digestibility is not a good predictor of amino acid bioavailability Indeed, only digestion in the small intestine is thought to supply amino acids to the body. Finally, all dietary proteins are potential sources of peptides with beneficial health effects.

Previous work on meat shows that modifications at the microscopic scale of the protein cellular network, for instance by protein aggregation during heat treatments, can slow down the enzymatic digestion of proteins (Bax et al., 2013). The rate of meat protein digestion can also be modulated by chewing efficiency (Rémond et al., 2007), probably in conjunction with meat macrostructure. However, the hierarchy between the effects on digestion parameters of the meat micro and macrostructure and mastication remain difficult to establish.

Furthermore, it has been shown that meat digestion reproducibly releases peptides containing amino acid sequences with antihypertensive activity (Bauchart et al., 2007a), and that a significant amount of carnosine is released in blood after a meat meal (Bauchart et al., 2007b). This dipeptide has numerous health benefits such as prevention of pathologies related to oxidative damage, or protein glycosylation. Nothing was known on the effect of the meat structure (micro and macro) on the bioavailability of meat derived peptides.

In this context we used in vitro and in vivo approaches (using minipigs and rats as animal models) to investigate the effect of structural modifications, on the bioavailability (kinetics and quantity) of amino acids and peptides by manipulating meat cooking conditions.

# Significance and benefits

We have shown that cooking pork meat for 45 min at 90°C accelerates protein digestion, compared to a 10 min cooking at 75°C, but has no effect on the total bioavailability of amino acids (Figure 1). Although these results underlined the importance of the structure of the ingested meat in the determination of amino acid absorption kinetics, they seem to be in disagreement with a previous study with beef, according to which meat protein digestion was slowed down by increasing cooking temperature from 75°C (30 min) to 95°C (30 min) (Bax et al., 2012). Beside a possible meat origin effect, this suggests that, more than the temperature, the time-temperature couple is important in the determination of protein digestion rate. Furthermore, in the beef study, meat was minced before meal serving, whereas in our study, meat was only sliced. An interaction between cooking conditions, and the efficiency of oral and gastric meat degradation, could therefore also explain the apparent discrepancy between the 2 studies. Indeed in the present study, prolonged cooking at 90°C produced a meat whose structure was much more sensitive to mechanical degradation in the mouth, leading to increased digestion rate.

Cooking conditions did not modify meat carnosine content, and in both animal models (rats and minipigs), the carnosine bioavailability was not affected by cooking.

In order to address the question of peptide bioavailability, we have developed an analytical approach, using an LTQ-Orbitrap Velos mass spectrometer and data extraction with XCMS, for the characterization of the plasma peptidome and the identification of selected peptides. The method developed enabled us to discriminate the plasma peptidome of the minipigs fed with the pork meat cooked according to the 2 conditions (Figure 2). We identified up to 33 peptides discriminating the cooking conditions. The identification of these peptides was validated by analysis of their fragmentation. Furthermore the kinetics analysis allowed us to show the postprandial trajectory of the plasma peptidome (Figure 3).



Figure 1: Amino acid absorption. Portal net flux of the sum of indispensable amino acids (IAA), in minipigs (n = 5) receiving two meals differing by the meat cooking conditions.







Figure 3: Peptidome. OSC-PLS score showing plasma peptidome trajectory during the postprandial kinetics after ingestion of meat, in minipigs.

#### Results and applications

Our work highlighted the difficulty of predicting protein digestion rate. Although it is clear that protein aggregation slows down the accessibility of digestive enzymes to their cleavage site within the proteins. This parameter appears to have minor effect when meat is not ground. Indeed in this case, the resistance of the meat structure to disruption by chewing activity could be the main determinant of the digestion speed. As it is attributable to the chewing efficiency of the consumer, it seems difficult to predict it only from meat derived measurements (chemical composition, structure characterization, and in vitro digestion). We have clearly evidenced that for a given meat, a simple difference in cooking conditions significantly modifies the postprandial plasma peptidome of the consumer, and therefore the potential health benefit of meat proteins.

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#### Prospects and challenges

This work is continued through the study, in human, of the interaction between meat structure (cohesiveness and tenderness), and chewing activity, in order to assess the final impact on the level of degradation of the swallowed bolus, and the consequences on protein digestion rate and the peptide release.

If we identify peptides that are reproducibly released from meat proteins, the next challenge will be to identify their potential biological activity (antihypertensive, immunomodulator, antioxidant, etc)



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# Industrial concerns and needs towards fungal risk assessment

# How to assess and take into account the ability of *Alternaria alternata* mould to grow and produce mycotoxin during tomato purée shelf-life

*Alternaria* species are reported to be the most commonly fungi affecting either tomato fruit and plant causing the so called black mould of tomato. Rapid infection of *Alternaria* in tomato may occur on the crop or post harvest yielding high economical loss due to spoilage of industrialized products such as tomato purée. Moreover, under specific growth conditions, *Alternaria spp.* may also produce various mycotoxins which represent a serious risk for human consumption of tomato-based products. Within the frame of this collaboration, the ability to grow and produce mycotoxins were determined as a function of tomato purée pH and temperature of storage. These boundaries and growth simulations will help food industrials to further optimize tomato-based food formulation and shelf-life.

#### Research aims and background

39°C

Predictive modelling and microbial risk assessment have emerged as a comprehensive and systematic approach for addressing the risk of microbial pathogens and spoilers in specific foods and processes.

Within WP6.4, food shelf-life or the impact of physico-chemical factors (pH, aw, Temperature) on microbial behavior were determined after (i) artificial inoculation of microorganism of interest in developed model food, (ii) fitting of experimental kinetics in food and (iii) prediction of shelf-life for various scenarii in static or dynamic conditions of storage.

Challenge tests in DREAM model foods were performed according to guidelines and standardized protocols based on the current NF V01-009 on guidelines to conduct microbial challenge tests. Experiment fitting and shelf-life determination were performed with recognized mathematical models available in Sym'Previus decision making tool (www. symprevius.org). While simulation with a large variety of characterized strains of pathogenic bacteria is possible, few spoilage fungal strains are available in the database. Similarly to the modelling approach reported by Huchet et al. 2013 predicting mould appearance time on pastries, an adaptation of the gamma concept of Zwietering (1992) was used to characterize fungal strain and to model its growth. In tomato-based products, *Alternaria alternata* represents a relevant microbial hazard since it is responsible for black mould spoilage (Figure 1) yielding high economical losses (Bottalico & Logrieco, 1992). In order to model the effect of environmental factors on the development of mould on tomato medium, deep characterization of the strain was performed for a wide range of temperature and pH values at a given water activity of 0.99. Moreover, to further screen the conditions yielding to mycotoxin production, a similar experimental set up was used and analysed.

#### Results and applications

A toxigenic strain of *Alternaria alternata* (ITEM8176) isolated from tomato fruit affected by black mould (Somma et al., 2011) and deposited at the ISPA collection, Italy (ITEM accession: http://www.ispa.cnr. it/Collection/) was used for growth and mycotoxin production assessment.

Growth ability and mycotoxin production of this strain were determined on similar samples, after inoculating fungal ascospores (7day-old culture) on WP2 cold break tomato purée supplemented with agar. Adequate controls and a complete factorial design with a total of 6 levels of pH (2, 3, 4, 5, 6 and 7) and 10 levels of temperature (6.5, 10, 15, 20, 25, 30, 35 and 37°C) were used (60 conditions in total), for 3 replicates, to define which conditions of pH and temperature supported (i) fungal development and (ii) mycotoxin production. Growth ability was determined on tomato-based medium by regular observations of fungal development and additional experimental data in broth were performed to determine the strain cardinal values, i.e. minimal, optimal and maximal values of pH and temperature allowing growth. When plates were covered by the fungus, mycotoxin quantification (tenuazonic acid, TeA; alternariol, AOH and alternariol methyl ether, AME) was performed by HPLC coupled with UV/DAD detection according to an adapted protocol from Solfrizzo et al. 2004.

After a maximum incubation time of 1 month, growth was observed in 35 conditions. A pH 3 was the lower pH values enabling growth. Lower and higher values of temperature allowing growth were further validated with experimental data. Among the investigated mycotoxins, only TeA was produced by Alternaria alternata ITEM8176 in studied conditions. The impact of pH and temperature on growth and TeA production of the strain on tomato based medium are indicated respectively in figure 2 and figure 3. Interestingly, growth optimum (24.5°C, pH5.5) and mycotoxin optimum production (15°C, pH3.5) occurred for opposite conditions suggesting a strong impact of stress conditions on the strain virulence that may represent a health issue for non adapted conditions of storage.



Figure 1: *Alternaria* species are reported to be the most commonly fungi affecting either tomato fruit and plant causing the so called black mould of tomato (Bottalico & Logrieco, 1992).



Figure 2: Impact of pH and temperature on growth ability of *Alternaria alternata* ITEM8176 strain on tomato based medium. For tested conditions, physico-chemical conditions supporting growth are indicated in red (>90% growth probability). For conditions close to growth/no growth boundaries, the impact of annex flora, texture or complex formulation might impact growth ability and experimental testing or challenge test is needed.



Figure 3: Impact of pH and temperature on TeA mycotoxin production of *Alternaria alternata* ITEM8176 strain on tomato based medium. For tested conditions, dark red dots refer to environmental conditions of temperature and pH yielding higher concentrations of mycotoxin (15°C, pH 3-4)

### Prospects and challenges

To our knowledge this is the first time that boundaries representing physico-chemical conditions yielding growth and mycotoxin production of *Alternaria* black mould spoilage were defined on tomato-based products. These results were obtained thanks to a fruitful

collaboration with complementary expertise and know how. These data are brand new and are currently being disseminated towards public, scientific and food industrial audiencefor future successful applications.

# Significance and benefits

Alternaria alternata is the major microbial contaminant causing tomato black spoilage yielding high economical losses. Thanks to these results the prediction of spoilage development is now possible for static or dynamic conditions of pH and temperature on tomato medium. Future work could address (i) validation for various recipe of tomato-based products, (ii) characterization of a larger number of strains to take into account biodiversity in growth prediction or (iii) further characterization of mycotoxin synthesis to predict health issue for various industrial relevant conditions.

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#### Task 7.1

Collecting and mapping the needs of the industry -Identification of the needs of the industry and barriers to using modelling methods in 4 sectors (vegetable and fruit processing; meat, fish and poultry processing; dairy and bakery industry) and 3 countries based on interviews and focus group discussions (partners involved: ACTALIA / France, CCFRA / United Kingdom, CCHU / Hungary).

#### Task 7.2

Assessing the practicability of the generic food models and their protocols -

The next step of WP7 was the testing of the practicability of the models developed by the relevant WPs. Tests was carried out by the WP partners (SOREDAB, ACTALIA, ADRIA, CCFRA, CCHU). After the adjustment the improved models and protocols were evaluated for practicality by involving industry partners

#### Task 7.3

Development of industry guides -To support the practical application of the models and to provide an overview on different food models and on modelling tools/software for the potential users a practical guideline was developed by involving industry partners including SMEs from each country. CCHU, SOREDAB and ACTALIA were responsible to consult national industry partners during the development of the guide.

#### Main outcomes

As it was emphasized for the model developer WPs by WP7, models developed within DREAM should consider the following aspects:

- Models should have practically applicable outputs, clearly described for the user.
- Models should be quick and as product specific as possible, easy to handle.
- Results should be as reliable and precise as possible.
- The modelling activity shall not require special equipment, but purchasing necessary materials and software is not a barrier. Companies could ensure a food engineer for operating the model.
- Confidentiality should be considered, regarding inputs.
- The main barrier of using models by the industry is the lack of knowledge, therefore a significant effort should be taken to remedy those failings.

Industry guide for Food Modelling:

After the consultation of the draft version with national industry partners the final version was discussed and adjusted.

# Introduction of WP7

András Sebők, Workpackage Leader Campden BRI Magyarország Nonprofit Kft., (CCHU, Hungary) a.sebok@campdenkht.com

#### Prospects

The use of existing modelling approaches as time saving and cost effective tools for assessing and optimising processes and their impact on product quality and supporting decisions during the process and product development activities is currently relatively limited in the industry, especially at SMEs.

Technology transfer was integrated into the project to promote that the model development is conducted in collaboration with food manufacturers through industry needs and feedback approach.

To ensure that the practical needs of the industry, especially those of the SMEs are considered during the development of the real model foods; To convert results of the research into appropriate format that can be used by the industry as simple process and product development tools, and To test their applicability at industry level and provide feedback for further improvement.

# Aims and background

According to the industry

- the economical advantages of using models should be emphasised and clearly explained
- models should be practically well applicable

• development of models should be focusing on food safety and the effect of addition or removal of components/ingredients to the product or applying new process conditions on shelf life, on texture and on flavour. Industry guide for Food Modelling:

The guideline is intended to give a technical aid to the users but also to create awareness and encourage the use of modelling in the food industry.



# Industry guide for Food Modelling

# A guideline which is intended to give a technical aid to the users but also to create awareness and encourage the use of modelling in the food industry

This guideline can be used as a manual, in which the potential user can find advice on questions related to the use of specific models and also for general considerations on the application and design of food models.

The content of the guideline, including the descriptions of the models, is based mainly on the models that were developed within the DREAM FP7 project and moreover on some models outside the DREAM project that are actually available and frequently used in the food industry. Brief descriptions of some general examples of successful practices, and hints for avoiding typical traps and failures are summarised in the guideline.

## Research aims and background

To support the practical application of realistic food models a Practical Guideline on use of models was developed. The main objective of the guideline is to provide an overview on different food models and on modelling tools/software for the potential users.

Models can be used in many different activities in the food sector considering the complexity of the food and their different applications. As changes in needs and requirements related to food products arise increasingly quickly and frequently, dissemination of available and effective models to the food industry and also to all the sectors who deal with food is therefore of high importance.

The target audience of this guideline includes several stakeholders of the food sector, particularly representatives of the food industry (including SMEs) and R&D teams, and decision makers on food safety, quality and nutritional questions. It is also recommended for food safety and regulatory bodies, nutritionists, food scientists and for marketing specialists who are particularly responsible for the industrial development of food companies.

Food models are useful tools for product and pro-



cess development, for the assessment of the safety of product/process design, and can help in understanding the impact of process parameters on final characteristics of the food and yield. However, their use requires an appropriate level of expertise, competence, skills and clear practical guidance.

# Significance and benefits

One of the main advantages of the realistic food models is to mimic the behaviour of real food products. Furthermore, models can predict the impact of changes to the ingredients, compositions and process parameters. Thus, they can reduce the number of necessary experiments in real conditions, which is particularly important in the case of experiments in factory environments. The use of models can save time and can reduce costs. Standardised physical modelling materials and calculations with mathematical models provide a more reproducible benchmark for the impact of different treatments on food properties than experiments with real foods. Because of the rapidly changing conditions and demands of the market, for the food producers it is required to have a safe but fast process for development. As experiments under real conditions can be expensive and time-consuming, frequently there are significant limitations for carrying



out a large number of experiments under such conditions. In these cases, food models and software models can definitely be good tools to screen options at low cost and to enable experiments with real foods to focus on the most promising test parameters. By using models, waste of the valuable real food product can be significantly reduced during the experiments.

Figure 1: The scheme of the food modelling process driven by the consumer  $$\ensuremath{\mathsf{per}}$$  / industry needs

## Results and applications

Although there is a wide range of models having different scope, the model development process is typically divided into five phases:

i) Defining the goal of the model: developing a statement of purpose

- ii) Designing and developing the model
- iii) Practical testing and verification
- iv) Making the model available for the audience
- v) Maintenance of the model

This guideline describes these five phases as a systematic procedure and provides a brief description of those steps that are essential to be considered during the model development. The most important facilities and requirements for application and operation of the models are discussed to help their use and to raise awareness among potential users. The model descriptions are grouped by four major generic structure groups representing vegetable, dairy, meat and cereal products. Furthermore, there is an additional group for models with general applicability, in which these models are discussed according to their function, such as predictive microbial models and heat treatment models.

# Prospects and challenges

However, finding satisfactory answers is a time consuming process. Models as time-saving and costeffective tools provide fairly good support when a company decides to develop a new product: food models can help to reduce the time needed to provide an initial protocol for a production process, and mathematical models can support the simulation of different processes and the changes of the parameters (see figure 1).

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# Management & Dissemination



communication planning and reporting project graphical identity

# Introduction of WPO

Monique Axelos, Workpackage Leader INRA, France monique.Axelos@nantes.inra.fr

Caroline Sautot, DREAM project manager NRA Transfert, France caroline.sautot@paris.inra.fr Management tasks The Management tasks were aimed at strategic steering, project monitoring, administrative and financial management of the project.

## Management

The management of DREAM met the – exciting – challenges posed by the project that:

i) adopts a bold scientific approach consisting of applying state - of - the - art modeling techniques that have been so far rarely used in food sciences;

ii) gathers a truly multi-disciplinary and multi-sectorial consortium made up of physicists, chemists, process engineers, microbiologists,

mathematicians, bioinformaticians, etc. in terms of expertises, and academia, technical centres;

iii) aims to provide concrete answers to an industrial sector facing somehow contradictory societal demands – taste and tradition, safety and nutritiveness, product affordability and sustainable production – by searching solutions in fundamental science. The management needed to be:

i) sufficiently flexible to enable its work programme to remain relevant throughout external events, whether scientific, technological, or even societal;

ii) transparent and focused enough to make the consortium work together in conformity with the work plan.

These main requirements have been achieved by a three-tiered management system integrating strategic steering (including survey of the societal background and the scientific state of the art),

project monitoring (including the development of a collaborative platform, a management guideline and a risk management plan) and consortium management (including financial and administrative issues).



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#### Task 8.1

Dissemination to the industry and food authorities: Trainings covered potential applications, the practical benefits, the functions and operation of the models, their limitations, explanation of the practical steps, practices and hints for their use, warnings about typical traps and malpractices. Trainings at national level on national languages created awareness and induce a multiplication effect (e.g. spread of use) also through development of the industry culture. A core training package was developed in English, covering each group of models.

#### Task 8.2

Dissemination to the scientific community: The publication in scientific journals and participation to scientific meetings as well as industrial fairs were found as good opportunity to disseminate DREAM developments and results through oral presentation, poster or discussion. We performed organisation of scientific workshops and sessions at EEFoST, IUFoST, CEFooD meetings and relevant ETPs aiming at exchange of best practices.

#### Task 8.3

Training and career development: For the efficient technology transfer it is important that young researchers understand the needs of the industry, the way how the industry thinks and operates, and the requirements of daily operation in the industry in terms of process, quality and food safety management, and also the specific priorities, conditions, resources and constraints of SMEs. We provided a practical training session for young participants attached to project events focused to the last advances in food model development, food process, industrial guality management.

#### Task 8.4

Communication with the general public: The objective of this task was to inform the general public about the context and achievements of DREAM, in terms of the benefits for society. European citizens were informed about the project values, with special attention to communication to public through invitation of the media and through the DREAM website, project popularized publications in brochures, newsletters, lectures.

# Introduction of WP8

Peter Raspor, Workpackage Leader UL-BF peter.raspor@bf.uni-lj.si

#### Dissemination

As DREAM meets important industrial and societal issues while addressing fundamental scientific issues, we played a particular attention to technology transfer since the consortium is convinced that the project results are to improve competitiveness of the European agrifood sector, which was one of the rationales of the project.

As the project was designed to respond to some of the most important issues listed by the European Technology Platform on Food for Life, which express industrial expectations from scientific research, industrialists will certainly be interested in appropriating the project outcomes.

Another prioritised target of the project is constituted by food authorities. Last but not least, European citizens will certainly be interested by the societal stakes of the project as it responds to some of the "hottest" societal topics that are food safety, nutrition patterns and food supply. For that reasons, it was important to disseminate outcomes as soon as possible to relevant stakeholders.

Our vision was:

-Make the best possible use of the project results by the food industry and concerned authorities.

-Ensure fruitful exchange with the scientific community, including individual scientists and initiatives.

-Inform European citizens about the societal stakes of the project and the way their money is used.

The intention of implementation of new concepts and techniques, which had not been applied by the industry widelly, such as modeling was enhanced significantly by practical training sessions, workshops provided to industry and to science. However, the special treatment for SMEs was done on their mother tongue. This was particularly important to achieve good understanding in the area of comprehensive dissemination activities, which is not as easy as it looks from the beginning since we had to induce awareness for this issue first with the media editors and finally with involved parties.


Figure 1: Active communication among partners Meeting of the chairs with coordinator dr. Monique Axelos fourth from the right in Nantes where project started in 2009.

The concept pushed to our research under terms Generic Model Foods, Basic Knowledge Models and Integrated Knowledge Models was very difficult to share with general public.

It looks very simple and strait, however when you ask even professionals about the details you face some barriers which can not be applied straightforward in daily practices and daily life. Let's analyse this particular case more in detail.

The term Generic Model Foods (GMFs) defined as realistic physical models in which several parameters can be varied represent structured statement but it has to be explained clearly that this type of model has to be established on particular food sample of the kind and is substantially different from the other which might look in consumer eyes and taste very similar. Additional explanation about structure and chemical composition makes this issue even more complex and complicated not just for general consumer, but sometimes even for practitioners in food supply chain when they are asked to explain some details in simply and transparent language. Additional obstacle is in research community. All this case development is based on lingua franca English which is not mother tongue for majority of researchers in the project and consequently we adopt this thinking for English, when we switch back to mother tongue we convert this to mother tongues and suddenly it get different emphasis or even different meaning. More scientifically complex statement has more possibilities to be transposed to other language with deformation, not just linguistically since researchers in this area does not posses this knowledge, but also professionally.

If we go to more simplistic Basic Knowledge Models or more sophisticated models like Integrated Knowledge Models this problem remains, actually It becomes even stronger. Just take a closer look to Basic Knowledge Model which is considered to be simpler since it is describing specific aspects of the model, through heuristic or mathematical approaches, what is not clear to average professions in food supply chain. For example description of the role played by temperature, pressure, chemical composition, etc. in formulation of food structure and (food) material properties is far to be simple and has many different implications in different foods.

Finally we addressed Integrated Knowledge Models the dynamic networks based on software systems integrating the operating rules of Basic Knowledge Models, technical expert knowledge, food properties and food processing data from the Generic Model Foods. This complex approach clustered initial experiments and simulations to improve models to reveal key parameters and material behaviour and help refine, optimise and establish the food model concept prior to the pilot stage.

All this issues ware elaborated carefully and we addressed potential professionals to create awareness about this new concepts and also to convey this message to general public. This was achieved through scientific meetings we participated (ie.: IUFoST in Brasil in the session with lectures at global meetings like.: "Food safety consequences arising from the Design and development of REAlistic food Models with wellcharacterised micro- and macro- structure and composition (DREAM)« or as organizers of some activities within the programe of regional meetings like CEFood in Serbia. Beside this we transferred DREAM experiences and messages also students and teachers in Ljubljana at joint workshop with EU USA ToBeSafe project. Moreover we approached general public also with contribution on world food day 16 of October 2012 at national event marking this day with DREAM contribution to profound knowledge on food models and food modeling. This is just to mention the main streams of our activities with dissemination in the project, which will continue via our web site and book of results for 5 additional years as planed.



Figure 2: Joint Workshop DREAM (FW7) & TU\_BE\_SAFE (ATLANTIS) Ljubljana 17 may 2011 gave opportunity to disseminate models to teachers and students in food area.



Figure 3: Novi Sad 2012 CEFood congress, Dream was disseminated to general food public in Central Europe at the meeting with 500 participants from academia and industry.



Figure 4: IUFOST (international union of food science and technology) gave possibility to DREAM to present their achievements in the area of modeling in Brasil 2012 to few thousand patricians from all over the world.



Figure 5: Marking importance of world food day 16th October 2012 Ljubljana (second from the left prof. dr. Peter Raspor, Responsible for dissemination in DREAM project)



Figure 6: The DREAM Project International Conference "From Model Foods to Food Models" held from 24th until 26th June 2013 in Nantes. At the event we had combination of dissemination activates: lectures, poster presentation, round table discussion and interviews.



Figure 7: The DREAM project last meeting held on 15th and 16th October 2013 in Bari, Italy. The DREAM project partners (upper image) and DREAM project WP leaders (bottom image) – from the left: Caroline Sautot (management WPO), Peter Raspor (leader of WP8), Kaisa Poutanen (leader of WP5), Matthijs Dekker (leader of WP2), Monique Axelos (coordinator), Alan Mackie (leader of WP4), Nathalie Perrot (leader of WP1), Carolina Realini (leader of WP3), Paola Lavermicocca representing Angelo Visconti (leader of WP6), András Sebők (leader of WP7).

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# Being curious?

Consumers clip for you.



## Food Modelling

Modelling in food science is used to predict/simulate an outcome regarding quality of food, production process or food shelf life. For example, if you are developing a new food product and you are missing certain information on what is going to happen with this food product in certain conditions (heating, etc) the food models can help you to find right answer to the question. What makes models to answer to this question? Food models are records of numerous laboratory experiments made by food experts and scientist and collated in a user friendly form that offers you to reuse them when needed.

Food modelling or food models reduce number of experiments; time, costs and provide process optimisation, improve process automation and control, due to results measured in previous experiments or production conditions. What has the DREAM project contributed to food modelling? The DREAM project used for its studies real food products, for example: dairy products, bakery products, meat, fruit and vegetables. The research demonstrated that mathematical models can be successfully adapted to complex food matrices (i.e.: food structure, composition) which are profoundly changed (i.e.: visually, nutritionally,) from entering substrate (i.e.: milk, flavour) to final product (i.e. Cheese, Bread).

### Model Food

Model food is a material that consists of or contains essential body nutrients, such as carbohydrates, fat, proteins, vitamins and minerals in exactly known amount. Model food is used for mimicking real food products. For example: the soft cheese model mimics real soft cheese characteristics. This kind of food models are used in research and development circumstances. Why to use food models rather than real food? Let's say that you would like to produce soft cheese with lower salt content. In the early stage of the new product development you can use model food to run the experiments. What means that you may use soft cheese model to mimic the real soft cheese characteristics. By using the model food you may reduce time to get comprehensive data for designing real new food product. This is achieved by reduction of number of necessary experiments, food material, food waste, packing material and consequently cost. Can model food completely replace experiments on real food? No. The model food cannot replace the real food it can only speed up the development of new products with designed nutritional and food safety characteristics; like less sugar, salt, fat, etc. What is the DREAM project contribution to the model food development? One of the main contributions of the DREAM project to the model food is linking mathematical modelling to the physical model foods from different foods (pork meat, soft and hard cheese, bread and biscuits, tomato and brassicas). Linking physical model foods with mathematical models gives good support tool for industry and for research.

by Peter Raspor, UL-BF & Lidija Baša, UL-BF

#### Food Safety

Food safety aims to assure a high level of food safety, animal health, animal welfare and plant health within food supply chain through coherent farm-to-table measures and adequate monitoring.

#### Food Quality

Food quality refers to the minimum technological, nutritional and gastronomical standards for substances to qualify as fit for human consumption

#### Process optimization

From a practical point of view, we define the optimization as approach which find the best solution for running particular process within constraints.

## Conclusion

by Peter Raspor, UL-BF

Since DREAM kick off meeting in May 2009 we have been working deeply on research but also on innovation aspects with permanent cooperation between different stakeholders (institutions, external industrial partners). DREAM as trans-disciplinary partnership in nine countries focused on development of realistic, physical and mathematical, food models for use as standards to be exploited across all major food categories to facilitate development of common approaches to risk/benefit assessment and nutritional quality in food research and industry.

Since DREAM kick off meeting in May 2009 we have been working deeply on research but also on innovation aspects with permanent cooperation etween different stakeholders (institutions, external industrial partners). DREAM as trans-disciplinary partnership in nine countries focused on development of realistic, physical and mathematical, food models for use as standards to be exploited across all major food categories to facilitate development of common approaches to risk/benefit assessment and nutritional quality in food research and industry.

Through 4 years, we have been constantly improving our capacity and skills in food models design and application. The multidisciplinary approach adopted by partners in the project integrated the diverse disciplines engaged in food science and engineering, mathematics, physics, chemistry, biochemistry, microbiology, process engineering, nutrition, etc. The knowledge gained on relationships between structure/formulation property functions and structure process functions allowed tailoring processes according to desired end properties such as safety, nutritive value, and sensory qualities.

The project delivered several prototypes of food models covering the four major food categories: bread, cheese, meat and vegetables. On brassica vegetables, the effect of thermal processing on colour, texture parameters and the effects of processing on bioconversion and bioaccessibility of glucosinolates were modelled. On apple, a model to predict the transfer of procyanidin from fruit to juice depending on processing conditions has been developed. On meat, a mathematical stoichio-kinetic model which assesses how much the kinetic parameters are affected by initial product characteristics and cooking conditions, has been developed.

On dairy dessert models, the impact of the interfacial composition and organization on the connectivity between fat droplets and the microstructure of oil in water emulsions were characterized depending on the protein type and concentration (casein micelle and whey protein) and heat treatments. The reproducibility of a soft-cheese model and a hard-cheese model developed is 3 times better than the reproducibility of classical platforms; it allows reducing the number of trials by 3/5 depending on the significant difference to prove and the alpha and beta risks. The generic bread and biscuit models were developed to study the influence of added dietary fiber on product quality and to develop mathematical models to describe dough formation and product quality. All these models were standardized and cross-validated and fully characterized in structure and composition. They were also used to assess nutritional properties as well as microbiological food safety. The in vitro digestion and in vivo studies highlighted the effect of the different processes in changing the physical and molecular structure of food and consequently the bioaccessibility and bioavailability of nutrients or bioactive compounds.

The model development was conducted in close collaboration with food manufacturers through a specification-based approach. All these models have been implemented thanks to the mathematical approach. This approach realized a complete dynamic description of food processing using an innovative strategy exploiting most recent advances in cognitive and complex system sciences to allow the generalised methodologies to be extended to other food products. Starting from the project outcomes, the industry is able to develop food matrices tailored to requested functions like controlled nutrient delivery, bioactive compound release as mentioned before. During the project, we had good attendance at the different DREAM meetings organized in France, in Spain, in Slovenia, in Hungary and in Italy and we got a very good feedback from the press and relevant technological and scientific

DREAM

community. Respecting this, we did not focus only on research but also we introduced dissemination of our findings to relevant environments such as: science, industry, consumers and regulators. Models and protocols developed within DREAM were efficiently disseminated by expert partners via existing scientific and research channels (CEFood, EFFoST, IUFoST congress) R&D channels (ETP and national platforms, CIAA and national federations, EFSA, national regulatory bodies) to be used by scientists, SMEs and multinationals to improve nutritional quality and benefit-risk management of the food supply networks. Finally, not just research but also industry is now able to develop food matrices tailored to requested functions like controlled nutrient delivery, bioactive compound release...

In short we can summarise our achievements as follows:

A) Industrial achievements: decision-support systems allowing realization of tailored microstructure in the food industry; protocols to produce operating procedures to standardize model foods and practical guidelines on the use of models for industry.

B) Technological achievements: realistic and easily-reproducible in-laboratory food models encompassing large structure variations; optimised methods to characterise relevant structural, chemical and biological characteristics and follow their changes during processing; critical assessment of the relevance of the developed models to determine relationships between matrix microstructure, biological and chemical composition, and activity, delivery and transfer of bioactive compounds (nutrients, phytochemicals, toxins etc) during static (storage) and dynamic conditions (processing, in vivo degradation, etc.); and critical assessment of the relevance of the developed models to valuate the impact of environmental changes on the microbial population of real products and conversely, the effect of microbial populations on food structures.

C) Scientific achievements: mathematical models linking food formulation, processing, resulting matrix tructure and material properties; cognitive mapping of technical know-how involved in construction of food matrices; unified integrated knowledge description of each selected food matrix, and description of the relationships linking molecular and macroscopic structures to functionality.



he DREAM Project International Conference "From Model Foods to Food Models" held from 24th until 26th June 2013 in Nantes.

## Perspectives

by Monique Axelos, INRA (DREAM coordinator)

During the last four years DREAM partners joined their efforts to develop models with in mind the idea that modelling will allow to mimic complexity and make progress in the process-properties relationship to be able to design tailor made food. Even though food processing is a field in which computer modelling is just emerging, the mathematical knowledge integration approach developed in the project appeared to be relevant and useful in giving interesting results as illustrated in this book. Three major issues must be pointed out.

The approach used in DREAM allows i) to integrate multidisciplinary skills, ii) to take into account a large number of parameters which could be integrated in decision support tools, iii) to gather heterogeneous and complex data and to face to the lack of data. Even though some progress has been done during the project further works must be undertaken. For the future, our findings suggest important avenues for further exploration. There are three that I will mention here.

Addressing issues on the collaboration between science and industry is of major importance to be sure that the models developed meet the real needs of the industry. For this purpose, interactions with stakeholders should play a key role and should be held sufficiently upstream of the model development in the laboratory so that opinions from both sides can genuinely be taken into account. Beside direct relationships among project partners our work showed that the main barrier of using models by the industry is the lack of knowledge of what a model could do. A significant effort should be taken to create awareness and encourage the use of modelling in the food industry especially for SMEs proving that models, as time-saving and cost effective tools, provide good support for innovation or for improving existing processes to reach new objectives. Work should continue on improving the industry guide and training tools that have been so far developed.

The second major avenue of interest is the use of this approach to answer complex, multi objectives new societal challenges like food sustainability. We know that the food system makes a significant contribution to the greenhouse gas emissions and gives rise to major environmental impact like biodiversity loss, water extraction and pollution, energy and material waste. In order to cope with this challenge, technologies are required to enable the management of elements (such as energy, conservation, waste reduction and efficient use of capacity) but other non-technical data such as consumer acceptance, economic impact, regulation modification, etc... must be taken into account. Knowledge integration as developed in DREAM appears a very adequate and realistic approach to take into account a large number of criteria in a dynamic way and to provide new way of conceptualizing this challenge.

The third one deals with the increase of knowledge acquisition and integration in laboratory at the interface between mathematics, cognitive and bio-technical sciences. Even inside the academic world this approach



is still poorly developed and implemented in daily work practices. It is a real challenge for public institutions to create awareness and encourage scientists to exchange at an early stage with mathematician to determine the lack of knowledge and to build together new approach of modelling. Food science has really much to gain to go along that route. Respecting new knowledge and skills in this area will contribute much to better quality, greater food safety and more economical food production, preservation and distribution.

#### The DREAM Project Consortium





Extension: 6 months (31 October 2013)

# Table of Content

Foreword Monique Axelos	6
Mathematical knowledge integration for food model numeric simulation (WP1)	7
Introduction of WP1 Nathalie Perrot	8
In silico comprehension and prediction of the structure and texture of a dairy dessert Descamps Etienne, Foucquier Julie, Gaucel Sébastien, Lutton lyne, Mackie Alan, Perrot Nathalie, Riaublanc Alain, Trelea Ioan Cristian	9
Reduction of complexity of an in-silico milk-gel model, using visualization and	
optimization Gaucel Sébastien, Lutton Evelyne, Perrot Nathalie, Riaublanc Alain, Tonda Alberto	12
<b>Coupling viability theory and active learning with kd-trees</b> De Aldama Ricardo, Alvarez Isabelle, Martin Sophie, Rouquier J.B., Reuillon Romain, P-H. Wuillemin	14
An integrative modelling of quality and safety of vegetable during thermal treatment: application to the cooking of Broccoli	
Dekker Matthijs, Gaucel Sébastien, Perrot Nathalie, Postollec Florence, Sarvan Irmela, Verkerk Ruud	16
Semi-supervised learning of a biscuit baking model, using symbolic regression and Bayesian networks	
Dekker Matthijs, Gaucel Sébastien, Perrot Nathalie, Postollec Florence, Sarvan Irmela, Verkerk Ruud	18
Filled cellular solid (WP2)	20
Introduction of WP2 Matthijs Dekker	21
Using mathematical modelling to optimise processing of fruits and vegetables with re- spect to nutritional and sensory quality Matthijs Dekker, Irmela Sarvan, Ruud Verkerk	22
Optimisation of mathematical model describing transfer of polyphenol from fruit to juice by understanding non-covalent interaction between procyanidins and pectins Aude A. Watrelot, Carine Le Bourvellec, Catherine M.G.C. Renard	25
Tomato processing method modify the bioaccessibility of its lycopene David Page, Stephane George, Catherine Renard	28
Proteinous cellular network model (WP3)	30
Introduction of WP3 Carolina Realini	31
Meat models and mathematical modelling to investigate how heating affects protein digestibility	
Astruc Thierry, Danon Jeanne, Daudin Jean-Dominique, García-Regueiro José Antonio, Gatellier Philippe, Gobert Mylène, Gou Pere, Kondjoyan Alain, Pérez-Juan María, Promeyrat Aurelie, Realini Carolina, Santé-Lhoutellier Véronique, Vénien Annie	33
Microstructure characterization of muscle tissue by quantitative imaging Astruc Thierry, Bonny Jean-Marie, Clerjon Sylvie, Damez Jean-Louis, Labas Roland, Vénien Annie	35
Combined gelled / dispersed / aerated systems model (WP4)	39
Introduction of WP4 Alan Mackie	40
The Dairy dessert: A model for designing tailored interfaces in food systems	
Claire Surel, Catherine Garnier, Alain Riaublanc, Marc Anton1, Alan Mackie	41

Coupled impact of product process and composition in lipids as emulsifiers on the structure and the texture of a cream cheese model in relationship with product oxidation Aliénor COUTOULY, Isabelle GAUCHER, Alain RIAUBLANC Open solid foams model (WP5)	
structure and the texture of a cream cheese model in relationship with product oxidation Aliénor COUTOULY, Isabelle GAUCHER, Alain RIAUBLANC Open solid foams model (WP5)	
Aliénor COUTOULY, Isabelle GAUCHER, Alain RIAUBLANC Open solid foams model (WP5)	
Open solid foams model (WP5)	
Open solid foams model (WP5)	
Introduction of WP5	
Kaisa Poutanen	
Structural challenges in nutritionally improved biscuits Kaisa Poutanen, Nesli Sozer, Martin Withworth, Guy della Valle, Lucio	
Creation of bread cellular structure	
Guy Della Valle, Hubert Chiron, Martin Withworth, Kati Katina, Kaisa Poutanen	
Model food applicability (WP6)	
Introduction of WP6	
Angelo Visconti	
Acting on micro- and macrostructure of meat, cooking conditions can modify the	
nutritional potential of meat proteins	
Didier Rémond, Marie-Agnès Peyron, Estelle Pujos, Jean-Louis Sébédio, Louise Salt,	
Emöke Szerdahelyi, Andràs Nagy, Eva Gelencser, Alan Mackie	
Industrial concerns and needs towards fundal risk assessment	
Florence Postollec, Véronique Huchet, Noémie Desriac, Annalisa De Girolamo, Francesca Valerio,	
Paola Lavermicocca, Angelo Visconti, Danièle Sohier	
Technology transfer (WP7)	
Introduction of WP7	
András Sebők	
Industry guide for Food Modelling András Schők, Ágnas Cyurá, Csaba Baár, Isaballa Cauchar, Olina Bustan, Jaan Baná Kariaan	
Management (WP0) & Dissemination (WP8)	
Management	
Monique Axelos, Caroline Sautot	
Dissomination	
Peter Raspor. Lidiia Baša	
Being curious? Consumers clip for you.	
Food Modelling & Model Food	
rood wodening & woder rood Peter Raspor, Lidija Baša	
Food Safety & Food Quality & Process optimisation	
Peter Raspor	
Conclusion	
Peter Raspor	
Perspectives	
Monique Axelos	

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