PROMETHEUS

PROJECT FINAL REPORT

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SEVENTH FRAMEWORK PROGRAMME

Project coordinator ACTIA

Contents

1 Final publishable summary report

1.1 Summary description of project context and objectives

1.1.1 Context

Since the discovery of trace amounts of acrylamide in a wide range of food products in 2002, great attention and concern has been raised on the potential safety issues associated with substances formed during heat processing of foods.

Acrylamide is a neurotoxic and carcinogenic compound well known by chemists and toxicologists. It can be formed naturally in foods such as baked cereal products and fried/roasted foods by reactions between the amino acid asparagine and reducing sugars such as glucose via the Maillard reaction, at a rate that is proportional to the time-temperature couple.

Furan, another carcinogen formed in the Maillard reaction has been found in heat processed food. Formation results from the heat degradation of sugars, vitamin C, amino acids and polyunsaturated fatty acids. Furan is formed in fruit, vegetables, meat or fish during heat sterilisation. It is very volatile but accumulates in jars, cans and bottles from where it cannot escape and can potentially reach levels of toxicological concern.

Another carcinogen, 3-monochloropropanediol (3-MCPD) known previously to be formed in protein hydrolysates produced with HCl, was also discovered at high levels in refined vegetable oils as 3-MCPD esters of fatty acids, along with the ester of the related carcinogen glycidol. Due to the likelihood that 3-MCPD and glycidol are released from their esters by enzymatic hydrolysis in the intestine, these esters are currently of great toxicological concern. The main question regarding these derivatives of glycerides is the potential role of heat treatment of food in forming these esters in addition to those preformed in the oil ingredients.

Finally and more generally, a mixture of familiar or less-known Maillard products called dietary glycation compounds or advanced glycoxidation end-products (AGEs) found in food could, after intestinal absorption, contribute to the endogenous pool of AGEs. Selected studies have proposed that AGEs are a risk factor that may trigger oxidative stress and microinflammation. Possible activation of the receptors to AGEs (RAGEs) by these food-derived Maillard products could explain the increased risk or aggravation of cardiovascular disease and diabetes in animals exposed to heat-treated food. Similarly, increase in early markers of cardiovascular risk and insulin resistance was observed to be significantly higher in humans ingesting severely heat-treated food. Carboxymethyllysine (CML) is one of the best-known dietary glycation compounds and seems a good marker of heat treatment of food. This neoformed Maillard compound is of particular importance in infant formulas, because of their high content of Maillard reaction substrates and the successive heat treatment applied during manufacturing.

The food industry behind FoodDrinkEurope, the European Technology Platform ‘Food for Life’ in its Implementation Actions and the food safety agencies/authorities such as FDA (Food and Drug Administration) and EFSA (European Food Safety Authority) have addressed the concerns raised by certain processing contaminants (PC) such as acrylamide and furan. For acrylamide the European Commission expects food industry to consider available mitigation strategies and has issued Indicative Values aiming to trigger further actions/research in cases where the acrylamide level exceeds the Indicative Value set for the respective food category.

Although the major reactions that form these contaminants from food substrates are known, the reaction rates and intermediary compounds are poorly understood. Nevertheless, mitigation can be investigated by comparing food models designed by well controlled recipes and characterized ingredients, and processed by various technologies using well-chosen parameters. The potential interest of some alternative technologies to conventional heat treatment can therefore be examined by comparing the PC level in the final product, while other quality parameters are measured for global comparison of risk-to-benefit interest. The economic aspects of such solutions must be evaluated, including the energy consumption, the running time and return on investment.

Assessing PC concentrations in the ingredients and food products at different steps of processing is possible thanks to chromatographic techniques. The European Normalization Commission has selected the most reliable techniques to be normalized, and interlaboratory studies (FAPAS) are running to evaluate each technique. However, such methods are expensive, complex and time-consuming and are not adapted to industrial scale. Rapid and simple methods must be developed to allow industrial plants to perform their own analyses and control their mitigation strategies or implement a routine quality control of their final products. Moreover, non-destructive techniques that could be implemented in-line are of great interest for future early control associated with possible corrective actions. Multicriteria analytical techniques are preferred to allow global evaluation of the final product quality, including sensorial, nutritional and of course microbiological quality.

1.2 Objectives

Any mitigation options proposed to the food industry must consider the impact on other food quality parameters: other undesired constituents, achievement of comparable (or better) nutritional and sensorial quality, whilst offering a microbiologically safe product. **This project aims at assessing the capabilities of alternative technologies and the value of using rapid analytical tools to help industry identify the optimal solution for their product(s).**

The easiest solution is to minimize the heat treatment applied to food. But a direct and potentially unacceptable consequence will be to decrease the margin of safety of the bacteriological quality in case of sterilisation, or to alter the sensorial profile in case of cooking, baking or roasting processes, with significant consumer impact. Such solutions are risky and an online precise control of the heat treatment received by the product is necessary to better fit the temperature profile to the specific needs. Although very accurate temperature sensors, allowing determining and control efficiently a time temperature profile, are available today the real impact on food is partly uncertain due to uncontrolled variability in the raw food material or ingredients. Moreover, a better understanding of the reactions taking place in the food product, leading to the formation of PCs will be necessary to orientate the reactions in the optimal way. Direct translation into specifications for ingredient quality or product recipe can be drawn from such comprehensive approach.

- **The first objective of the project is to implement an online sensor to control the heat charge absorbed by the food product and monitor the main heat-influenced quality indicators.**
- **The second objective is to obtain a precise understanding of the reaction mechanisms leading to PC and other quality parameters when the food is exposed to this heat charge.**

The combination of the two first objectives will allow the food industry to have a better control on the food processes and orientate the time temperature profile at the point where PC are decreased while maintaining acceptable product quality attributes, with minimal cost and investment.

In some cases however the decrease in PC achieved will not be sufficient, thereforenew technologies improving the time-temperature profile and heat transfer to the product, such as ohmic heating, or a

decrease the temperature needed to obtain the same result, such as under vacuum heating or high hydrostatic pressure preservation, should offer significant mitigation of PC.

- **The third objective is to develop and validate the efficiency of new alternative processing technologies regarding PC mitigation.**

In addition, a protection of the sensitive nutrients or components of the food product will be possible with the microencapsulation technique. Vitamin C and iron form a very reactive mixture when in solution and isolation of at least one of these ingredients via an easy digestible polysaccharide capsule should impede the interaction between them. Moreover the heat and oxygen sensitive vitamin C or polyunsaturated fatty acids (PUFA) could be protected against these two interacting degradation sources by including an oxygen and water barrier at the capsule periphery.

- **The fourth objective is to explore the technological feasibility and potential of microcapsules for isolation of reactive substrates to limit their destruction and their interaction with other food components.**

Finally each solution to be tested in the project will be optimized and validated. Beyond the PC mitigation aspects, all other quality parameters will be studied including economic aspects related to the process to ensure the sustainability of the solutions proposed. The performance of the solutions will be tested throughout the product shelf-life, so that storage conditions will also be taken into account and optimized. A sensor will be developed to monitor the main quality parameters of the final product throughout the shelf-life. In order to limit the number of experiments to be performed at pilot plant, modelling of the reactions in the food products following processing will allow simulation of the impact of different process parameters. The optimised conditions derived from simulation will be performed to confirm the efficiency of the new parameters at pilot plant followed by an assessment of the possibility to scale up to semi-commercial scale.

- **The fifth and last objective is to propose sustainable processing technologies and analytical control, taking into account the multiple aspects of food quality throughout the shelf-life of the product in a risk-benefit like approach.**

This will include not only the different quality dimensions of the food but also the process energy consumption level and cost.

2 Description of the main scientific and technological results

2.1 Innovative monitoring strategies

2.1.1 Front face fluorescence analysis to monitor food process contaminants

Fluoralys technology

Fluorescence is a natural phenomenon of light emission when a molecule has received light energy, with 10% of light-absorbing molecules being fluorescent. In food products a wide range of molecules, such as proteins, various vitamins and pigments, have this capability. Maillard products are highly fluorescent, therefore fluorescence can be detected with exceptionally high sensitivity, making it possible to detect trace amount of PCs in heat-treated food products.

To detect the natural fluorescence emitted by food products, a compact and robust analyzer, Fluoralys, was developed by Spectralys (Figure 1). It comprises the optical system required to perform front face fluorescence measurements. Four fibre coupled LED light sources for sample excitation, and one for fluorescence recovery are embedded in a probe. The probe is inserted into a chamber containing the sample at a given distance from a solid sample (biscuit) or inside the puree or milk (baby food). The fluorescence light is decomposed by a spectrometer and analysed using software in an embedded computer. The software sends the measurement to a remote database and applies decomposition and prediction models producing a result, such as PC concentration, in 30 sec.

Scheme of the optical design of the compact fluorimeter Fluoralys.

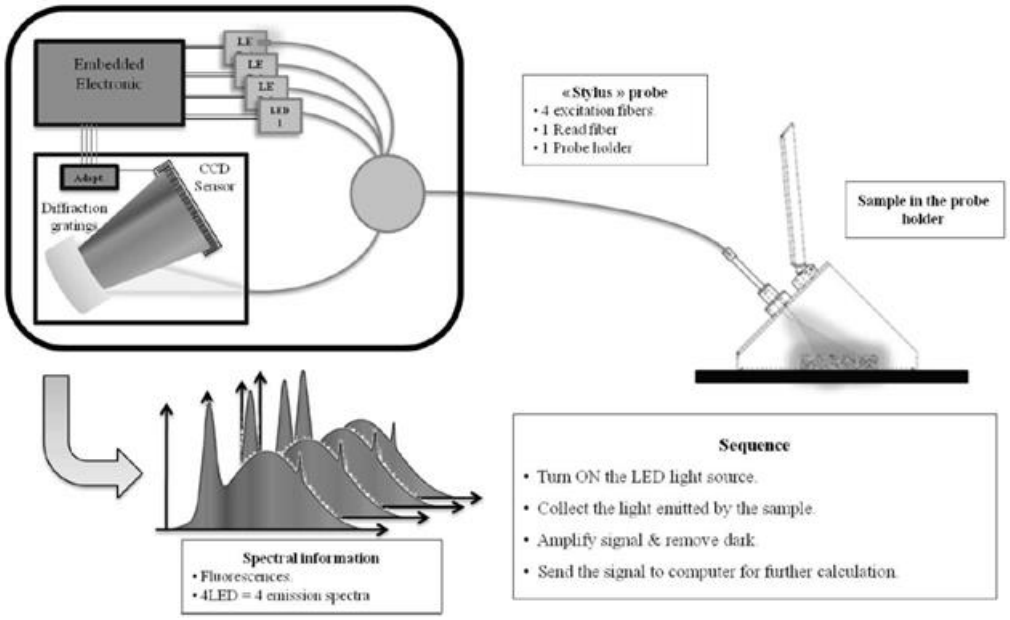

Figure 1. The Fluoralys analyzer: principle.

The Fluoralys analyser allows fast optimization of recipes and process parameters by real-time diagnosis of the impact on final product quality. The analysis can be made at a laboratory by sampling the product and analyzing it in the black chamber or on line (near the production line). The apparatus can also be used in line, using temperature and pressure resistant probes (Figure 2). A

control map in real time, allows implementation of corrective actions when necessary to ensure stable product quality.

These various applications are of great advantage to the food industry to help deal with new safety issues such as control of PCs, and more generally to face the high and uncontrolled variability of the ingredients. The simple and real-time analysis of product is an efficient tool for improvement of the quality, which is not possible when samples have to be sent to external laboratories in an expensive and time-consuming approach.

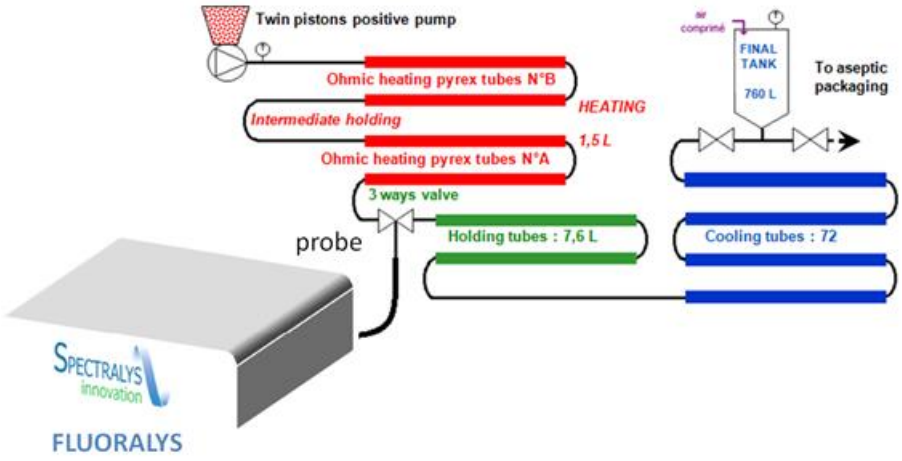

Figure 2. Example of Fluoralys in line implementation on an ohmic heating system for baby food puree sterilization.

2.1.1 Application to the objectives

a. A global and rapid diagnosis to compare the efficiencies of different technologies

A simple way to get a rapid view on the efficiency of an alternative technology compared to the conventional one is to calculate the difference between the two fluorescence images of the corresponding products. This difference is calculated on statistical basis as the **Distance from reference index (DFR)**. DFR measures both the degradation of native components such as vitamins, or protein denaturation, and the formation of contaminants including PCs. The method calculates the DFR using the raw product before thermal processing as reference. The DFR index increases proportionally to the severity of the heat charge and damage suffered by the food product. In the Prometheus project, we aimed at selecting alternative technologies able to ensure the microbiological and sensorial qualities of the final product whilst decreasing the negative impacts of the process, including formation of PCs. We show in Figure 3 how the real-time assessment of DFR using Fluoralys provided a reliable diagnosis of the positive impact of ohmic heating compared to retorting on carrot puree quality parameters, as confirmed by furan assessment. The two evolutions are not perfectly similar, because of the global quality assessment of the DFR index. Beyond the specific impact measured on furan as a model PC, many other quality parameters were shown to evolve, but to a different extent. Hence, carotenoids and some phenolic compounds were also increased by ohmic heating compared to retorting, but to a much lesser extent than furan.

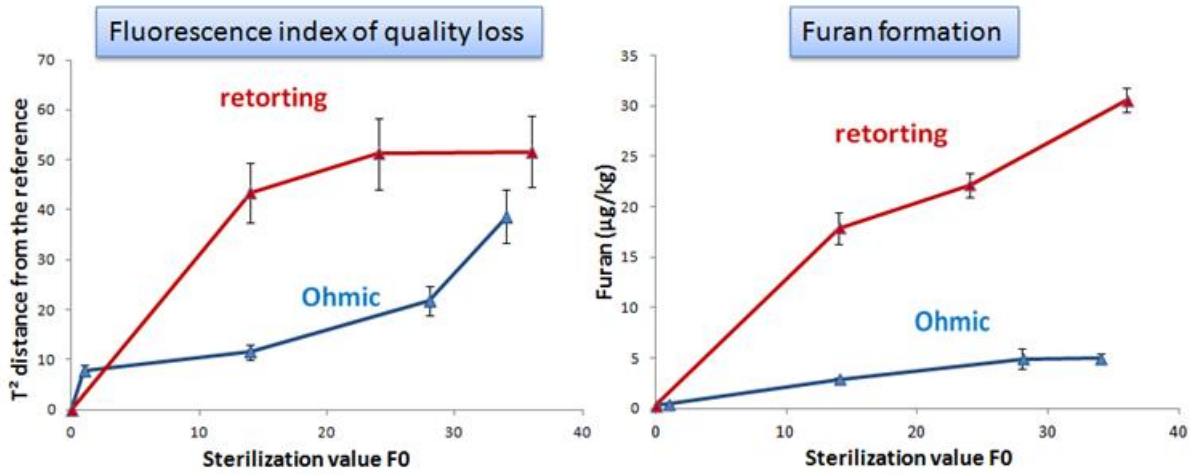

Figure 3. Comparison of retorting and ohmic heating impact at 129 °C on carrot baby food using the fluorescence parameters DFR and the chemical assessment of the PC furan.

b. *Assessing product quality parameters including PC in real time*

The other aim of Fluoralys was to enable easily real time measurement of several compounds of interest (vitamins, neoformed compounds, moisture, etc.) present in very low amounts. The concentrations of these molecules should be strongly influenced by the heat charge absorbed during the thermal treatment. Other quality parameters, such as texture or colour, evolving under the same conditions could also be assessed in this way.

First, a calibration model is built relating the fluorescence information with quality indicators measured using standard chemical techniques (Figure 4). Based on such calibration models, fluorescence measurement on any food product of the same type as the calibration will allow assessment of the quality parameter of concern. For example, measurement of acrylamide in biscuits can be made without any preparation, by inserting a biscuit in the black chamber and waiting for approximately 30 seconds. The result has an error slightly higher than that of the conventional method due to the additional calibration error. The calibration model must be checked and updated regularly, at least whenever changes in the recipe or process are made. A control map allows monitoring of the data obtained through different batches, and a database including the results together with all sample characteristics for traceability and further analyses can thus be built.

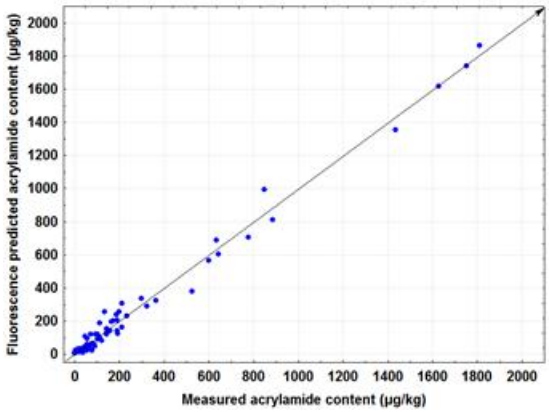

Figure 4. Calibration model for acrylamide (mean relative error 8%).

2.1.2 Some major results obtained within the project

We applied the analytical technology with success to the four food products for PC monitoring, or process/recipe comparison regarding their susceptibility to produce PCs.

We show here two examples: detection of the undesirable Maillard product CML in infant formulas (IF) and inline quantification of the impact of ohmic heating time-temperature parameters on the PC, and monitoring of acrylamide in biscuits with other quality parameters.

a. 3.1 CML in infant formulas

A basic IF recipe was chosen and some variations were introduced on the basis of their known impact on Maillard reaction development. For example, docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid (LC-PUFA), is a critical ingredient in IF because of its beneficial influence in brain development. However it is very prone to oxidation on heat treatment. A second ingredient of interest is iron, because of its high importance for prevention of anemia, while activating oxidative reaction.

We compared formulas with and without long-chain PUFA in the presence and absence of iron, using Fluoralys in the DFR approach to give a global view on the quality of the product (Figure 5). The DFR evolution during the sterilization treatment was compared with the CML concentration measured by conventional analysis. We observed much faster product damage in presence of LC-PUFA, especially in presence of iron, in agreement with observations based on CML. We confirmed that fluorescence provides a reliable and real-time diagnosis on the recipe impact throughout the heat process. A calibration model was also built to allow CML prediction in the various recipes using Fluoralys. The mean relative error was 8%.

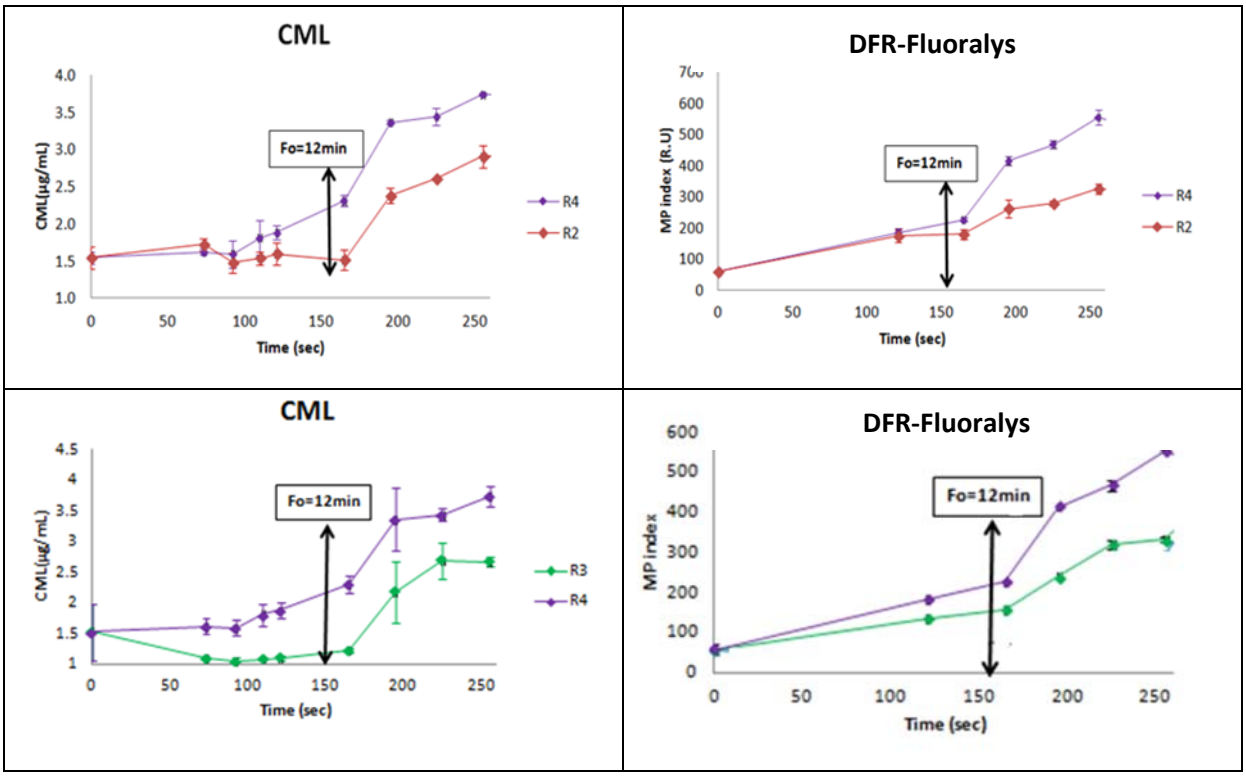

Figure 5. DFR evolution using Fluoralys and CML concentrations of infant formulas including or not LC-PUFA in the presence of iron (R3 without and R4 with) and the effect of iron in the presence of LC-PUFA (R2 without and R4 with 12 mg/L).

b. *A multi-criteria quality assessment of biscuits using Fluoralys*

In partnership with the Spanish company SIRO, a proof of concept was developed at industrial level to demonstrate the potential of Fluoralys to provide a multi-criteria comprehension of the interactions between different batches of dough of two recipes after baking process.

This industrial study was carried out to provide 43 independent batches produced with 2 different recipes of black biscuits (Z and M). The 2 recipes studied were very similar differing only in few ingredients. For the different batches of a same recipe, different ingredients batches and even suppliers were also considered. Samples were taken after baking (final product). The water content is the parameter used to control baking with a target final level of 2-3% depending in the recipe. Baking temperature and ventilation were adapted to reach this standard, the variability on this parameter thus being very low (approx. 10%).

Various calibration models were built, over acrylamide, colour (L ;a ;b), humidity and texture, with a average relative prediction errors of 8%, to provide a multicriteria quality control on the final product in real time. Figure 6 shows batch variability of the different quality criteria. While color (here factor b in Lab assessment) and humidity were relatively stable (less than 10% variation), acrylamide and texture strongly varied, from 71 to 358µg/kg and from 1600 to 2100 g respectively. Such variability is probably due to variation in the ingredients and dough composition and to fluctuations in process parameter intentionally implemented to control the final water content.

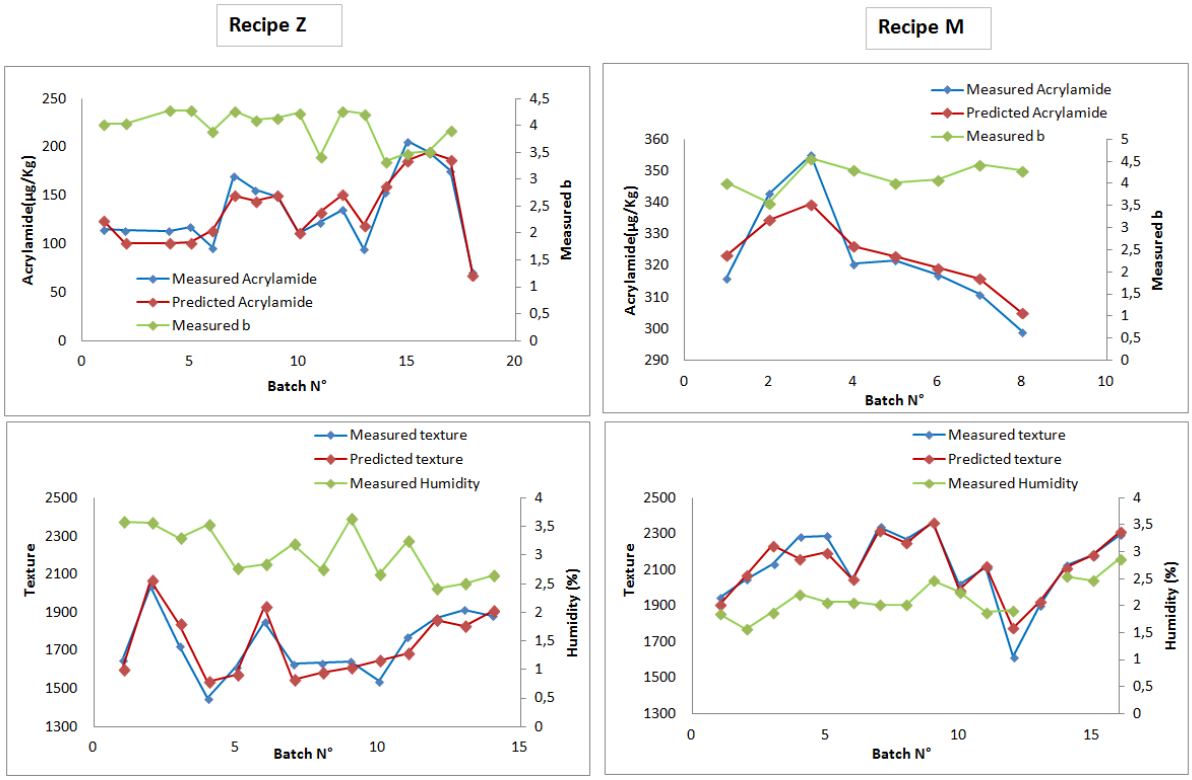

Figure 6. Acrylamide and texture monitoring using Fluoralys in the final biscuits: comparison with conventional analyses. Color (b factor) and humidity are standardized by adaptation of process parameters.

2.1.3 Conclusion

- **Front face fluorescence is an accurate, sensitive, and non-destructive real time analytical tool**
  - ✶ To control the raw material variability and impact on the final product quality
  - ✶ To assess the impact of processing steps and storage
  - ✶ To control the compliance of each batch regarding the targeted quality
  - ✶ To compare different technologies

- **Fluoralys can be used with different methods**
  - ✶ **Global quality index** without calibration to get a rapid screening of quality compared to a reference
  - ✶ **Calibration** to monitor in real time a specific quality parameter
  - ✶ Such methods are automated and can be used at line and in line

- Thanks to this innovative tool, it is now possible to get a **rapid diagnosis of the effect of alternative technologies, such as under vacuum baking, ohmic heating, or high pressure**. Not only is the potential to mitigate PC provided but also the possibility to evaluate their impact on other quality parameters, such as water content, colour or texture.
- Furthermore, this multicriteria approach **allows correction of the process parameters in line** to achieve final product standardization at the desired quality level.

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2.1.2 Ambient mass spectrometry

Direct analysis in real time (DART) is new ambient ionization technique for mass spectrometry (MS), which enables rapid analysis of liquid, solid or gaseous samples in the open atmosphere, without any separation of sample components. DART-MS has a high throughput and needs minimal sample preparation. When coupled to a mass detector it is an excellent tool for instant chemical characterization of various biological samples, including foods.

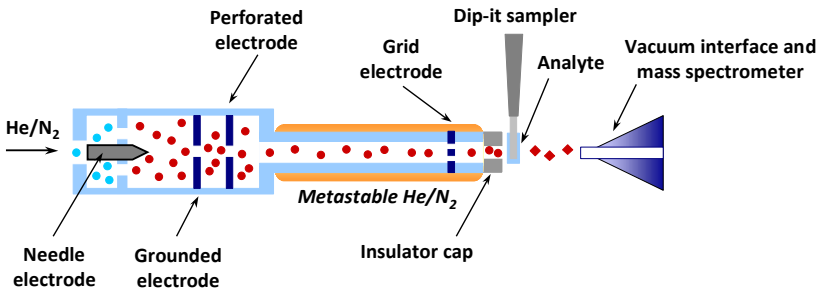

Figure 7. Layout of the DART ion source.

The scheme of DART ion source is provided in Figure 7. In DART, metastable excited-state atoms are formed via a glow discharge in a compartment separated from the sample desorption/ionization

area. Charged particles are removed from the gas stream carrying metastable species through the perforated electrode. Then, the gas is heated to desorb the analytes from the sample. In the sampling region, metastable ions react with atmospheric components to form reactive species, ionizing the analytes. DART produces mass spectra, dominated by protonated molecules in positive-ion mode, or deprotonated molecules in negative-ion mode. Although DART represents a soft ionization technique, fragments can also be observed in DART mass spectra.

2.2.1 Methodology

The applicability of the DART technique for the purpose of the project was demonstrated with the use of four representative food matrices, supplied by the partners. The samples were as follows:

- Infant formula prepared without ohmic heating; samples prepared by heating at 90 to 140°C without a holding time and IF heated at 130 and 140°C for time corresponding to F0; and overheated samples.
- Biscuits prepared within atmospheric baking in laboratory scale oven under different conditions of temperature and time (180-200°C, 10-14 min).
- Baby food puree (carrots) prepared by 3 heating/freezing regimes) and sterilized at 2 different temperatures (117 and 127°C).
- Canned fish (tuna in brine; tuna in sunflower oil; sardines in sunflower oil) prepared by high pressure treatment under different conditions temperature and holding time 115°C, time 28 min or 121°C, time 7 min.(tuna in brine: temperature 115°C, time 28 min.

Two complementary sample preparation approaches were used to isolate and analyse consecutively both polar or non-polar compounds. For infant formula, dilution of the samples with methanol (1:5, v/v) was used to enhance the DART ionization of polar components . Non-polar compounds were simply extracted with toluene. For biscuits and baby food puree isolation of polar and non-polar compounds was performed via extraction by a methanol-water mixture (1:1, v/v) and toluene, respectively. For canned fish isolation of both, the polar and non-polar compounds, was performed simultaneously in one step by homogenising in deionized water and cyclohexane and centrifuging.

For statistical evaluation of the data, group t-test (differences between contrast samples) and principal component analysis, PCA (visualization of multivariate fingerprint data) were used (Statistica, version 8.0; Statsoft, USA). The input data for PCA were the intensities of the ions pre-selected from DART fingerprints, normalized to the most abundant one.

2.2.2. Results

Infant formula

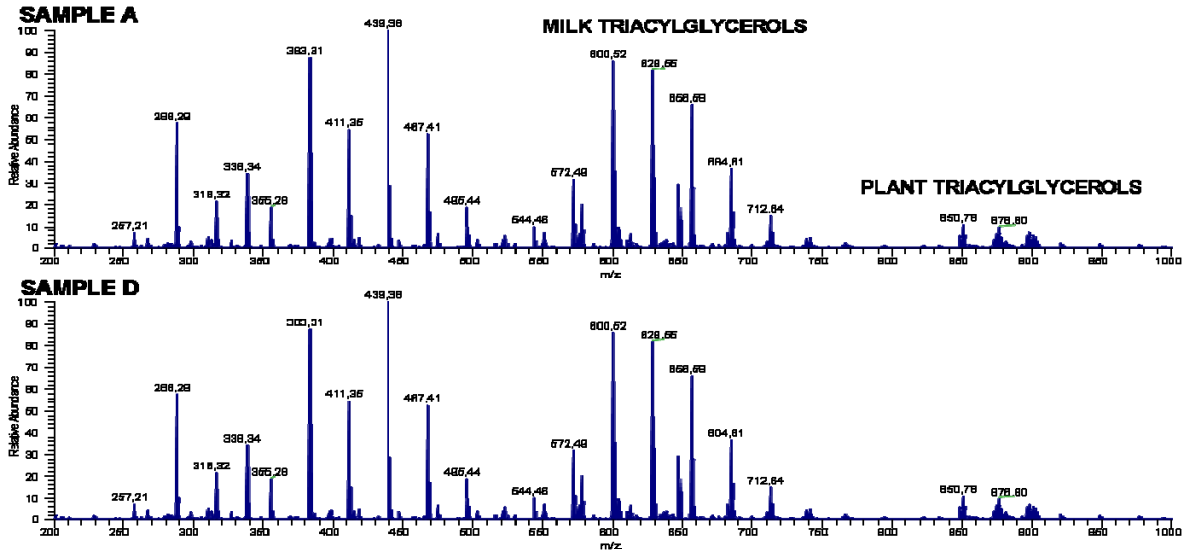

Figure 8. DART–MS spectra of infant formula toluene extracts of different samples.

Infant formula samples were analyzed in both the positive and negative DART ionization mode. The positive ionization mass spectra of the infant formula toluene extracts enabled monitoring and identification of milk and plant triacylglycerols (Figure 8). No statistically significant differences were observed, between non-treated and heat-treated samples, indicating that mild heating did not cause any changes (oxidation) of lipid In negative ionization mode, no abundant signals could be observed.

Principal components analysis (PCA) was used to show differences between the samples. It produced a visual plot of the data by transforming the input variables (relative intensities of 16 pre-selected ions) into principal components. A PCA plot constructed for the infant formula data is shown in Figure 9. As can be seen, the objects representing sample A and sample D are well resolved each other and from the objects in samples B and C. One of the marker signals was identified as HMF.

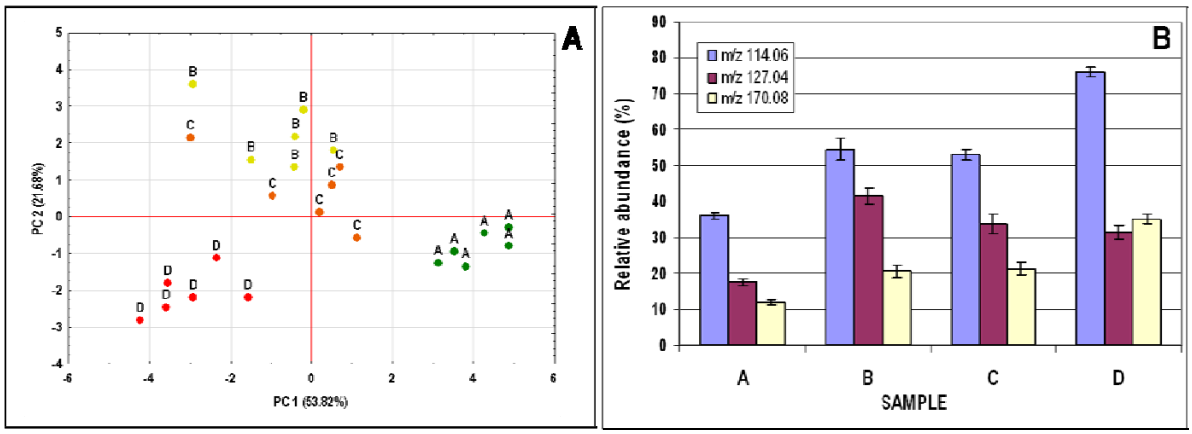

Figure 9. (A) PCA plot of positive ionization data obtained by analysis of methanolic-extracts of infant formulas, (B) relative intensities of selected marker signals in respective infant formula samples.

Biscuits

Analysis of toluene extracts of biscuits also showed differences between DART fingerprints of different samples. DART–MS spectra obtained in either positive or negative ionization mode exhibited much higher potential for monitoring of changes within experimental heating time/temperature combinations compared to the non-polar fraction fingerprints.

Numerous signals corresponding to various compounds are changing their relative intensities (both increase and decrease can be observed), some of them being formed or disappearing from the mass spectra. Such changes in chemical composition are clearly linked to a different extent of the Maillard reaction taking part in the samples during heat-treatment (baking). The extent of the alteration of the DART fingerprints during prolonged heating, and at increasing temperatures, is shown in Figure 10 and via PCA in Figure 11.

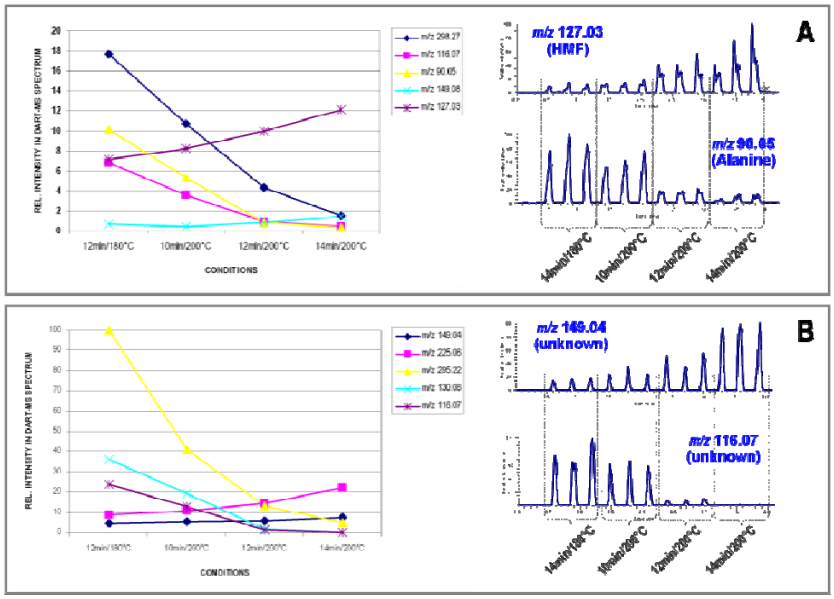

Figure 10. Changes in relative intensities of selected signals in biscuit samples (left) and example of extracted ion records (right). (A) Positive ionization mode data, (B) negative ionization mode data.

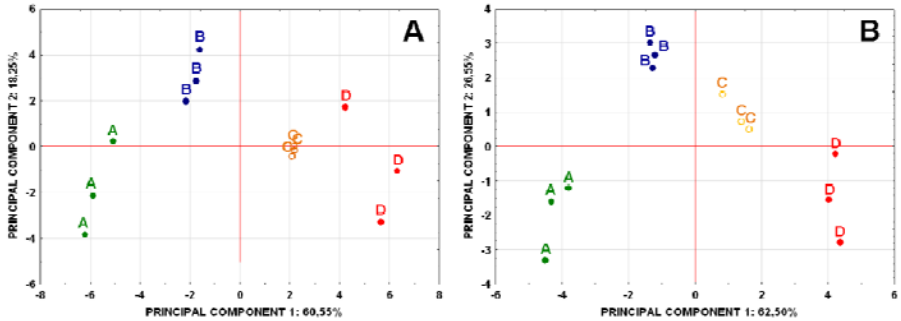

Figure 11. PCA graphs of DART fingerprints obtained by analysis of methanol-water extracts of biscuits samples. (A) Positive ionization mode data, (B) negative ionization mode data.

Statistical t-tests enabled the selection of the most discriminant ions. The intensities of some signals increased while others decreased, mimicking the dynamic changes in the concentrations of reactants, intermediates and end-products of Maillard reaction. The identification of some of these compounds was achieved by the accurate mass measurements by DART–MS.

Baby food puree and canned fish

The relative intensities of the ions present in baby food puree fingerprints (both polar and non-polar fractions) were not influenced neither by production process nor by the sterilization temperature. Similar results were obtained also for high pressure-treated canned fish. Differences related to the fish and/or oil type could be observed in cyclohexane extracts, however discrimination of the test samples according to the temperature-time combinations were not possible.

2.2.3. Discussion and conclusions

DART–MS-based fingerprinting of extracts prepared from four representative food matrices investigated within the project, enabled efficient fast monitoring of polar and non-polar compounds. The novel approach was able to monitor temperature-induced changes in chemical composition of infant formula (ohmic heating used for sterilization) and biscuits (baking).

Statistical analysis enabled selection of signals that changed during heating. These "marker" signals derived from precursors, intermediates and products of reactions in the food (mainly Maillard reaction). A tentative identification of "marker" substances could be made by estimation of elemental formulae, based on the knowledge of their accurate masses. The high-pressure treatment/sterilization and freezing/thawing/sterilization applied to baby food puree and canned fish, respectively, did not cause any notable changes in DART fingerprints.

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2.1.3 Computer vision based image analysis

Colour is an important feature of food products takes critical part in buying decision as it communicates with the consumers. Change of colour in biscuits during baking is a dynamic process in which certain colour transitions occur as the baking proceeds. Browning development in biscuits begins when sufficient amount of drying has occurred. Moreover it is associated with the recipe (reducing sugars, leavening agents, salt, amino acids, etc.) and baking conditions (temperature and time). For a fixed recipe, increasing the thermal load also increases the degree of browning on the surface of biscuits. Previous findings have revealed that colour can be correlated with the concentration of thermal processing contaminants such as acrylamide and 5-hydroxymethylfurfural (HMF) in bakery products.

Computer vision technology has been used for many years in food industry. In this project, **the objective was to develop a computer vision-based image analysis tool to monitor the development of surface browning in biscuits, and hence to predict the changes in the concentrations of acrylamide and hydroxymethylfurfural in biscuits during the baking process.** Being an objective, rapid and non-contact tool, computer vision-based image analysis is considered as a powerful technique for quality inspection and safety evaluation purposes for bakery products like biscuits.

A computer vision based image analysis system is composed of a camera to acquire digital images, daylight lamps to illuminate the object, and a personal computer to process the image data. With the camera, it is possible to register the colour of any pixel of the image of biscuits using three-colour sensors. The changes in the surface colour of biscuits can be determined by using two approaches;

1. Mean colour information (i.e. CIE a* value),
2. Featured colour information (i.e. browning ratio)

A typical image captured by a digital camera consists of an array of vectors called pixels. In the digital images of biscuits, pixels can be categorized into two or more groups based on the typical colour transitions occurred in biscuits during baking. These reference values are used for the segmentation of biscuit images. The pixels are classified into sub sets based on their Euclidian distances to the

representative colour reference values. The segmented image is then used to calculate browning ratio as featured colour information to establish a correlation with other quality features of biscuits. Another approach is to extract mean colour information from the digital images of biscuits. In this application, it is possible to calculate average colour information in different colour space parameters (i.e. CIE Lab, RGB) for a selected region or entire image.

In order to test the capability of computer vision based image analysis, the biscuits prepared from a basic recipe (Table 1) by baking at different temperature (180-220^{o}C) and time (6-20 min) combinations were analyzed for surface colour.

Table 1 The basic recipe used to prepare model biscuits for computer vision based image analysis

Ingredient	Amount, g
WHEAT FLOUR (T55 / W150)	80
REFINED PALM OIL	20
SUCROSE	35
NaCl	1
WATER	17.6
SODIUM BICARBONATE	0.8
AMMONIUM BICARBONATE	0.4

The digital images of biscuits are shown in Figure 12. As an alternative tool, different computer vision based analysis algorithms were applied to biscuits in order to validate the potential of this technique for online monitoring.

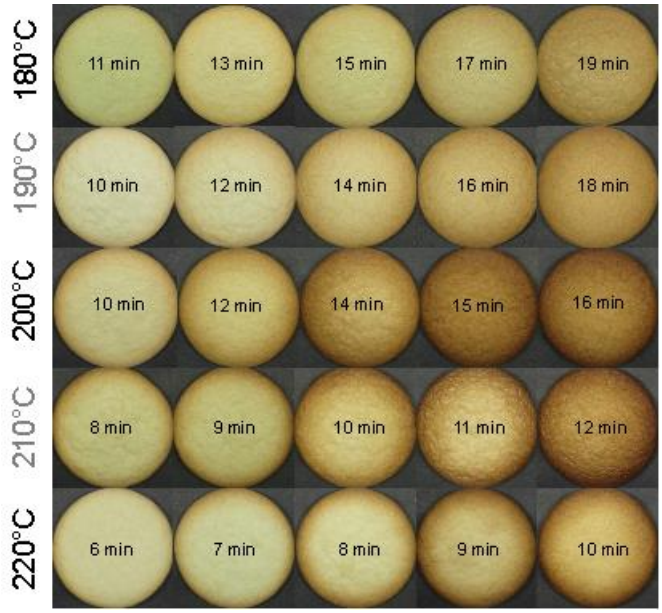


Figure 12. Digital images of biscuits baked at different temperature-time combinations used to build a calibration model for the prediction of acrylamide and HMF in biscuits

An algorithm for the determination of mean colour information was developed to measure surface colour of biscuits in CIE Lab colour space. Among the colour space coordinates, CIE a* value better indicated the development of browning on biscuit surface during baking. Since browning develops as a circle during baking, different colour regions occur on the biscuit surface. The image is first converted Lab from RGB, and mean L, a, and b values are calculated for the region of interest (centre,

middle, edge). Mean colour (CIE a*) determinations were performed on biscuits to validate the computer vision-based image analysis tool for the prediction of acrylamide concentrations. The algorithm developed in the preliminary assays was used to correlate mean CIE a* value with acrylamide. As shown in Figure 13, change of CIE a* value with time was significantly different in centre, middle, and edge regions of biscuit. As expected, the edge of biscuit discs became darker more rapidly than the middle and centre regions.

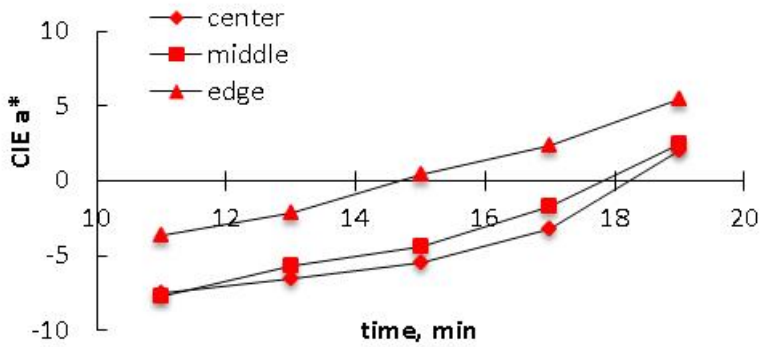


Figure 13. Change of CIE a value in the center, middle and edge regions on biscuit surface during baking at 180 °C.*

The CIE a* values measured in the edge correlated well with acrylamide concentrations of biscuits. As shown in Figure 14, there was a linear correlation between CIE a* value and acrylamide concentration with a high correlation coefficient (r2=0.927). Based on this correlation, a CIE a* value of 4 indicates an approximate acrylamide concentration of 100 ng/g in biscuits prepared from the basic recipe. Similarly, CIE a* value of 11 indicates an approximate acrylamide concentration of 200 ng/g in biscuits. These results indicated that mean colour information taken from the digital image of biscuits could be used to predict acrylamide concentration in biscuits. However, this correlation is specific to the recipe given in Table 1. Any significant changes (removal or addition of ingredients, change in the ratio of certain ingredients) made on the recipe may require recalibration of the model as these changes may influence both the rates of browning and acrylamide or HMF formation. However, the calibration model exemplified here reflected very well the changes in baking temperature and time.

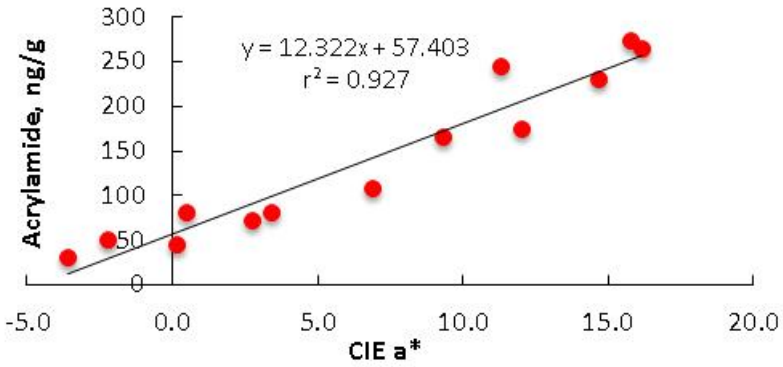
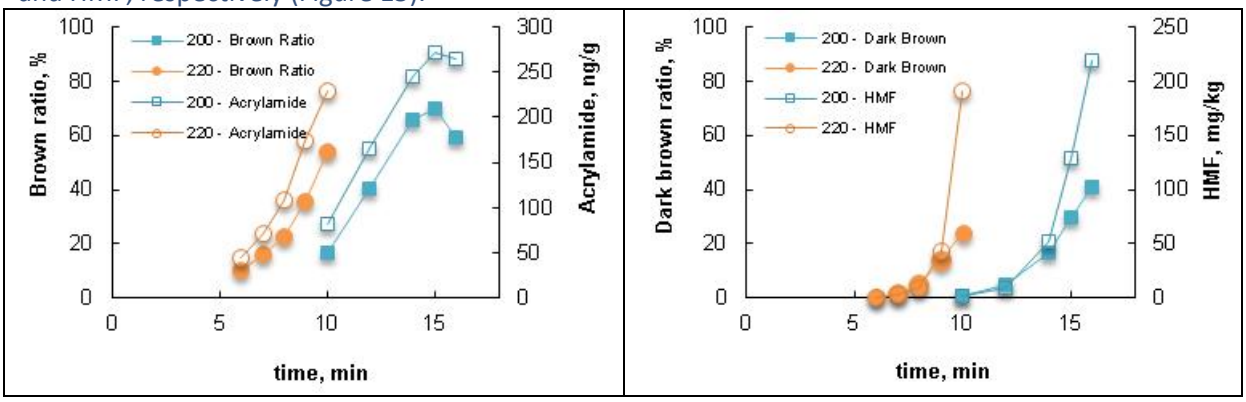


Figure 14. Correlation between the CIE a value and acrylamide concentration of biscuits prepared from the basic recipe at different temperature-time combinations.*

Another algorithm developed for the determination of brown ratio and dark brown ratio was based on the colour segmentation of digital biscuit images. The developments of brown and dark brown

ratios, the new features defined in this study, have typical kinetic patterns resembling to acrylamide and HMF, respectively (Figure 15).



(a) (b)

Figure 15 (a) Change of brown ratio and acrylamide concentration with time in biscuits baked at 200 and 220°C. (b) Change of dark brown ratio and HMF with time in biscuits baked at 200 and 220°C

These kinetic pattern similarities allowed us to build a correlation between brown ratio and acrylamide concentration, and between dark brown ratio and HMF concentration for biscuits. There was a linear correlation between brown ratio and acrylamide concentration (r^2=0.963). A correlation between dark brown ratio and HMF concentration was also built for biscuits (r^2=0.964). The assays performed on the biscuits confirmed the potential of computer vision based image analysis algorithms for predictive monitoring of acrylamide and HMF during baking.

The following points were highlighted from the experimental results:

- Two computer vision based image analysis algorithms were developed for the extraction of mean colour and featured colour information from biscuits. These algorithms were successfully applied to the biscuits baked at different time-temperature combinations.
- Mean colour information as CIE a* value and featured colour information as brown ratio were found to correlate well with the change of acrylamide concentration in biscuits during baking. Dark brown ratio, another featured colour information was also defined and found to correlate with the change of HMF concentration in biscuits.
- **Surface colour of biscuits can be monitored online by means of computer vision based image analysis to predict PCs under real processing conditions.**
- The calibration models are specific to the recipe used. Any changes or modifications in the recipe would require validating the existing calibration model. If required, new calibration should be built for the modified recipe.

In conclusion, computer vision based image analysis offers rapid, accurate, non-contact, and non-destructive analysis of foods. Additionally, it provides a high level of flexibility and repeatability at relatively low cost and high throughput. Besides, it can be implemented online as an integral part of processing plants for real time monitoring of product quality.

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2.2 Mitigation strategies

2.2.1 Vacuum baking

Baking is a complex process in which certain chemical and physical changes take place simultaneously. It is important not only in terms of the shelf life stability of biscuit, but also in terms of eating quality, taste and texture. Beside its desirable properties, baking come with certain food safety concerns caused by PCs such as acrylamide and HMF. Temperature, cooking time, and final moisture content are closely related in the baking process. Temperature is one of the most significant parameters affecting acrylamide and HMF formation in biscuits. Apart from the processing conditions, ingredients play also an important role in the formation of PCs.

Mitigation strategies propose modifying the product formulations or processing conditions. **It is hypothesized that reducing the thermal energy load during baking may generally lower the formation of harmful compounds in biscuits.** Although lowering the temperature may generate less acrylamide or HMF, prolonged cooking time is usually required to achieve desired moisture content and textural properties in the final product. The literature is lacking in investigation of the effects of low-pressure at elevated temperatures on the formation of thermal PCs in bakery products. **The objective of this project was to investigate the effect of baking under vacuum on acrylamide and HMF formations in biscuits.** The principle of vacuum baking was to decrease pressure in the oven, thus to decrease boiling point of water during baking. Baking under vacuum allowed us to decrease cooking temperature without retarding the drying process, because moisture evaporation was accelerated under vacuum. Reducing atmospheric pressure in the oven by half enables to decrease baking temperature by 20^{o}C with approximately same drying rate.

The basic recipe given in Table 2 was used to produce biscuits using conventional atmospheric baking and vacuum baking technologies. It was adapted from AACC method 10-54. The dough prepared by mixing the ingredients has been rolled out to obtain the discs having a diameter of 5 cm with a thickness of 3 mm.

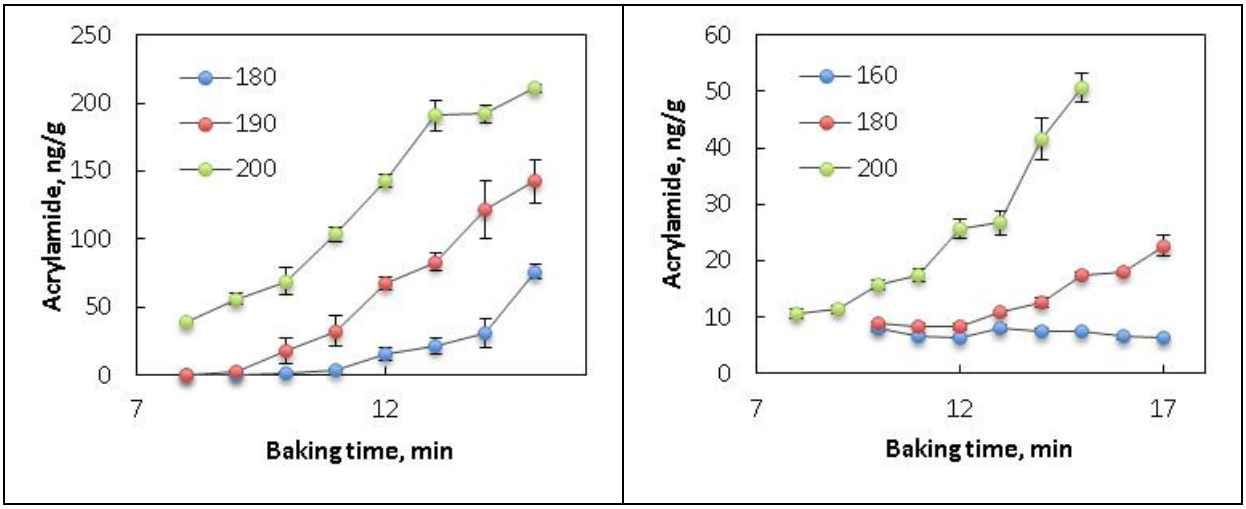
Biscuits were baked using three different processes, namely conventional baking, vacuum baking, and combined conventional-vacuum baking in order to determine their effects on acrylamide and HMF contents of biscuits. Conventional baking process was performed using an oven at 180, 190, 200^{o}C for different times up to 15 min. Vacuum baking process was performed using a vacuum oven at 160, 180, 200^{o}C and at 500 mbar for different times up to 17 min.

For combined conventional-vacuum baking process, a set of biscuits was first partially baked in the conventional oven at 220^{o}C for 2, 3, and 4 min, and then they were post baked in the vacuum oven set at 180^{o}C and 500 mbar for 6, 5, and 4 min, respectively, keeping a total baking time of 8 min for final products. Control biscuits were baked in the conventional oven at 220^{o}C for 8 min.

Table 2. The basic recipe used to produce biscuits under atmospheric and vacuum baking conditions

Ingredient	Amount, g
WHEAT FLOUR (T55 / W150)	80
REFINED PALM OIL	20
SUCROSE	35
NaCl	1
WATER	17.6
SODIUM BICARBONATE	0.8
AMMONIUM BICARBONATE	0.4

Figure. 16a shows acrylamide formation in biscuits at different temperatures during conventional baking. Expectedly, increasing baking temperature or time significantly increased the amounts of acrylamide formed in biscuits during conventional baking. As shown in Figure 11b, similar kinetic trends were obtained in biscuits during vacuum baking at 180°C and 200°C. Interestingly, no significant acrylamide formation (<LOQ) was observed in biscuits during vacuum baking at 160°C. Obviously, the heat load was limited to form acrylamide in biscuits under these conditions. The results indicated that the levels of acrylamide concentrations attained during vacuum baking were significantly lower than those attained during conventional baking at all temperatures studied ($p<0.05$). This was in parallel to less browning of the vacuum baked samples which indicates a lower degree of desired baking (Maillard) reactions and accordingly a different flavour profile. Similar to acrylamide, HMF formation had also an increasing trend with increase of temperature and time. This exponential increase was remarkable at 200°C due to high heat load. However, there was no HMF formation (<LOD) in biscuits during vacuum baking at a temperature range of 160 and 200°C. It is a fact that sucrose hydrolysis leading to the formation of HMF during baking requires higher thermal load at elevated temperatures.



(a) (b)

Figure 16. Change of acrylamide concentration in biscuits with time during (a) conventional baking and (b) vacuum baking at different temperatures.

Biscuits had a typical time-temperature profile during baking at 200°C under conventional baking conditions. The biscuit temperature rapidly rose to the boiling point of water (96.8°C in Ankara) within 2 min of baking and remained constant at the range of 97 and 103°C for 3 min until the moisture of biscuits largely evaporated. After a critically low moisture level was attained, the biscuit temperature began to rise again reaching to 200°C at the end of baking. The time-temperature profile of biscuits was different during vacuum baking at 200°C and at 500 mbar. The biscuit temperature rapidly rose to 71°C in 2 min, and then slowly to 81°C. It continued to rise very slowly reaching to 105°C at the end of baking. In comparison to conventional baking, noticeably low time-temperature profile of vacuum baked biscuits was the main reason of reduced formation of acrylamide and HMF. Loss of moisture in biscuits gives also insights on differences between conventional and vacuum baking processes. As expected, the low pressure of vacuum baking accelerated the drying rate of biscuits. The drying rate of biscuits in conventional baking at 180°C was found to be similar to that in vacuum baking at 160°C.

As shown in Figure 17, there were significant differences in the development of browning in biscuits during conventional and vacuum baking processes ($p<0.05$). Since oven air was partially removed in

vacuum baking, convective heating was limited, but conduction and radiation took place inside the oven. A recent study indicated that adding Maillard reaction products to the dough could solve lack of browning in cookies.

Figure 17. Biscuits baked at 180°C in conventional and vacuum oven

In the combined process, the dough was partially baked at 220°C for short times (2-4 min) in the conventional oven. Then, partially baked biscuits were post baked in the vacuum oven for accelerated drying at 180°C and 500 mbar for 4-6 min until the desired final moisture content was attained. In the combined process, exposure of biscuits to high temperature long time conditions, which were essential to facilitate the chemical reactions leading to the formation of thermal PCs, was prevented. There was no acrylamide or HMF formations (<LOD) in biscuits baked in the combined process. Control biscuits that were baked at 220°C for 8 min in the conventional oven were found to contain acrylamide content of 140 ng/g.

In conclusion, **vacuum baking allows production of biscuits with very low PC content linked to the lighter colour of the biscuit due to a lower degree of desired Maillard reaction which also results in different sensorial profiles.** It is a new technology to produce biscuits with lower acrylamide levels as a result of the effect of lower temperatures. As to be concluded from the lighter colour of the vacuum baked samples, other chemical reactions including the desired Maillard reaction determining the flavour profile may get effected in a similar manner.
. Since it lowers the thermal input without extending total processing time, vacuum baking limits significantly the formations of acrylamide and HMF in biscuits. Although lack of browning development of biscuits appears as a disadvantage of this technology, the light coloured biscuits may be particularly preferable for chocolate-coated products. Combination of conventional partial baking of biscuits followed by vacuum post-baking process can improve surface colour of biscuits. Moreover, adding brown-coloured Maillard reaction products can modify the colour characteristics. As a promising technology considering PCs, the vacuum baking process may be of importance for the production of baby biscuits in which the highest level of product safety is required in terms of thermal PCs.

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2.2.2 Ingredient microencapsulation

Microencapsulation has been investigated as a potential method to reduce consumer exposure to undesirable compounds formed during food processing without affecting food quality or microbiological safety. Microencapsulation is not only a convenient way to protect hydrosoluble molecules from water, but it is also a smart approach able to prevent reactions with other compounds present in food products, specifically in the Maillard reaction. Microencapsulation strategies were applied to two food models: biscuits (microencapsulation of sodium chloride) and infant formula (microencapsulation of ascorbic acid, mineral blends containing iron, and

polyunsaturated fatty acid). Three microencapsulation techniques, adaptable at industrial scale, namely fluid bed coating, prilling (also known as spray-cooling) and spray-drying, were selected.

Fluid bed coating, a microencapsulation process where a coating material is sprayed onto particles of a fluidized compound was applied to sodium chloride (particles size ≈ 500 µm; NaCl content > 750 mg/g), with the objective to avoid chemical reactions during the baking of biscuits, which could increase amounts of HMF and acrylamide, and the formation of 3-MCPD esters.

The ability of three selected coatings (fatty acids blend, candelilla wax and carnauba wax) to prevent dissolution of NaCl in water was assayed by conductivity measurement (monitoring of NaCl release). Figure 18a highlights the efficiency of the coatings. The particles coated with carnauba wax released less than 15 % of NaCl after 2 hours under stirring in water. Figures 18b and c show respectively the general aspect of NaCl particles coated with carnauba wax (optic microscopy) and a detail of the smooth coating obtained (scanning electron microscopy). Microencapsulated sodium chloride was compared to pure sodium chloride in the biscuit model. After incorporation in biscuit dough and subsequent baking, the HMF content of the biscuits was assessed by chromatographic analysis. HMF content decreased according to the melting point of the coatings (Figure 18d); data demonstrated that the more heat-resistant the lipid-based coating was, the more pronounced was the reduction of HMF formation, and the best result was obtained with the carnauba wax. In addition, sensory analysis revealed that the salty taste was still present in the biscuits; NaCl was released after melting of the coating in the late stage of baking.

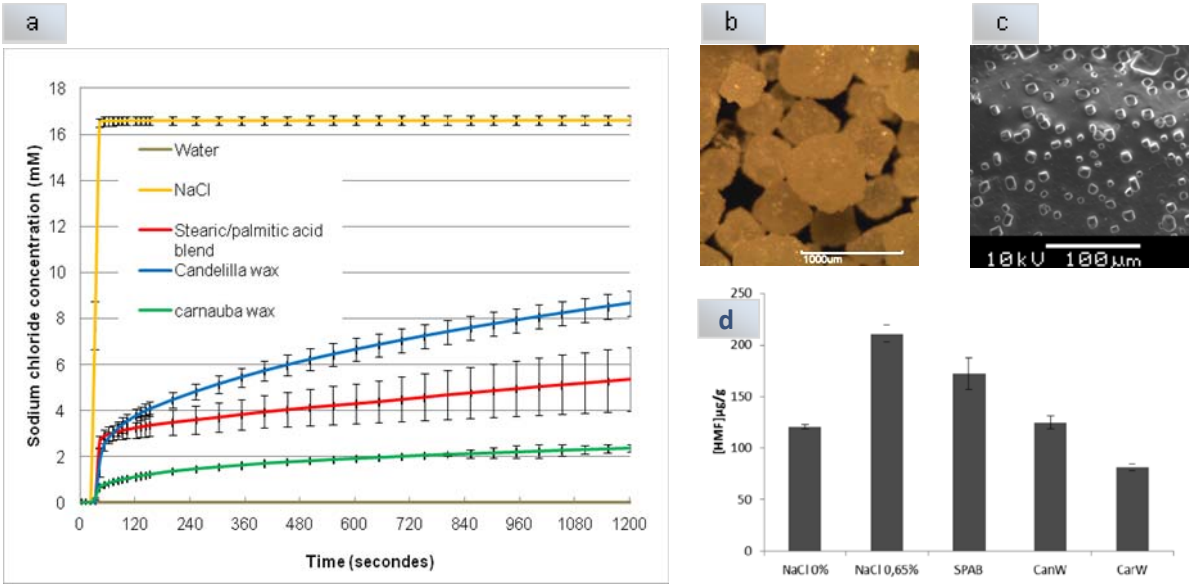

Figure 18. Properties of the microencapsulated particles.

Prilling is a microencapsulation technology where the compound is dispersed or solubilized into a melted material. The formulation is then pulverized, and the micro-droplets formed are converted into microparticles by solidification. Prilling was applied to the microencapsulation of ascorbic acid, iron sources and mineral blends, in order to mitigate the formation of contaminants during the sterilization process of infant formula. The actives were dispersed as fine powders in the encapsulation material (microparticles size ranging from 100 to 500 µm, active content up to 400 mg/g). The powder particles entrapped inside the microspheres produced were clearly visible under optical microscope (Figure 19e). Prilling technology allowed a close control of the size and size distribution of the microspheres, whatever the encapsulated active ingredient (Figure 19f: mineral

blend; Figure 19g: iron fumarate). A subsequent fluid bed coating of the microparticles was also performed to further limit the dissolution in water.

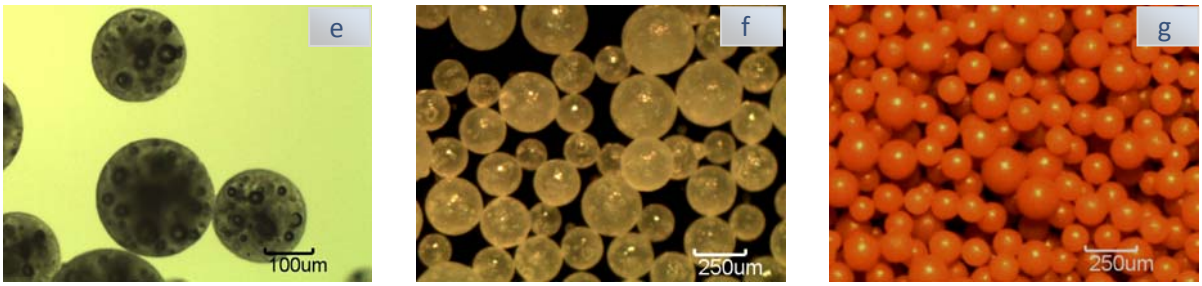

Figure 19. Microscope images of the microparticles

Spray-drying technology allows the combination of atomization and drying of solutions. This technology was applied for the microencapsulation of polyunsaturated fatty acids, which were first emulsified in presence of whey proteins and maltodextrin. The resulting emulsion was spray-dried and a fine dry powder was obtained (particle size < 50 µm, active content up to 250 mg/g).

To conclude, three technologies (namely, fluid bed coating, prilling and spray-drying) were evaluated to protect actives compounds from heat induced reactions during the manufacture process of two food models (namely, biscuit and infant formula). Microencapsulation allows limiting of substrate availability for process contaminants formation. The most significant effect was obtained with the encapsulation of sodium chloride in biscuits. The selected microencapsulation processes are fully available at the industrial scale, with production capabilities up to hundreds of tons per year of encapsulated actives (microparticles with an active content ranging from 200 to 750 mg/g, are typically incorporated between 0.1 and 5 % in final product mass). In this extent, such microencapsulation processes were found particularly relevant to mitigate the formation of contaminants in industrial food processes.

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2.2.3 High hydrostatic pressure

The use of high pressure processing (HPP) in the food industry was developed as an alternative to common thermal processes, such as pasteurization and sterilization, to produce a microbiologically safe food while avoiding and reducing undesirable changes in sensory, physiochemical and nutritional properties of foods. The use of high pressure at high temperatures as a tool for sterilization (high pressure thermal sterilization - HPTS) may lead to benefits in terms of food safety and food quality when compared to conventional retorting. This promising technology is not yet available at industrial scale level, however recent research may trigger the use of this application for certain foods. HPTS combines the synergistic effects of elevated temperatures (90 - 121°C) and pressures above or equal to 600 MPa to realize a quick and sufficient inactivation of endospores.

An additional benefit is the compression heating, which is caused by the compression work against intermolecular forces if pressure is applied. Depending on the food system this temperature increase can range from 3 – 9 °C per 100 MPa and helps to additionally heat up the product to the required temperatures; whereas the thermal load applied to the product can be reduced. Within the Prometheus project this promising emerging technology was used as a tool to mitigate and reduce the carcinogenic food PCs, which are the result of a high thermal load applied to the product during the processing. The aim was to increase the food quality without influencing the food safety of the product.

2.2.3.1 Results of the HPTS for the selected food systems:

Formation of furan and MCPD-ester in the fish systems:

Retorted cans were treated at 115°C for 28 min (total process time 55 min), which equals an F0 = 7 min. The analyses of furan in fish samples showed that significant amounts could only be found in canned sardines in olive oil. In all pressure treated and retorted samples of tuna in brine (0.23 – 1.5 µg kg -1) and tuna in sunflower oil (0.37 – 1.1 µg kg-1) furan levels were nominal and close to the detection limit of the analytical method. The amounts of furan formed during retorting were 58 µg kg-1 (28 min, 115°C). The data for the formation of furan clearly show that one of the main sources of furan, is the olive oil, in which PUFAs are probably the precursor. The formation of furan is also dependent on the treated food system and the treatment conditions. The reasons for the lower amounts of furan in the high pressure treated samples could be the shorter overall processing times which result in a reduction of the thermal load, and Le Chatelier's principle, which states that under high pressure conditions only reactions are favoured who have a negative reaction volume. The comparison of the retorted and the high pressure treated sardines in olive oil showed that depending on the treatment conditions, a reduction of furan in the high pressure treated samples is possible between 71 to 97 %. Even at sterilization conditions of 121°C, 600 MPa a reduction of 78 % is possible.

Formation of MCPD / MCPD-esters:
In all samples tested only very low amounts of free MCPD were found. The results of the preliminary trials indicate that the main focus concerning the formation of MCPD-esters should be put on tuna in sunflower oil. Since here the highest amounts of MCPD-esters are found. The quantities found in tuna in brine and in sardines in olive oil were nominal in all HP treated samples, the untreated sample and the retorted sample as well. In addition, the untreated sample of tuna in sunflower oil already contained quite high amounts of MCPD-esters (167 µg kg^{-1}) which are derived from the refined oil used (672 µg kg^{-1}). The quantities found in tuna in sunflower oil for the different temperature time combinations at 600 MPa showed no clear trend for any combination. Otherwise the results indicate that the use of non-refined oils or no oil in the tested food systems result in no or a low formation of MCPD-esters. Here the main aim should be to reduce the amounts of MCPD-esters in the food by changing the recipe towards non-refined oils.

Formation of furan in the baby food puree:

The quantities of furan formed in retorted baby food puree were 30.11±1.6 µg kg^{-1} at an F_0 = 7 min (115°C, 28 min). These values confirm the amounts of furan found in commercial available vegetable baby food purees with a mean concentration of 37 µg kg^{-1}. The levels analyzed by both solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) and static headspace GC-MS (HS-GC-MS), were much lower than the levels common found in retorted vegetable baby food puree. The results of both methods are in most cases very comparable, and within the uncertainty of the methods (at such low levels ≤ 5 µg kg^{-1}, 25-30 % is considered uncertainty), although the amounts analyzed by HS-GC-MS are a little bit higher than the ones analyzed by SPME-GC-MS. The main reasons for this can be twofold: the use of a different method to collect the volatiles and the samples that were sent to the two labs were not from the same batch and therefore variations/differences in the ingredients of the food system cannot be excluded. A reduction of furan within a range of 81 to 96 % is possible under HPTS conditions in comparison to the retorting. Even under sterilization conditions F_0=7 min (115°C, 28 min; 121°C, 7min) at 600 MPa a reduction of 81 to 86 % is possible.

Endospore inactivation:

To gain a better understanding of the T, t dependencies at 600 MPa a modelling of the spore inactivation, based on the inactivation kinetics obtained, for the selected food systems and the ACES

buffer was conducted for an extrapolated 12 $\log_{10}$ inactivation of *Bacillus amlyoliquefaciens* (12 D-concept as for thermal retorting) for isothermal and isobaric conditions. The temperature-time combinations at 600 MPa, for the processing of the different food samples at pilot scale, were based on calculated and extrapolated isokinetic lines of the inactivation kinetics of *B. amyloliquefaiens* obtained at lab scale. Figure 20 shows the isokinetic lines in a temperature-time range of 90-115°C and 0-40 min. The dotted and dashed line above the ACES-buffer (straight) (mainly stable under HP-conditions) represent as follows: sardine in olive oil (dotted) and tuna in sunflower oil (dashed). The progress shows that the oil within these systems has a protective effect on the spore inactivation. Whereas tuna in brine (dashed-dotted line) and the baby food puree (dot-dot-dashed line) are running beneath the ACES-Buffer and therefore represent food systems that have a rather low influence on the spore inactivation.

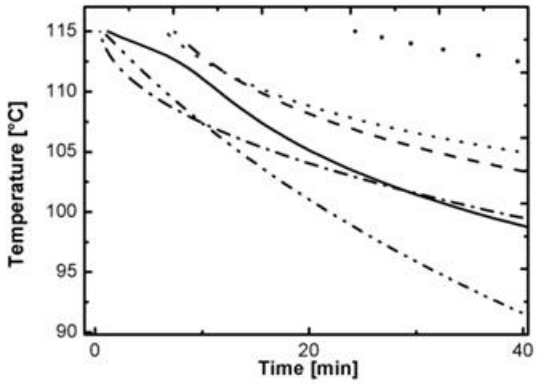

Figure 20. *Isokinetic lines of selected food systems* for an extrapolated 12 log inactivation of Bacillus amyloliquefaciens at 600 MPa in a T,t-range of 90-115°C and 0-40 min. Sardine in olive oil (small black dotted line); Tuna in sunflower oil (black dashed line); ACES-Buffer (solid black line); Baby food puree (black dash dotted line); Tuna in brine (dash dot dotted line); border of the thermal inactivation (big black dots).

This clearly indicates that the composition of foods plays an important role in the inactivation of spores. Due to this independent optimal treatment conditions could be obtained for the different foods without having to deal with an over-processing of the foods.

Upscaling of the HPTS-process from 4ml at lab scale to 55 L at pilot scale level:

Although pilot scale high-pressure systems as well as high-pressure-high-temperature stable packaging for HPTS are available on the market, up to now there is no HPTS treated product on the market. There has not yet been an approach to go from lab scale based modeled inactivation kinetic data of a high pressure high temperature resistant spore strain into an pilot scale system with economical T,t combination (t ≤10 min) in connection with storage trials. Based on inactivation data derived in a 3.5 ml vessel under isothermal, isobaric conditions during pressure dwell-time a scaling up of the HPTS with tuna in brine, tuna in sunflower oil, sardine in olive oil and a baby food puree was conducted with the 55 L vessel HT from Hiperbaric at the AZTI-Tecnalia (Derio, Spain) to verify the findings in a large scale and non-uniform temperature. The foods were not inoculated with spores they were only treated at the calculated conditions for a 12 $\log_{10}$ inactivation of *B. amyloliquefaciens* (T 100-115 °C; t 0.45-28min at 600 MPa) and later on stored after the French norm "Standard NF V 08 408" at room temperature and 37°C for 21 days to see if the calculated T,t-combinations at 600 MPa lead to a shelf stable product. The aim was 1) to use extrapolated temperature-time combinations obtained at lab scale; 2) Evaluate the formation of FPCs in comparison to lab scale; 3) Feasibility of HPTS as a pilot scale process.

Formation of PCs under Upscaling-conditions:

MCPD-esters in tuna in sunflower oil:

Due to the findings at lab scale only tuna in sunflower oil was investigated for the presents of MCPD-esters. The results at pilot scale level confirm the results at lab scale. The untreated samples already contained 84±19 µg kg^{-1} (lab scale 120 ± 19 µg kg^{-1}) and in comparison to the thermal sterilized samples with 64±15 µg kg^{-1} and 97±24 µg kg^{-1}, the samples treated with the same T, t regime but at 600 MPa with 108±26 µg kg-1 were not significantly different. Nevertheless, the levels of MCPD-esters present in the samples were the same for a treatment at pilot and lab scale level. No significant formation could be detected for these two approaches and the retorting, since it is mainly due to the use of the refined oil.

Furan in sardine in olive oil and baby food puree:

Furan was found only at levels close to the detection limit (≤ 0.1 µg kg^{-1}) of the method in tuna in brine and tuna in sunflower oil for the retorted and high pressure sterilized samples and so will not be discussed further. The analysis of furan in sardines in olive oil showed that similar amounts of furan were obtained in the retorted samples at lab scale and pilot scale. The results also indicated the same trends as seen for the lab scale experiments that for the same F_0-value (7) lower amounts of furan are measured under HPTS conditions as for normal retorting. At sterilization conditions under pressure (600 MPa) the amounts of furan were 17±2 µg kg^{-1} (lab scale 115°C, 28 min, 600 MPa) and 28±1 µg kg^{-1} (pilot scale 115°C, 28 min, 600 MPa). Overall the trials at pilot scale validated the trials conducted at lab scale. A reduction of furan in sardine in olive oil in comparison to the retorting was possible even for scaled up processes. For the pilot scale trials the reduction of furan was similar and between 42 % (115°C, 28 min, 600 MPa) to 77 % (113°C, 9.4 min, 600 MPa) in comparison to the retorting. The amounts of furan found in the baby food puree were similar to those for the formation of furan in sardine in olive oil. Although in general lower amounts of furan, between 30.1 to 0.4 µg kg^{-1}, were detected in comparison to sardine in olive oil. The reduction of furan in baby food puree was 94-98 % in comparison to retorting, similar to the reductions obtained at lab scale, where the reduction for a broader temperature time range (T: 90-121 °C, t: 0-30 min) was between 81 and96 %. The results of the formation of PCs under pilot scale conditions overall agree with the results obtained at lab scale

Storage trials:

The results of the storage trials revealed that altogether three temperature-time combinations (107.5°C, 9.9 min; 115°C, 0.45 min for baby food puree and 107.5, 9.9 min for tuna in brine) were not suitable to produce a stable product. All of the undertreated (100°C, 10 min) and untreated samples were not stable and these are not discussed further. All other treatment conditions for the other tested foods proved to be suitable for the production of a high pressure sterilized stable product. To be certain that no spore recovery had occurred, all pouches were checked for revivable microorganisms. This shows that a storage trial performed after the treatment is a powerful tool to validate the safety of the applied extrapolated T, t – combinations at 600 MPa. The process window for the temperature-time domain at 600 MPa for the different food systems is shown in Figure 21. Here the assumption was made that if a sample at given T, t – combination would be stable it would also be stable if the temperature would be held constant and the time would be extended to a time ≤ 10 min. The connected symbols (for selected T, t-combinations) represent the treated and stable temperature-time combination at 600 MPa for the different food systems. In all cases the temperature-time combinations under HPTS-conditions that would lead to a safe product in terms of microbiological safety are lower in terms of time and temperature in comparison to the thermal retorting. Furthermore treatment conditions result in a lower formation of carcinogenic food PCs.

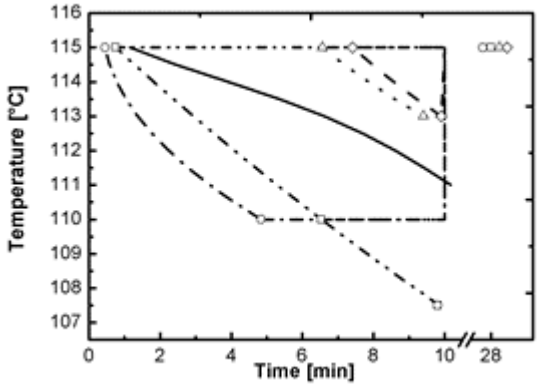

Figure 21. Process windows for the selected food systems for a 12 log inactivation of B. amyloliquefaciens that lead to stable product at 600 MPa in the temperature time range of 107.5-115°C and 0.45-28 min. Sardine in olive oil (Δ and small black dotted line); Tuna in sunflower oil (◊ and black dashed line); ACES-Buffer (solid black line); Baby food puree (○ and black dash dotted line); Tuna in brine (□ and dash dot dotted line).

2.2.3.2 Conclusion:

The industry can use the gathered data and new insights into the HPTS to produce food of a better overall quality without affecting the safety of the product. The results obtained at pilot scale verified the results at lab scale. It was possible to go from lab scale based modeled inactivation kinetic data of a high pressure high temperature resistant spore strain into an pilot scale system with economical T,t-combination (t ≤ 10 min) in connection with storage trials for the selected food systems. The experiments showed that the storage trials were successful and that a suitable and that a feasible temperature-time combination at 600 MPa could provide a safe product. Furthermore over-processing can be avoided if HPTS is used as sterilization technique, resulting in a double benefit in terms of food quality and food safety. In the future more research needs to be conducted with more food systems and target microorganisms for the HPTS-process. Also since pilot scale and small industrial systems are available these need to be optimized to guarantee an economical process for the food industry. This signifies that the process line needs to be fine-tuned in terms of output, the heat up time of the vessel needs to be shorten and tools need to be developed to guarantee safe and constant temperature-pressure contribution in the packed food. Hence, the HPTS-process could lead to a new principle of application for high pressure processing, where the benefits of this emerging technology merge to create safer, healthier and high quality foods.

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2.2.4 Ohmic heating

2.2.4.1 – Main objectives

Ohmic heating is an innovative thermal technology based on High Temperature Short Time treatment (HTST), which reduces thermal damage of the product, has low residence time dispersion, improved preservation of sensory characteristics (colour, texture etc.) of the ingredients and preserves nutritional compounds (vitamins, polyphenols, carotenoids etc.) (Novel Q, 2009).

Unlike conventional treatments, ohmic processing heats products internally by passing an electric current through the product, rather than relying on heat transfer from a heated vessel. Ohmic heating was applied to baby puree to reduce the PCs that are formed during sterilization.

Ohmic heating was chosen because it was expected to reduce PC formation on account that:

- Ohmic heating is well known to reduce the Cook Value (used to compare cooking procedures) with respect to microbiological safety. This means that for the same sterilization value (Fo value), products processed with a retort system are “overcooked” (de Alwys A.A.P. et Fryer P.J., 1990; Godereaux S., 2000).
- Ohmic heating is also known to avoid fouling and reduce deposits formed in vessels compared to traditional technologies such as heat exchangers and the retort system (Ayadi M.A. et al., 2003).

The following pages present the main results obtained on PCs, sugars, and micro-nutrients of baby purees. All the sterilized purees presented in this deliverable are shelf-stable at room temperature according to the Standard NF V 08-408.

2.2.4.2 – Processing contaminants

Firstly, we compared the evolution of furan in purees sterilized in a retort and by ohmic heating for a range of sterilization values (Fo) (Figure 22) .

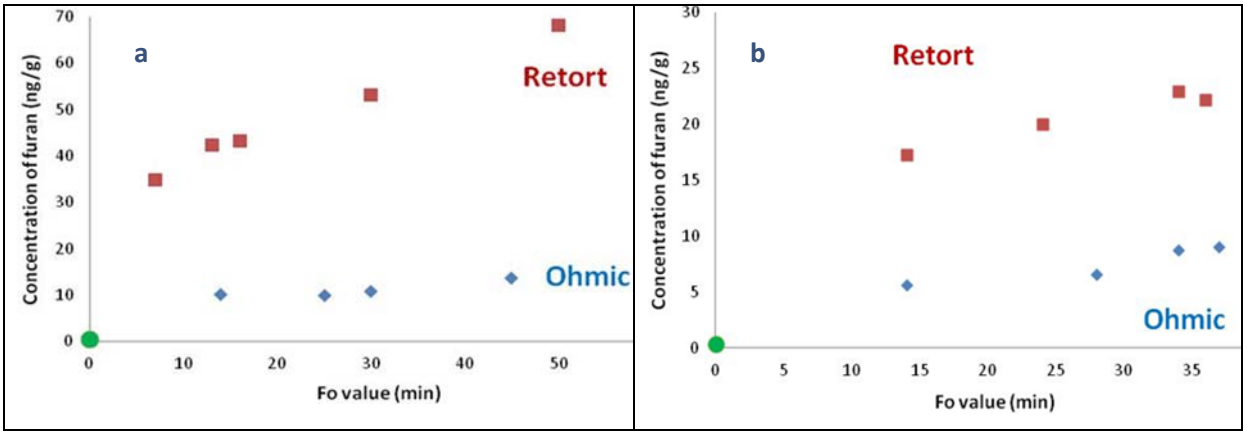

Figure 22. Amount of furan in vegetable mix (a) and chicken mix (b) after sterilization in retort or by ohmic heating for different Fo values

The most important finding was, as expected, that for all the recipes tested **the products sterilized by ohmic heating had a lower furan (3-7 times less) content than those sterilized in retorts** . We also demonstrated that agitation during retorting had a low impact (<5%) on reducing the level of furan in the purees, especially for high viscosity foods which reduced the effectiveness of the convection.

We also saw that for the products sterilized in retorts, the furan content increased when the Fo value increased. On the other hand, for the products sterilized by ohmic heating, there was no significant increase when the Fo value increased. **This result is interesting because it confirms the value of alternative heating methods such as ohmic heating.**

For the products sterilized by ohmic heating, the temperature had no impact on the furan content. For the retorted products, the furan content was slightly higher at 123°C than at 129°C and the 129°C was slightly greater than 135°C. The results tend to confirm that the formation of furan depends more on the time (longer at 129°C than at 135°C) than the temperature.

The differences in the level of furan observed in various tested recipes were related to amount of sugar content in these recipes (see paragraph C). Vitamin C and sugar are the two known precursors of the Maillard reaction in the baby purees. To learn more about their influence on the rate of PCs formed during sterilization, we added sugar and/or vitamin C. We observed that an increase in the two precursors was necessary to obtain a significant increase of the level of furan, HMF, and furfural.

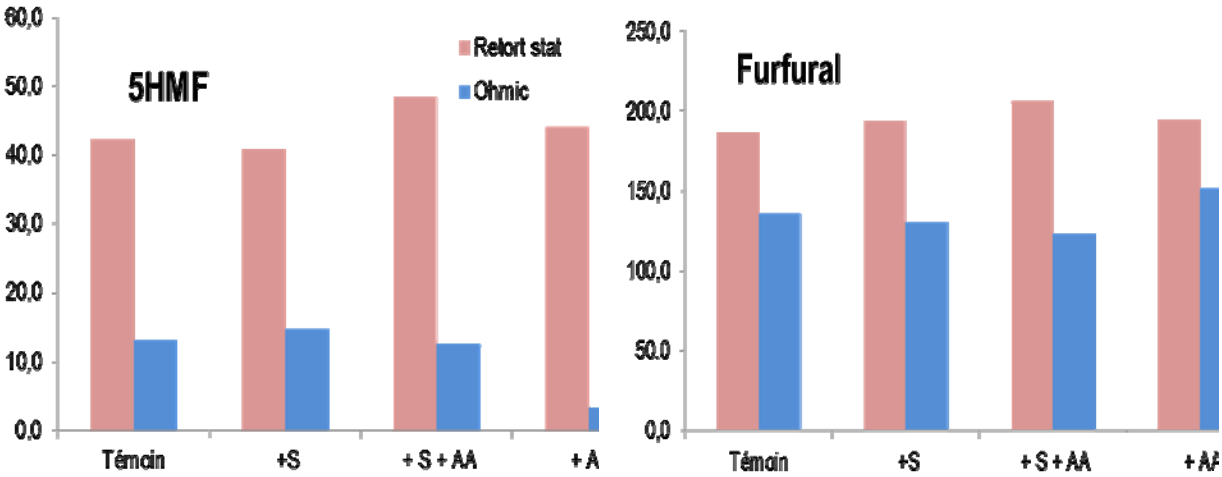

Figure 23. Amount of HMF (a) and furfural (b) in chicken mix with and without different supplementation after sterilization at 129°C for the same Fo value

Levels of all the PCs (furan, HMF, furfural) were higher in retorted puree than in ohmic sterilized product. **These results confirm that the ohmic heating reduced the volatile and non-volatile PCs formed in baby puree during sterilization.**
We studied the evolution of the PCs in purees sterilized in retorts and by ohmic heating. The few variations observed were not significant. The level of PCs in baby puree did not change during the storage at room temperature.

2.2.4.3 – Sugar

The total sugar content (Figure 24) did not depend on the heat treatment in either type of process, nor the intensity, in any recipe. There was twice the amount of sugar in the vegetable mix than in the chicken mix. This probably explains the higher amount of PCs measured in vegetable mix than in chicken mix (sugars are precursors of furan in the Maillard reaction). There was no impact of temperature or storage on the sugar level.

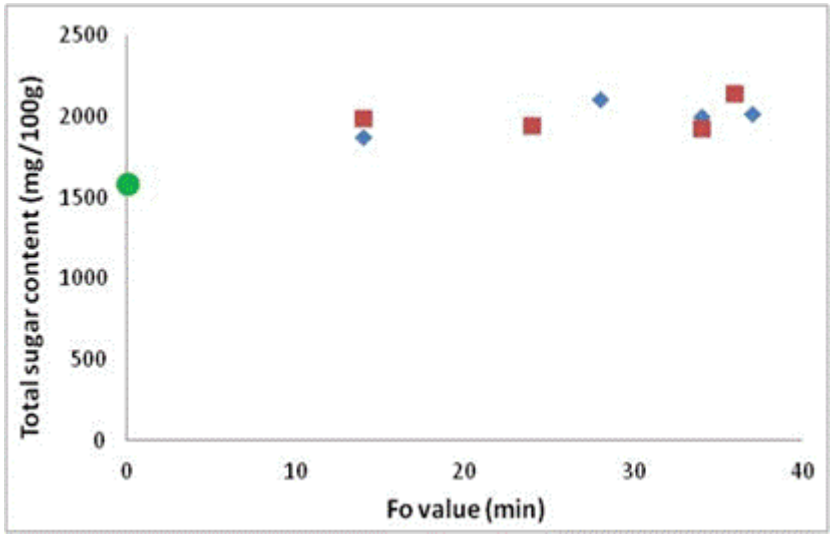

Figure 24.Total content of sugar in vegetable mix after sterilization in retort or by ohmic heating for different Fo value

2.2.4.4 – Nutritional compounds

The amount of vitamin C was very low in all recipes even before sterilization; it was completely destroyed by sterilization. The carotenoids and polyphenolic compounds in all puree recipes were not affected by the manufacturing process. There was also no impact of the intensity of sterilization on the amount of carotenoids content in purees for two technologies. We observed the same effects for different polyphenolic compounds. Therefore **ohmic heating provides a very much better means of preservation of carotenoids than retorting.**

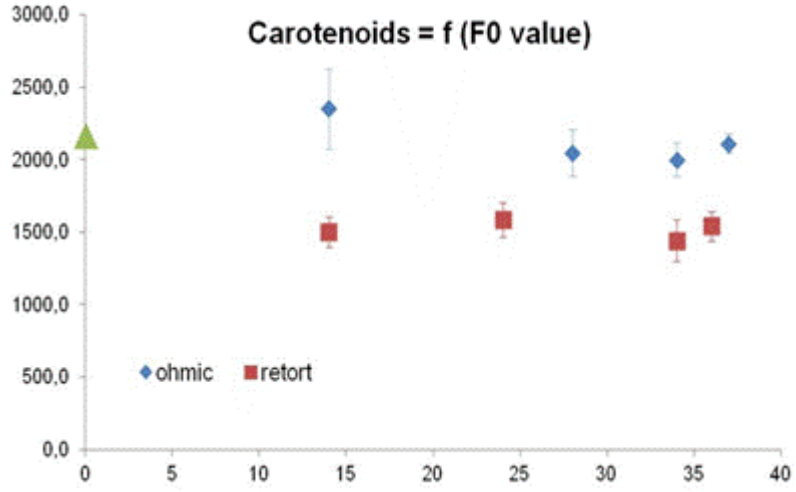

Figure 25. Total content of carotenoids in chicken mix after sterilization in retort or by ohmic heating for different Fo values

There was no impact of the storage on carotenoids or polyphenolic compounds whatever the process of sterilization used for all recipes tested.

2.2.4.5 – *Conclusion*

The most important result was that whatever the matrix, the products sterilized by ohmic heating had a lower furan (2-7 times less) content than those sterilized in retorts. **This result demonstrates that the use of an alternative technology such as ohmic heating greatly reduces the amount of PCs (furan, HMF, furfural) produced during a heat treatment for microbiological decontamination to obtain a shelf-stable product.**

We also found that the amount of furan formed was greater for the vegetable mix than for the chicken mix. We can relate this information to the higher level of sugars, phenolic compounds and carotenoids in the vegetable mix. This is consistent with their role as precursors of furan in the Maillard reaction.

In the case of products sterilized by heating ohmic, we did not observe the impact of treatment intensity or temperature on the formation of PCs for two recipes. This is consistent with the same behaviour observed for sugars and carotenoids and phenolic compounds. In contrast, in the case of products sterilized in retorts, we observed a significant impact of treatment intensity and temperature on the formation of PCs for two recipes, while we did not find a decrease in the amount of precursors in these purees. A higher temperature, allowed a shorter sterilization duration and so a lower furan content for the same Fo.

We observed that an increase of the two precursors of Maillard reaction in baby puree (sugar and vitamin C) induces an increase of different PCs.

The study of purees during storage of 6 months demonstrated no chemical evolution. The products are very stable.

All these tests have shown the relevance of alternative technology (ohmic heating) to significantly reduce the formation of PCs (volatile and non-volatile) such as furan, HMF and furfural and to preserve the nutritional compounds (carotenoids, polyphenols etc.) in purees for baby food.

These results were obtained with semi-industrial line of ohmic heating in CTCPA (200 to 1000 l/h). The maturity of the technology ohmic heating is excellent. There is no technical difficulty in deployment of ohmic lines in industry plants for baby puree aseptically filled into glass jars.

For a company that manufactures small volumes of puree, a continuous line of heat treatment has a higher initial cost than a retort process. However, when the output of the company is high the continuous heat treatment becomes more competitive financially. The cost of a thermal treatment by ohmic heating also depends on the price of electricity in each country.

Current major industrial applications for the stabilization of food by ohmic heating are:

- HT-ST flash sterilization of low acid foods: prepared meals (i.e. pasta with sauce, chili con carne etc.); baby foods, vegetables with sauce; soups with particles; chestnut cream.
- HT-ST flash pasteurization of acid foods: fruit based products, tomato sauces.

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2.3 Modelling

Kinetic modelling of the formation of PCs during food processing is a valuable tool to obtain insights into the chemical pathways for their formation, and possible effects of food processing and ingredients. These insights can be used to develop mitigation measures for the presence of the PCs in the foods.

As part of WP3 of the project, an attempt was made to use a multi-response kinetic modelling approach to model the kinetics of the formation of PCs in food products. Multi-response modelling is an approach based on the fundamental chemical reaction pathways. Several chemical compounds (ingredients, intermediates or products that are related to the compound of interest) are measured at certain time-temperatures points of food processing and their kinetics are modelled at once. On the contrary, the classical single response approach focuses only on the chemical compound for which the kinetics is sought. The advantage of multi-response modelling over the single response approach is that it improves the precision of the estimates of the kinetic parameters while providing insights into the actual reaction mechanisms for the formation of the compounds of interest.

However, kinetic modelling requires an extensive set of kinetic data, with information on precursors and PCs during processing, and preferably at different processing temperatures. Data collection and analyses in the course of WP2 allowed the approach to be applied to data for baking *biscuits* and heating of *infant formula*. More specifically, multi-response modelling was applied to understand the formation of acrylamide and HMF during the baking of biscuits, and for the formation of CML in infant formula. In this report, the design and results for the biscuits are summarized; a full description can be found in Van der Fels-Klerx et al. (2014).

For the multi-response modelling of acrylamide and HMF formation in biscuits, data from the basic formulation (see Table 3) baked at 200°C were available. The biscuits from the basic formulation were baked for different times and samples were collected between 8 and 15 minutes. The baking experiments were performed in duplicate. Seven target compounds (herein called “responses”) were

measured in the above mentioned samples: sucrose, fructose, glucose, asparagine, total amino acids (AA), acrylamide and HMF. Temperature profile and moisture loss were also measured.

Table 3. Composition of the biscuit recipe

Ingredient	Amount (g)
Wheat flour (standard T55/W150 flour)	80
Refined palm oil	20
Sucrose	35
NaCl	1
Water	17.6
Sodium bicarbonate	0.8
Ammonium bicarbonate	0.4

The kinetics of the formation/degradation of each response is presented in Figure 26. The temperature profile in the biscuit crust and the evolution of the moisture content in the biscuit are also depicted.

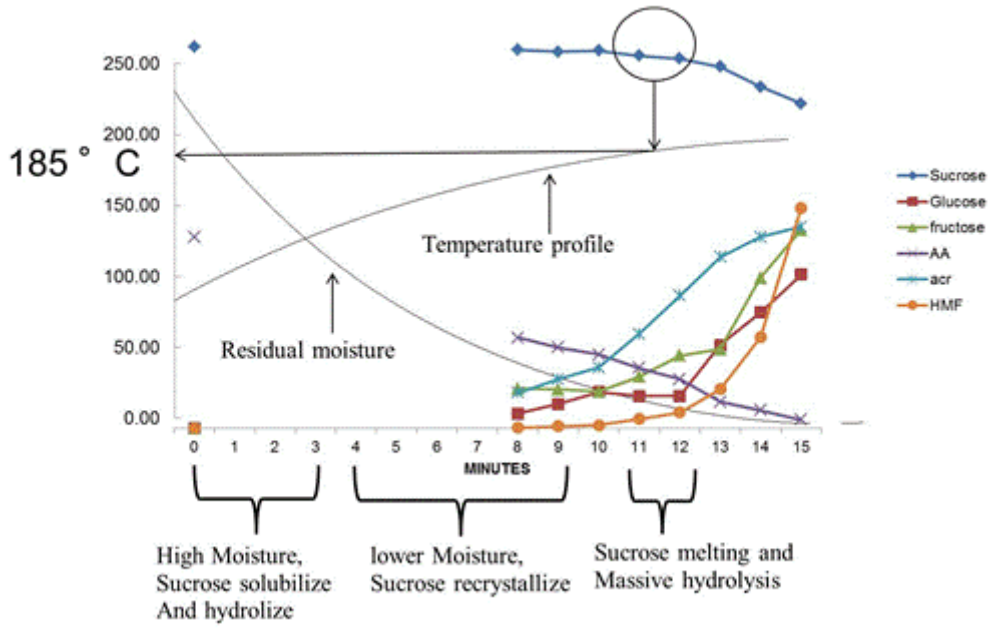

Figure 26. Evolution of the measured responses during biscuit baking at 200 ˚C, and the temperature profile of the biscuits (crust). The concentrations of each single compound are not to scale.

Based on the data collected (Figure 26), it was postulated that reducing sugars - present during the first part of baking - drive asparagine degradation and the formation of the amount of acrylamide that is measured after 8 minutes. At around 10-11 minutes, when the temperature inside the biscuits has reached approximately 180 °C, sucrose would start melting which results in the steep increase of the reducing sugars (glucose, fructose) content and the change in the rate of acrylamide formation as well as in the onset of the HMF formation. This would indicate that the *physical state* of sucrose has a primary role in the kinetics of acrylamide and HMF formation in biscuits. In dry systems, crystalline sugars first have to melt before they can react with asparagine (or form carbonyl

intermediates) and yield acrylamide. This would explain why fructose is more reactive (it will form more acrylamide upon comparable heating times) than glucose in dry systems despite ketoses are less reactive than aldoses towards asparagine.

Based on the data obtained from baking the basic recipe at 200˚C, the formation of acrylamide and HMF could not be modelled over the entire baking time range (0-15 min). The reason was that the variation in the concentration of reducing sugars could not be modelled based just on fundamental chemical reactions as it also depended on sucrose hydrolysis which exhibited a defined lag time to occur which could not be included in the kinetic model. We therefore decided to kinetically model only the data collected between 12 and 15 minutes, i.e. right after the onset of sucrose hydrolysis to the end of baking. In this selected time range, the change in moisture content was negligible. Therefore, the effect of water loss on reactants and intermediated concentration needed not to be incorporated in the model.

We started with a very detailed chemical reaction network which took into account all the possible pathways and intermediates for acrylamide and HMF formation. This reaction network was simplified to comply with the number of available data points. We constructed and tested a variety of possible kinetic models, and selected the model that best fitted to the data. This model is presented in Figure 27.

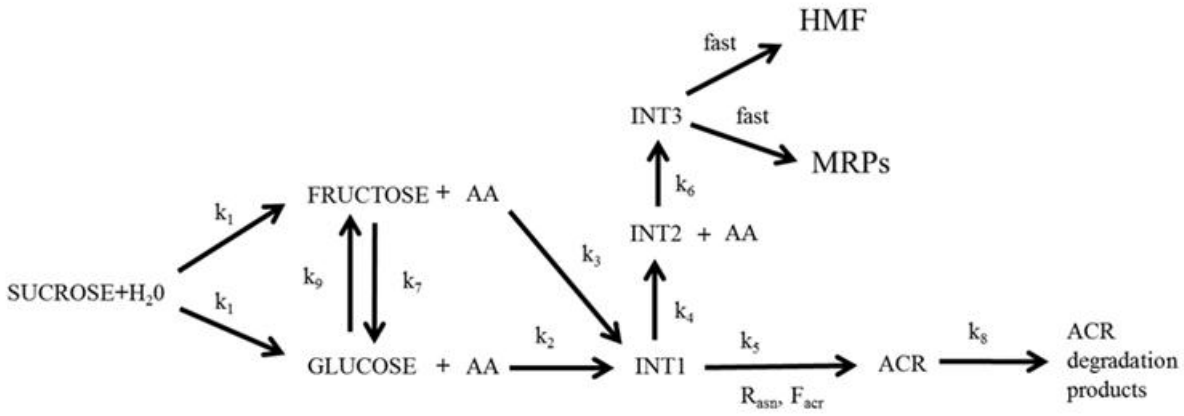

Figure 27. Simplified kinetic scheme selected by model discrimination analysis

In this model, sucrose hydrolyses to yield glucose and fructose which interconvert into each other through isomerization reactions. INT1 is an intermediate which is common to both acrylamide (ACR) and HMF formation pathways. It forms from the reaction of glucose and fructose with amino acids (AA) and can undergo two distinct pathways. In one pathway INT1 yields a second intermediate (INT2) in the same time regenerating the amino acids. INT2 would in turn yield a transient intermediate (INT3) which does not build up to any extent in the system but rapidly undergoes reaction to yield HMF and other Maillard reaction products (MRPs). In another pathway, INT1 will yield degradation products of amino acids, including acrylamide from asparagine (asn). Finally acrylamide undergoes elimination reactions to yield acrylamide degradation products. The rate of acrylamide formation depends on the molar ratio of asparagine to total free amino acids (Rasn) and the fraction of asparagine converted to acrylamide (Facr). Rasn was constant throughout the baking time (≈0.15). The fraction of the overall loss of asparagine that is converted to acrylamide (Facr) was set at 0.62% based on literature data.

The fit of this model was satisfactory for all of the measured compounds even though a slight underestimation of sucrose hydrolysis appears.

Globally the model suggests that (1) both the reducing sugars are involved in acrylamide and HMF generation, (2) the rate of acrylamide generation through the generic amino acids pathway is slightly higher for glucose than for fructose, and (3) the rate of conversion of glucose to fructose is higher than that from fructose to glucose. Multi-response kinetic modelling was considered to be a very useful tool to understand in depth the underlying reaction mechanisms for the formation of PCs. It requires a set of very carefully designed experiments, and related analytical results. Once a best fitting model is obtained it can be used for scenario analyses to optimize processing, and reduce analytical resources.

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3 Potential impact

3.1 Perspective for market up-take

Thermal processing of foods is a major technology used to increase shelf-life and maintain food safety with a reasonable processing cost. Traditionally, food safety and palatability issues require the use of thermal processes for modulation of food raw materials during food processing at industrial and household levels (*van Boekel et al., 2010*). Some disadvantages of conventional thermal processing technologies are well known, particularly in products where the organoleptic quality need to resemble those of the unprocessed one. There are for example chemical reactions leading to off flavours, destruction of thermolabile nutrients, and other losses of product quality such as textual and colour changes. Conventional thermal food processing produces both desired Maillard reactions products to confer taste and aroma to foods, as well as undesired PCs. The PROMETHEUS project investigated the feasibility of application of alternative technologies to reduce the formation of PCs. Alternative and new processing technologies are necessary to offer chemically and microbiologically safe foods, retaining the sensory and nutritional quality of fresh ingredients, and finally to improve the convenience of processed foods to consumer expectations (*Jaeger et al., 2010*). Two novel technologies such as ohmic heating and high pressure processing have revealed as successful for scaling up .The PROMETHEUS project aimed to map out the main limits and advantages of the selected alternative technologies for reduction of targeted PCs in baby food purees and canned fish, in terms of

a. microbial stability
b. nutritional quality and content of process contaminants
c. technical feasibility, cost effectiveness, and consumer acceptance

a. Microbial stability

Assessment of food safety in terms of microbial stability of the product to prevent food spoilage is the first mandatory step for any scaling up process, including any novel technology. Sterilization guarantees the absence of pathogenic and food spoilage bacteria capable of growing in food products under non-refrigerated conditions of storage and distribution.

Ohmic heating of baby food puree. The mechanisms of microbial inactivation in baby food puree treated by ohmic heating are exclusively thermal in nature. The effect of storage on baby food puree samples was carried out over 6 months and evaluated in a stable low acid product using a standardized method (NF V 08-408). Results confirmed that all baby food purees produced at pilot scale were stable at 37°C and 55°C where ohmic heating mets the expectatives for maintaining microbial safety during shelf-life.

High pressure processing of fish. A precise evaluation of microbial inactivation for high pressure processing is needed since it is not possible to assume that the most heat-resistant spores are also the most baroresistant. High pressure processing inactivates microorganisms by interrupting cellular functions responsible for reproduction and survival. Although pressure levels in the range of 400–800 MPa inactivate the vegetative forms of pathogenic and spoilage bacteria, the inactivation of bacterial spores by pressure alone is not assured. Alternatives such as PATP (pressure assisted thermal processing), termed HPTS (High pressure thermal sterilization), can inactivate bacterial spores since it is based on the combined application of high pressure and high temperature, typically in excess of 600 MPa and 100 °C. Feasibility of this alternative technology has been compared with conventional retorting.

The results of the storage trials revealed that HPTS did not produce a stable product for tuna in brine. For sardine in olive oil and tuna in sunflower oil, HPTS treatments were effective for microbial inactivation, and 110 °C/600 MPa for 6.53 min.

b. Nutritional quality and process contaminants

Impact of the alternative technologies on the main quality parameters have been evaluated for baby food puree and canned fish including after shelf-life.

Ohmic heating of baby food puree.

Infant nutritional requirements are increased after an age of four months, and breastfeeding alone is not enough to covering these needs. Complementary foods are needed to provide a suitable energy and nutrient intake. Purees, with their soft texture play an important role in infant nutrition.

Sugars, protein, fat, carotenoids, total polyphenols, vitamin content were evaluated in the baby food purees produced in the project. In addition, the reduction in the levels of targeted PCs (furan and HMF) recorded at lab/pilot-plant level was confirmed during scaling up.

For some samples studied we observe a better preservation of carotenoids and polyphenols content after sterilization by ohmic heating than after retorting.

No significant differences in sugars, fat, protein, vitamin C, and fatty acids profile were detected when comparing ohmic heating sterilization and conventional retort sterilization for equivalent sterilization values. However, there was a significant decrease in total amino acids content (36.6 % as compared with unprocessed sample) and essential amino acids including arginine and histidine during retort sterilization. On the contrary, baby food puree treated by ohmic heating did not show differences in essential and non-essential amino acids content as compared with unheated control. Ohmic heating applied to baby food purees has a significant protective effect on the destruction of essential amino acids as compared with the conventional sterilization process for baby food puree.

In regards to PCs, ohmic heating was an effective strategy for the reduction of HMF and furan formation both in vegetables mix and in chicken mix. The concentration of HMF and furan in ohmic treated samples was always significantly lower than the retorted counterpart. Levels of HMF and furan did not change during the storage at room temperature of the baby food puree.

High pressure processing of fish.

The consumption of fish is particularly recommended because of the contribution of fatty acids, where an important part of the fish consumed is in the form of canned fish. Recently in Europe, canned fish preparations accounted for around 20-30% of total seafood consumption. Some of the most commonly canned fish are tuna and sardines, together with albacore, and salmon. Tuna and sardines are represent a great percentage of the total amount of blue fish capture. Only around 35% is sold fresh whereas the rest is destined for making preserves. Tuna is canned in edible oils, in brine,

in water, and in various sauces. Sardines are canned in many different ways, including packing in olive or sunflower oil, water or difference sauces.

The major difficulty encountered in the project for the canned fish production is the seasonality of the raw material. This phenomenon is observed within all species but it is more significant for fat fish during spawning period or migration. Variations of composition mainly affect water and fat fractions, since these components may represent around 80% of the composition of the flesh.

Nutritional quality assessment was focused on the fat and fatty acid profiles. The individual fatty acids were classified by saturated, monounsaturated, polyunsaturated, as well as the sum of EPA and DHA for tuna in brine, tuna in sunflower and sardines in oil treated by HPP. No significant differences were observed in the fatty acids profile of fish regardless of the extent of the process and type of treatment.

In regards to PCs, 3MCPD and esters were not detected in alternative and canned samples. But furan content was significantly reduced after high pressure thermal sterilization compared with conventional retorting for sardine in olive oil.

c. Technical feasibility for scaling up, cost effectiveness, and consumer acceptance.

From the industrial side, replacing conventional technologies with one of the alternative technologies applied in the project is a decision that must be approached carefully where many variables should be considered. In the case of novel technologies, the added value to the product (improved quality, safety, and shelf-life) should be balanced with the beneficial effect on other important issues, such as packaging, transportation, storage, insurance, labour costs, or consumer convenience, among others. It is not the aim of the project to carry out a prospective study of the market.

However, it has been identified a number of limitations/beneficial technical features of the alternatives technologies for implementation at industrial scale as summarized in tables 4 and 5.

Table 4. Assessment of limitations vs benefits of the ohmic heating.

Ohmic heating	
Limitations	**Benefits**
• Food must be easy to be pump at a constant flow • The electrical conductivity of the food must be comprise between 0.01 S/m and 10 S/m to allow the electric current to pass through and heat the food. • Potential disruption in the current flow by presence of air in the food • Feasibility depends on the food matrix, where sterilization by steam injection is still more efficient for liquid products.	• The process is able to heat materials very rapidly and uniformly with reduction of the cooking time • The limited fouling rate will allow the equipment to remain operational for longer • High energy efficiency where almost 100% of the electrical energy is converted into heat • Feasibility to adapt aseptic fillers to large flow rates from the ohmic heaters • Highly effective for foods with particles • Operational costs are comparable to those for freezing and retorting of low-acid products

Table 5. Assessment of limitations vs benefits of the high pressure processing.

High pressure processing	
Limitations	**Benefits**
• Product should be vacuum-packed to reduce the pressurization time • No commercial high pressure thermal sterilization unit is currently available, although there is an available system operating with a large vessel capacity (55 L) • Initial high cost of the installation	• Technically is very simple to scale up since high pressure processing effects are independent of the equipment and product geometry and size • Design of high pressure units is not restricted to spatial considerations as they can be vertical or horizontal

Alternative food technologies could create some level of consumer concern because consumers are in some cases unaware of the processes applied to foods. Thus, effective communication regarding their benefits is essential for the successful marketing of novel and conventional technology processed foods. Current consumers' expectations are linked to food products that provide convenience, variety, adequate shelf-life, low caloric content, reasonable cost, and environmental soundness.

In general, ohmic heating and high pressure thermal sterilization are mature technologies with feasibility for scaling up and not adverse response from consumers to be implemented in the European market. However, the initial high investment required for the high pressure thermal sterilization installation could be balanced in production of gourmet-like or tailored-made foods. Applications of ohmic heating for baby foods and high pressure thermal sterilization to canned fish not only fit important consumers' demands concerning to high quality foods with naturalness characteristics, maintaining nutritional value and shelf-life (microbial stability) but also in terms of reducing the levels of processing contaminants.

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