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ICARE

- Impeding neo-formed Contaminant Accumulation to Reduce their health Effects -

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Content

Project ICARE

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Impeding neo-formed Contaminant Accumulation to Reduce their health Effects

1. Project execution

Introduction

Heat treatment of foods is a key process in the agro-food industry; it mainly results in the development of a large range of flavours and tastes through the **Maillard reaction**. However, some of these Maillard products called **Neo-Formed Contaminants** are currently suspected to have deleterious health effects. The recent discovery of neo-formed acrylamide in a variety of fried and baked foods has highlighted the fact that agro-food enterprises and especially SMEs are poorly prepared to face this emerging issue. Because of a lack of knowledge and inappropriate analytical and technological tools, SMEs are presently not able to control the impact of the different steps of the process on the NFC level.

Specific project objectives

In this context, the ICARE project is dedicated to:

- Reinforce the technological basis of the agro-food sector in order to understand and control NFC formation in industrial foodstuffs;
- Develop a rapid and cost-effective analytical method in order to enable SMEs to monitor the NFC level all along the production line;
- Increase the knowledge base of SMEs by providing them a customized training;
- Perform pre-normative research in order to provide EU regulatory authorities with:
    - a fast method for NFC quantification
    - a data base of NFC levels in food and their impact on health.

ICARE will provide European agro food SMEs with appropriate means to produce foods with reduced NFC rate and valorise such added-value products: guidelines of manufacturers' practices, alternative solutions to heat treatments, analytical methods to control the process and quantify the NFC levels, related training for IAGs and SMEs, clinical data and economical studies providing elements to implement a communication strategy toward consumers.

Consortium

Partic. Role*	Partic. Type**	Participant name	Participant short name	Country
CO	RTD	Association de Coordination Technique pour l'Industrie Agroalimentaire	ACTIA	France
CR	RTD	CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS	CSIC-IF	Spain
CR	RTD	DEUTSCHE FORSCHUNGSANTALT FUR	DFA	Germany

		LEBENMITTELCHEMIE		
CR	RTD	Institut National des Sciences et Industries du Vivant et de l'environnement	AGROPARIS TECH	France
CR	RTD	Institut Polytechnique LaSalle Beauvais	LaSalle Beauvais	France
CR	RTD	Research Base of the Slovak Medical University - Institute of Preventive and Clinical Medicine	SMUIPCM	Slovakia
CR	RTD	UNIVERSITA DEGLI STUDI DI NAPOLI FEDERICO II.	UNINA	Italy
CR	IAG	Association Nationale des Industries Alimentaires	ANIA	France
CR	IAG	Federalimentare Servizi S.r.l.	FS	Italy
CR	IAG	Federation of the Food & Drink Industries of the Czech Republic	FFDI	Rep. Cz.
CR	IAG	Federacion Espanola Industrias de Alimentacion, y Bebidas	FIAB	Spain
CR	IAG	Federaçao das Industrias Portuguesas Agro Alimentares	FIPA	Portugal
CR	SME	BAGETA Gottschall	BAGETA	Slovakia
CR	SME	Emmepiemme SRL	MPM	Italy
CR	SME	Argonix Sprl	ARGONIX	Belgium
CR	SME	SAIREM S.A.S	SAIREM	France
CR	SME	Progeotech s.r.l	PTECH	Italy
CR	SME	Laiterie de MONTAIGU	MONTAIGU	France
CR	SME	Union des des Coopératives Agricoles Laitières de Villefranche sur Saône et ses environs	UCLV	France
CR	SME	JOGIAFRA S.r.l.	JOGIAFRA	Italy
CR	SME	CHIPS LEBON	LEBON	France
CR	SME	SARL HUILES CAUVIN	CAUVIN	France
CR	SME	APERITIVOS MEDINA 3 SL	MEDINA	Spain
CR	SME	PATATAS SAN JERONIMO SL	PSJ	Spain
CR	SME	RUDOLF s.r.o	RUDOLF	Rep Cz.
CR	SME	VITAGERMINE	VITA	France
CR	SME	TRANCI DI BONTA	TRANCI	Italy
CR	SME	INDUSTRIAS RODRIGUEZ SA	IRSA	Spain

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Contractors

IAGs

- Federalimentare Servizi S.r.l. (I)
- Association Nationale des Industries Alimentaires (F)
- Federation of the Food & Drink Industries of the Czech Republic (CZ)
- Federacion Espanola Industrias de Alimentacion, y Bebidas (ES)
- Federaçao das Industrias Portuguesas Agro Alimentares (P)

RTDs

- Association de Coordination Technique pour l'Industrie Agroalimentaire (F)
- CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS (SP)
- DEUTSCHE FORSCHUNGSANTALT FUR LEBENMITTELCHEMIE (G)
- Institut National des Sciences et Industries du Vivant et de l'environnement (F)
- Institut Polytechnique LaSalle Beauvais (F)

- Research Base of the Slovak Medical University - Institute of Preventive and Clinical Medicine (SK)

SMEs

- BAGETA Gottschall (SK)
- Emmepiemme SRL (I)
- Argonix Sprl (B)
- SAIREM S.A.S (F)
- Progeotech s.r.l (I)
- Laiterie de MONTAIGU (F)
- Union des Coopératives Agricoles Laitières de Villefranche sur Saône (F)
- JOGIAFRA S.r.l. (I)
- CHIPS LEBON (F)
- SARL HUILES CAUVIN (F)
- APERITIVOS MEDINA 3 SL (SP)
- PATATAS SAN JERONIMO SL (SP)
- RUDOLF s.r.o (CZ)
- VITAGERMINE (F)
- TRANCI DI BONTA (I)
- INDUSTRIAS RODRIGUEZ SA (SP)

Work performed, partners involved

Research activities

WP01: **NFC profiles and spectral data base of a large range of products**

A data base was constructed including information on the country origin (France, Italy, Spain and Tchequia), the packaging description for each product type, the concentration of different NFC and the intensities (PARAFAC scores) of the main fluorophores composing the fluorescent fingerprint. 107 cookies from the 4 countries (90HMF, 34 CML+furosine, 81 acrylamide, 10 furane and 10 trans FA were analysed); 40 bread crisps from the 4 countries (34 HMF, 32 CML+Furosine, 26 Acrylamide, 2 furan); 38 infant formulas from France, Tchequai and Slovaquia (14 HMF, 38 CML+furosine), 70 potato crisps (14 HMF, 12 CML and 61Acrylamide) and 22 malts from France (22 HMF, 11 CML+Furosine, 22 Acrylamide, 11 3-MCPD and 22 furane and 22 furfural).
From this three section data base, a statistical analysis was performed. The main objectives fulfilled were to obtain :
1- The distribution of each NFC amongst the food types studied in the project inorder to identify the ones at higher risk of contamination for a given NFC
2- The distribution of NFCs in each food product in order to evidence the NFC at higher risk of accumulation or variation for a given food type
3- The correlation between NFC in each and all food types
4- Some correlation between known ingredients and NFCs

From the multivariate analysis, information has been obtained on the type of product associated to major risk of NFC formation, on those where the variability is highest indicating the need for identifying the ingredient or process at the origin of such a variability. For

example, those contaminants such as acrylamide and HMF which tend to increase exponentially with time-temperature, need a particularly strict control. Moreover, correlations between NFC will allow better understanding the chemical mechanisms leading to their formation; from positive or negative correlation. Generally, all NFC are correlated because of the common major influence of the heat treatment. However, CML is more influenced by time than temperature, while acrylamide and HMF are mode sensitive to the temperature in the product. This observation orientates the strategy of mitigation regarding the heat treatment. Furthermore, the sugar content in the product appears as another very important parameter. Bread crisps contain less acrylamide than biscuits, lactose-based infant formulas have higher levels than maltodextrin containing formulas. Playing on such simple parameters could help significantly improving the mean NFC level of european food products, especially because this mean level is often strongly increased by some samples with very high levels of NFC, probably because of particularly severe heat treatment and high sugar content.

WP2: **Building of calibration models for NFC prediction by fluorescence**

Calibration of the fluorimetric method over conventionally measured NFC needs those latter methods to be accurately validated. The quality of the reference analytical method for NFC assessment conditions the reliability of the result given by the indirect fluorimetric technique. Consequently, great attention was paid on the standardization and evaluation of the analytical methods used in the project. Two ring tests were performed on real samples of infant formulas, bread and potato crisps, malt and biscuits. This study was also used to define which NFC was of interest for the food product concerned. Acrylamide was assessed by LC-MS in CSIC laboratory, which is participating to the ring tests organized by CIAA. HMF was analyzed by three laboratories, and the mehtod developed by UNINA was selected as the most accurate. Regarding furosine and CML, 2 to 3 laboratories were participating and the method developed by AgroParisTech appeared as the most sensitive and reliable. Concerning lipid derived NFC, ITERG was the only partner involved and the analysis of such NFC allowed identifying that potato crisps were the only food product where such NFC were formed significantly enough to be of interest in the project.

The different NFC were therefore analysed by the selected and validated methods in the food models produced at laboratory or pilot scale, as well as in the real food provided by the core SMEs.

Fluorescence images were acquired on a laboratory fluorimeter. Analysis of the 2D excitation-emission matrix corresponding to such images allowed evidencing that the heat process modifies the fluorescence signal with sufficient sensitivity and reproducibility to use it as indirect tool for predicting the process impact on NFC formation. The multiway decomposition tool, PARAFAC, was therefore applied to obtain the main fluorescence profiles characterizing the specific food product. The intensities of each fluorescence profiles was calculated for all the samples and a calibration model was constructed over the NFC of interest. A satisfactory calibration model was obtained for each food model, but also for the commercial food products analyzed in WP1, although the quality of the regression line was less (R^2 around 0,80 and mean calibration error of 15-20%).

WP03: **Adaptation and validation of the fluorimetric method**

The calibration models built in WP2 were enriched for each NFC of interest by adding new information obtained in new samples. We were successful in predicting acrylamide in bread and potato crisps as well as in biscuits; furfurals, acrylamide, furan and HMF in malt, CML and furosine in infant formulas. The quality of the model was compared for different type of

signal pretreatement including way of light scattering removal and spectra normalisation. The reliability and sensitivity of the prediction method were totally satisfactory, as the error was decreased up to 5% for certain NFC-food product couples.
Both food model systems and real food products either provided by the core SMEs or purchased on the european market gave similar fluorescence landscape, indicating that specific food composition, type of ingredients and process/technology applied induced only minor differences in the overall fluorescence image. We experimented however that, by constructing more specific models for a given family of products more accurate prediction models were obtained with lower prediction error (lower than 10%). For example, biscuits were divided according to the type of sugar added, hexose or sucrose; potato crisps were analyzed separately depending on the potato variety, and bread crisps were modelled differently according to the type of flour used, whole or refined wheat … We also evidenced that the limit of sensitivity of the method was not conditioned by the fluorescence detection limit which is very sensitive but rather by the conventional one. Similarly the repeatability of the processed fluorescence signal was most often better than that of the conventional chemical analysis.
In conclusion, the prediction models developed to measure indirectly NFC in processed food samples, using the fluorescence image obtained on the laboratory fluorimeter, exhibit a high robustness, accuracy and sensitivity. All NFC were predicted very well, sometimes still better when submodels were built taking into account some important parameter influencing the NFC kinetic in the product (potato variety, type of flour ...). These models may be used at lab scale for routine analysis purposes. However, it must be considered that acquisition of a complete fluorescence image takes 30-45 min, and at least double image must be acquired. Furthermore, image treatment using PARAFAC decomposition is a complex task that only specialized researchers can manage using the Matlab software. So the method in this state does not fulfill the objectives of a rapid and simple method for NFC assessment by SMEs.

WP04: **Industrial validation of the adapted fluorimeter**

The conclusions drawn from WP3 explain whay a simple fluorimeter must be constructed and a new simplified method developed to apply the principle of the new analytical technique for non destructive assessment of NFC in food products.
To achieve this objective, it was necessary to conceive a simplified device allowing to excite the fluorophores in the food sample, detect and amplify the emitted light, decompose the emitted light to obtain spectral resolved information, and develop a software allowing to implement the calibration models and to calculate in real time the concentratin of NFC in the sample.
A prototype was therefore constructed by Argonix composed of light sources allowing exciting the major fluophores in the sample. A cuvette holder was included to receive the crushed sample to analyse. An optical fiber allowed relating the cuvette to a spectrometer and a cmputer was equiped with an adapted software to analyze the signal and convert it by the calibration models in the corresponding NFC concentration. The result is shown on the screen once the model specified : type of food product and type of NFC to analyze.
Considering that the optic and method used with the simplified fluorimeter prototype is completely different to that of the laboratory fluorimeter, all the calibration models have been developed again. In a first step, the signal obtained on the portotype for a given excitation wavelength was compared to that of the lab fluorimeter. The sensitivity of the signal was considered as sufficient, but the repeatability was not high enough. As the analysis is very rapid (1 min), several analysis of a same sample could be proposed to imporve this

repeatability. In addition, new signal pretreatment tools were applied until the repeatability error lower than 5% was obtained.
The prototype has been validated for two food products, where the demand of SMEs was highest: infant formulas and biscuits. Regarding infant formulas, two calibration models were developed, one on a standard formula used for lab and pilot scale assays, the other on powdered formulas produced by the SME Montaigu. The stake was very requiring for this last model because each infant formula had a completely different composition, and was processed differently. Similarly for biscuits, a calibration model was developed for the simple biscuit developed for lab experiments, but also a model for the different biscuits produced by the two core SMEs, Vitagermine and Rodriguez. Again, the receipes as well as the baking process differed considerably between the three biscuits provided by the SMEs.
Despite these difficulties, a very satisfactory model was obtained in each case with calibration errors lower than 8-10 %. The prototype was then used to predict NFC in experimental infant formulas and biscuits allowing avoiding the strong time consuming and expensive chemical analysis. The quality of the kinetic models obtained using the indirect rapid method proved the reliability of the prediction obtained.

WP05-06: **Understanding the effect of formulation and processing steps on NFC formation at laboratory and pilot scale**

The objective of these two WP was to describe the kinetic of NFC formation and evidence the major factors influencing their accumulation in the final procuct using laboratory as well as pilot plant experiments. The work was divided according ot the food models, infant formulas, bread crisps, biscuits, potato crisps and roasted malt.

1– Infant formulas

A simple model was developed which was based on either pure whey proteins or microfiltrated milk homogenized with a mixture of vegetable oils and vitamin-minerals in order to meet the nutritional requirements of infants. Production of microbiologically safe and stable IF is possible through milk atomization or heat sterilization. However, consequently to the heat processes, a wide range of chemical reactions occur, especially the Maillard reaction. The numerous neoformed compounds (NFC) which are formed by these reactions affect the product quality, nutritional value and safety. The Maillard reaction starts with the irreversible reaction between amino acids and lactose. The first consequence of this reaction is a significant decrease in lysine bioavailability, which is generally quantified by the Furosine content, indicative of the Maillard product lactulosyllysine. At high temperatures, lactulosyllysine may be further degraded by different processes during the advanced step of the Maillard reaction. Hydroxymethylfurfural (HMF) and carboxymethyllysine (CML) are produced, and considered as undesirable compounds as the former is mutagenic, and the latter could mediate the production of inflammatory cytokines, oxidative stress and lower insulin sensitivity. Vitamin C can also give rise to CML, especially in the presence of iron salts. A first objective of the task was to compare the kinetic of the different reactions during heating of the formula model. VitaminC oxidation and CML formation appeared to be the most heat-sensitive reactions. They can therefore be considered as good indicators of the heat impact on the formula quality.

Improvement of infant formula quality parameters such as the nutritional value and chemical safety, is an important challenge for the dairy industry. Besides the continuous progress achieved regarding the nutritional composition of the formulas by trying to better fit the

nutritional needs of newborns, another important aspect of infant formula quality concerns the better control of the heat process impact on various quality parameters.
The objectives were to study potentially interesting technologies, including microwaves, ohmic heating and microfiltration, to decrease the level of undesirable Maillard compounds in infant formulas.

The sterilization level was characterized by the heat treatment necessary to reach a 12-decimal reduction number of spores of *Clostridium botulinum*. However, for security problems, the non pathogen germ *Geobacillus stearothermophilus* was selected as indicator and a 5-decimal reduction number was considered as equivalent to a 12-decimal reduction number of *Clostridium botulinum*.
For both conventional and microwave sterilization, a Thermal Death Time (TDT) approach was used. It consisted in determining kinetics of nutritional spoilage (vitC and protein denaturation and modification by the Maillard reaction), and of spores destruction in order to find an optimal zone where nutritional and safety loss are minimized while spores of *G. stearothermophilus* are acceptably destroyed.
Unfortunately, the optimal conditions found in terms of specific microwave power (W/mL) needed for MW sterilization was not reachable by our MW pilot plant. Therefore, only a MW pasteurization process could be investigated and optimized.

Ohmic heating was also explored to test the advantage of increasing the temperature and cooling very rapidly. Formation of CML and furosine was found to increase linealy once the holding temperature reached. CML and furosine were essentially formed during this step and the more rapid increase in emperature, the lower the formation of Maillard products. The sterilization system was found to be very efficient, especially when the increase in temperature, from ambient temperature to 130°C was between 2 and 5 sec. In such conditions, CML formation was the lowest, suggesting that this technology could be of high interest to improve the chemical and microbiological safety of liquid infant formulas.

2 – Bread crisps model

In bread crisps the attention was focused on acrylamide and 5-hydrossymethylfurfural (HMF). The kinetic of formation of these hazardous compounds were studied at laboratory scale by means of a simple bread crisp model system made up of water, flour, salt and leavened by bakery yeast. The dough were kneaded, manually shaped in baguettes and baked. The bread was then cut in slices that were toasted at different temperatures for several times. The effect of different type of flour (wheat, whole-wheat, rye, rye flour with different extraction rate) as well as the effect of salt concentration, malt extract, antioxidant extracts, glycine and asparaginase addition on acrylamide and HMF formation was thoroughly investigated. Acrylamide content in untoasted bread was very limited but it accumulated during the toasting step following a zero-order kinetic regardless of formulation. Its final content in bread crisps was found to be correlated to the free asparagine content of flour. Rye bread crisps exhibited the highest acrylamide content followed by whole-wheat and then by wheat formulation. This order parallels that in free asparagine content of flour which is higher in rye. For the same reasons, the flour extraction rate (ER) has a strong effect on acrylamide content of bread crisps as well. The higher the ER, The higher the free asparagine content of the flour and thus the final acrylamide content. Free amino acids are mainly located in the outer layer of the grain thus a less refined flour contains more free asparagine than a more

refined one. Finally, no significant effect of salt concentration, antioxidant extracts as well as of malt extract on acrylamide formation was pointed out.

HMF formation followed a first-order kinetic at each temperature and in each formulation tested. Its final content was found to be strictly depending on final water content of the slices and highly correlated to browning development. The flour ER was found to have an effect on HMF formation likely because of the different amino acids content which affects the rate of Maillard reaction. Rye based bread crisps showed the highest HMF content followed by wheat and then by whole-wheat based bread crisps. No effect of salt concentration and antioxidant extracts was pointed out whereas addition of malt extract slightly increased HMF formation regardless of formulation because of the extra reducing sugars provided. Asparaginase and glycine were both effective in reducing acrylamide. Asparaginase addition at a concentration of 2000 U/kg of flour reduced acrylamide formation by up to 90% in bread crisps processed at laboratory scale. Regardless of concentration, asparaginase addition had no significant effect on HMF formation and browning development, thus making asparaginase addition the most promising tool to mitigate acrylamide formation in bread crisps. On the other hand, glycine addition was less efficient than asparaginase in reducing acrylamide levels. The addition of 1gram of glycine per kg of flour reduced acrylamide formation by 30% in wheat bread crisps and by 50% in rye and whole-wheat bread crisps. Moreover glycine addition had a clear effect on both HMF formation and browning development. In particular HMF content increased by an amount ranging from 20 to 50% depending on formulation and temperature.

Concerning process, Toasting time-temperature was recognized as the key parameter to keep under control in order to have a limited NFC formation. Results showed that prolonged toasting times at lower temperatures allow to achieve considerably lower NFC content while reaching the same final moisture content. Toasting at low temperature drastically decreased browning development of bread crisps as well. The final colour is often the most important parameter to identify the end point of the heating process in bakery industry, being the browning level recognised as one of the main quality attribute by consumers. However, In bread crisps, we pointed out by a wide consumer test that texture is more relevant than colour and this attribute mainly depends on the moisture content.

3 – Cookies model

The objective of this work was to analyse the influence of the ingredients and of the heat treatment, on NFC formation in cookie types of products. Four NFCs were selected for this study. *Furosine* (FUR), being a marker of early Maillard reaction, has been used to give an estimate of the extent of protein damage caused by heating in cereal products. *Carboxymethyllysine* (CML), *hydroxymethylfurfural* (HMF) and *acrylamide* (ACR), are relatively stable advanced Maillard reaction products with possible negative health impact, which presence has been related to formulation and processing conditions in cereal products.

A simplified model cookie system was developed for this study. Three recipe variables were observed: sugar type (sucrose, glucose, maltitol), fat saturation level (high, low) and leavening agent (presence, absence). To model the thermal effect on NFC formation, kinetic data were generated under three baking temperature (150, 200 and 230 °C). Three physical responses (water content, color and core thermal input) were observed for all samples. The chemical responses (FUR, CML, HMF and ACR) were partially recorded due to the high number of chemical analyses required.

The main results of the study showed that NFC production is dependent on *thermal input*, either baking temperature for HMF and ACR or baking time for CML and FUR (figure 1). HMF and CML follow respectively a first and pseudo-zero order kinetics with both an Arrhenius type of temperature dependence. Water content and color are linked to the production of advanced Maillard products.

The type of sugar is of main importance for the final NFC content: glucose tends to generate more HMF than sucrose at low baking temperatures and more CML whatever the baking temperature. However, at higher temperature, 230°C, sucrose wa even more effective in producing HMF than hexose. This suggests that heat-induced sucrose degradation favours the formation of a reactive intermediate in the HMF reaction pathway. The use of a sugar alcohol allows limiting HMF production. Surprisingly, HMF appeared to be affected by the presence of ammonium carbonate whereas no significant effect was obtained on ACR. This observation might be partly due to the limited number of ACR analysis being insufficient for discrimination purposes.

4- Potato food model

During industrial processing of potato crisp, many different factors are accounting for the final formation of NFC, specifically acrylamide, and they should be globally considered and properly weighted. As opposite to other food commodities, potato fresh tuber cannot be modelize in order to obtain a reproducible sum of constituents (water, starch, sucrose, reducing, amino acids, proteins, pectin, minerals, etc) in a well-defined structure. Then experiments in models are limited to the variety of potato harvested in that period. Taking into accounts former limitations, three main factors were investigated at lab-scale with the aim to asses their contribution to the overall formation of acrylamide, being, levels of reducing sugars, and effect of pre-treatment and frying conditions (thermal input and type of oil). Three different potato tuber varieties (Agria, Hermes and Bintje), soaking step with citric acid, kinetics at different temperatures (150 – 190 ºC) and frying times (3 to 7 min), and four types of oils (olive oil, sunflower oil, palm oil and palmolein fat) were tested. It was concluded that:

a) Levels of reducing sugars in the potato tuber will directly affect the final amount of acrylamide in the potato crisp.
b) Colour is directly related to the final concentration of acrylamide and burned hot-spot should be removed.
c) There was not statistical significant effect ($P<0.05$) of the type of frying oil used and final levels of acrylamide by taking all data at once (T/t).
d) Formation of acrylamide is dramatically enhanced at temperatures higher than 170ºC for similar frying times, being the frying time less determinant in its formation.
e) Acrylamide levels were reduced at nearly 50% by soaking potato sliced in citric acid solution at 1% but some organoleptic attributes appreciated by consumers were modified, being negative the taste (sour after-taste), but positive a consumer-appreciated golden colour.

A lab scale study was performed in ITERG to compare the level of different compounds neoformed in drastic frying conditions with different vegetable oils that contain different ratio of saturated and mono- or poly-unsaturated fatty acids: palm oil, linoleic sunflower oil, high oleic sunflower oil, grapeseed oil, rapeseed oil and a blend of Huilerie CAUVIN composed with linoleic sunflower, high oleic sunflower and palm (oleic fraction) oils.
The study included 200 frying cycles performed at 180°C with bintje variety potato crisps.

Different quality parameters were determined in the frying oil to evaluate the thermo-oxidative degradation : polar compounds, polymer of triglycerides, free fatty acids, vitamin E, oxidation products. The following NFC were analysed in the frying oil: trans-fatty acids, oxysterols, cyclic monomers of triglycerides, polycyclic aromatic hydrocarbons. The level of acrylamide was determined in the crisps.

The more the number of fryings, the darker the color of oil and of crips. The main conclusions of this study are:

1. Palm oil (oleic fraction) , oleic sunflower oil and rapeseed oil are the most resistant oils considering all thermo-oxidative degradation parameters. The polar compounds and the polymers of triglycerides are still at the limits fixed by France after 50 frying cycles : 25 % for polar compounds and 14 % for polymers.
2. The NFC formation at 180°C is very low for any oil:
   - the content of TFA (trans fatty acids) in the frying oil is less that 1% even after 100 frying cycles,
   - no PAHs (Polycyclic Aromatic Hydrocarbons) are generated during frying even with severe conditions,
   - the formation of oxysterols and cyclic monomers are not linked to the nature of the oil and are mostly dependant on the frying conditions; nevertheless the levels of these NFC found in the frying oils are acceptable
3. It's difficult to link the acrylamide level in crisps with the type of oil and the number of frying cycles.

WP7: **Diagnosis and Industrial validation of possible strategies to improve food safety**

The industrial step of the project aimed at providing a diagnosis of the impact of industrial processes on NFC levels in the final product manufactured by each SME, examining the possible improvement strategies to decrease if necessary the NFC levels, testing some of these mitigation strategies or the interest of the NFC analysing sensor for NFC monitoring.

1– Infant formulas

A microfiltrated infant formula intended to 10-24 months old infants has been produced by UCLV, with new ingredients allowing maintaining the CML content at the lowest possible level (10-50 times lower than the mean level observed in commercial powdered IF) throughout the storage period of 3 weeks at 4°C. The study of packaging impact on CML content as well as vitamin levels, has demonstrated that the polyethylene bottle already used by the SME for regular cow's milk is convenient for this new formula.

The analysis of the powdered formula produced by Montaigu has confirmed the important variability of the content in Maillard products depending on the receipe. However, the factors known to affect the reaction rate were not sufficient to explain the NFC variability. The role of the quality of the ingredient, including the whay or milk protein isolates was evidenced. The impact of the pasteurization, concentration and drying process were also shown to strongly influence the final product quality.

Three new formula including new ingredients expected to limit the Maillard reaction were produced for the project. Unfortunately, we did not observe a significant decrease in NFC formation, and no satisfactory interpretation could be proposed.

Another factor was tested concerning the impact of storage at room temperature. It was concluded that the quality of the powder significantly decreases after 3 months of storage, but not before.

A specific prediction model was developed to control NFC formation in the first pasteurization step, as well as another model to predict the final furosine and CML content in the final powder. The models were included in the prototype software and was used to analyze part of the samples during storage, to study the impact of different concentration levels of the liquid formulated milk, and to test the impact of a new drying process.

2– Cookies

The two cookies producing SMEs, have received information on the NFC level in their cookies at the different steps of the process, as well as on the stability of the process. The final level could be compared to that obtained in the european market. For one of them the acrylamide levels were at the median range, but for the other one, the levels were lower, almost at the range of the first quartile. Regarding the CML levels, they were rather high as compared to the levels measured in commercial samples.

3– Bread crisps

The experiments performed at industrial scale by BAGETA and TRANCI DI BONTA confirmed results achieved from lab scale experiments on the effect of the type of flour on HMF and acrylamide formation in bread crisps. Wholemeal flours produced more HMF and acrylamide then refined ones because of the different free asparagine and free amino acids content. The addition of malt extract has no effect on acrylamide formation, but it slightly increases the rate of HMF formation because of further reducing sugars provided. Sodium chloride concentration has no significant effect on both NFC under investigation.
Asparaginase and glycine were both effective in reducing acrylamide content of bread crisps. Asparaginase is an enzyme able to hydrolise free asparagine in aspartic acid and ammonia. Asparaginase addition at a concentration of 2000 U/kg of flour reduced acrylamide formation by an amount ranging from 60 to 75%. Lower concentration (1000 U/kg of flour) was also effective in reducing acrylamide formation but to a minor extent whereas only a limited further reduction was achieved when a concentration of 4000 U/kg of flour was used. Regardless of concentration, asparaginase addition had no significant effect on HMF formation and browning development. For these reasons, asparaginase addition can be regarded as the most promising tool to mitigate acrylamide formation in bread crisps. On the other hand, glycine addition is less efficient than asparaginase in reducing acrylamide levels. At a concentration of 1 g/kg of flour acrylamide content is 20-30% lower than control whereas at a concentration of 5 g/kg of flour acrylamide content is 30-50% lower than control bread crisps. The main drawbacks of glycine addition are the increasing in HMF formation (up to 40%) and the higher rate of browning development which makes this straregy not suitable for crisps processed at industrial scale.
Experiments performed at industrial scale showed also that the introduction of a drying step prior to toasting at high temperature resulted in a reduction in acrylamide content by up to 30% and in a similar reduction in HMF formation. The shorter the toasting step at high temperature the lower the final content in NFC . Of course, toasting at low temperature drastically decreased browning development of bread crisps as well. The final colour is often regarded as the most important parameter to identify the end point of the heating process in bakery industry, and the browning level is one of the main quality attributes by consumers. This problem could however be overcome by introducing a limited amount of malt extract in the formulation despite of the very slight increasing in HMF formation.

Regarding bread crisp production by te two core SMEs, clear results were obtained allowing proposing a strategy to decrease the crisps HMF content. Rye and maïs flour should be avoided as enhacers of both HMF and acrylamide. Furthermore, introduction of a previous drying step at 100°C for at least 45 min before toasting allowed to decrease by 50% the final HMF content. It remains to verify that such new conditions also allow limiting the acrylamide level.

4– Potato crisps

During industrial processing of potato crisp, many different factors are accounting for the final formation of NFC, specifically acrylamide, and they should be globally considered and properly weighted. It is particularly difficult to translate lab-scale or pilot scale experiences into a continuous production line where about 15000 kg of fresh potatoes are processed daily by gibing about 4000 kg pf potato crisp and consuming more than 1000 L of sunflower oil. However, an integrate approach should be considered to minimise the final levels of acrylamide in the product without affecting the sensorial attributes appreciated by consumers. In parallel, the ALARA concept for mitigation, as well as the caution concept is applied since, by today, there are not official maximum levels regulated for acrylamide in potato crisp. Different steps has been identified for quality inspection which are, raw material (storage conditions and levels of reducing sugars), pre-treatment (soaking), and frying conditions (type of oil, frying temperature and time, ratio oil:potato).

There are some conclusions at industrial level:

a) Quality of the raw material in critical and will drive the success of further mitigation strategies in the process. Level of reducing sugar should be below 0.3 % as declared by CIAA. SMEs should implement the adequate internal quality control to meet that requirement, mainly during the winter period.
b) Excessive soaking time will not reflect a net reduction of acrylamide in the potato crisp but there is a risk of softness apart of loss of micronutrients.
c) To reduce the temperature of frying as much as possible but extending the frying time to ensure proper organoleptic characteristics in the product since acrylamide formation has higher temperature dependence. However, it should be evaluated the effect the side effect such as the oil uptake in the crisp and shelf-life.

The company Chips LEBON is a very small French producer of “traditional” crisps, equipped with a pilot-industrial plant semi-continuous (25 kg/h). The crisps are produced with Spanish AGRIA variety potatoes and linoleic sunflower oil. The marketing objective of this company is to produce small volumes of a high sensorial quality product with a fruity taste, a yellow gold color and a crispy texture. The shell life proposed is short (3 months) to have only fresh products on the market.
The process includes: storage of potatoes in a regulated room at 9°C, a washing step, an abrasive pealing, a 1,5 mm slicing, a cold fresh water washing to eliminate fecule, a frying at 176°C, a static deoiling, a light salting and the packaging.

A diagnosis of the process was done by using the CIAA toolbox given for the reduction of acrylamide level in crisps. All the key points of the process are totally in accordance with the recommendations of the toolbox.
All the quality parameters analysed in the frying oil and in the crisps of CHIPS LEBON are very good and in conformity with regulation and all the NFC levels are correct. The end of

frying is determined with the colour of the crisps and all the brown or dark crisps are eliminated before packaging. The mitigation of the NFC in this commercial product is not necessary.
To can increase the nutritional value of the crisps with a higher level of omega 3 poly-unsaturated fatty acids and to can get a longer shell life, a test was performed with a new blend oil produced by HUILERIE CAUVIN. This new frying oil is a mix of 80 % oleic sunflower oil (very stable oil with high level of oleic acid) and 20 % of rapeseed oil (that contains 10 % of omega 3 linolenic acid). The challenge was to not change the process, the potato variety (Agria), the packaging and the sensorial quality of the crisps. The quality of the crisps produced with the new blend and the NFC levels are correct.

WP8: **Comparative study of the impact of a standard diet versus boiled diet in healthy human volunteers**

The specific objective of the WP08 (Comparative study of the impact of a standard diet versus boiled diet in healthy human volunteers) is to evaluate the impact of ingestion of NFC on biological indicators related to glucose and lipid metabolism, oxidative stress and inflammation.

Dietary neoformed compounds (NFC) resulting from heat treatment of food are thought to be involved in diabetic complications, atherosclerosis and impairment of renal function. Carboxymethyllysine (CML), a Maillard NFC, is considered to be the most pertinent indicator of dietary as well as endogenous exposure to NFC because of its stability, its recognized bioactivity through the receptor for advanced glycation end products and its ubiquitous presence in aging and diseased tissues.

The aim of the present study was to determine whether a diet that is low in NFC, and specifically low in CML, has a biological impact on carbohydrate and lipid and fatty acid metabolism, inflammatory variables and oxidative stress in young healthy adults.

Sixty-two non-smoking subjects (mean age 20 years) were randomised successively to 4-week periods on each of the two experimental diets in a cross-over design. The diets were nutritionally equivalent and differed only in the cooking techniques (steam cooking for the low-NFC diet; frying, roasting and grilling for the high-NFC diet that corresponded to the normal diet with minor adaptations).

CML bioavailability data have been reported elsewhere. Compared with the steam-cooked diet, which contained three times less CML, a higher (P<0.002) HOMA index was observed with the normal diet, resulting from an increase in fasting insulinaemia (P<0.01) and glycaemia (P=0.07). Plasma TAG (P<0.01), total cholesterol (P<0.005) and HDL-cholesterol (P<0.0001) were higher with the normal diet, but LDL-cholesterol was similar for the two diets. A lower plasma EPA (P<0.0001) and DHA (P<0.0001) was observed with the normal diet, without any change in the linolenic acid content. Both plasma vitamin C (P=0.015) and vitamin E:cholesterol (P<0.0001) were decreased with the normal diet, whereas plasma ubiquinol (P=0.013) was higher. No change in inflammatory variables or lipid peroxidation was found.

The results indicate that after 1 month on a high-NFC diet compared with a low-NFC steam-cooked diet there is significant impairment of the *n*-3 PUFA profile as well as a trend to insulin resistance associated with higher oxidative stress, which has been shown for the first time in healthy subjects. The direct involvement of NFC is strongly suggested by the similarity between the two diets in relation to confounding factors, the evidence of NFC bioavailability and the significant correlations between biological NFC levels and metabolic variables. The specific role of CML must be clarified.

WP09: Evaluation of the impact of infant formulas and maternal milk in infants

The objectives of this second clinical study were to compare the inflammatory status, oxidative stress, renal function and cardiovascular risk factors in formula/breast-fed infants and toddlers; to measure the circulating and urinary AGE levels and finally to evaluate the evolution of these parameters when milk is the exclusive food, and when it is only a part of diversified diet

During March 2006 to December 2008 blood samples were obtained from 247 healthy infants. Dietary questionnaires served to allocate the infants according to the type of feeding. The 3-7 months old babies (n=113) were assigned into 2 feeding categories: exclusively breast-fed (n=53) or formula/mixed fed (n=60). From among 134 older infants (age range 8-19 months) on diversified infants' diet, 67 were still partially breast fed, while 67 drunk either formula or cow milk. Spot urines were obtained from 185 children. Blood and urine samples were obtained from 136 healthy mothers of 130 infants.

1. *Inflammatory status*: No profound differences were revealed between the breast- and formula-fed infants.
2. *Oxidative status markers:* Breast- and formula-fed infants differ in the markers of oxidative status. Some of them are attributed to a unique composition of human breast milk, e.g. higher total antioxidant capacity of plasma in the breast-fed infants may be contributed by higher plasma bilirubin levels. This results from high activities of β-glucuronidase in mother milk, which facilitates the intestinal reabsorption of bilirubin (Gourley and Arend. Lancet. 1986;1:644). Antioxidant properties of bilirubin (Neuzil and Stocker. J Biol Chem 1994;269:16712) may contribute to better antioxidant defense in the breast-fed infants. On the other hand, fortification of formulas with fat-soluble vitamins may show beneficial effects on lipid peroxidation. Moreover, differences in plasma concentrations of advanced oxidation protein products (AOPPs; marker of myeloperoxidase reaction), nitrotyrosine (marker of protein nitration), and urinary excretion of 8-hydroxydeoxyguanosine (marker of oxidative damage to guanosin/DNA) were recorded.
3. *Renal function*: no major differences were revealed.

We found higher plasma concentrations of homocysteine in the breast-fed infants, which are sought to be normal finding, due to low serum cobalamin (Hay et al. Am J Clin Nutr 2008;88:105). Further studies are required to determine whether this pattern is solely attributed to the cobalamin content in breast milk or whether metabolic effects of breast milk or breastfeeding cause a change in cobalamin homeostasis.

Human breast milk contains much lower amounts of Maillard reaction products (MRPs) than infant formulas (ICARE study paper: Šebeková et al., Annals N Y Acad Sci, 2008; 1126: 177). Industrial heat processing of infant formulas, inevitable to guarantee their microbial safety and prolonged shelf-life, results in formation of MRPs, e.g. N^{ε}-(carboxy-methyllysine) (CML, excellent marker of MRPs formation in foods). Feeding with infant formulas is associated with mild rise in plasma CML concentrations, and marked rise in urinary CML excretion in infant formulas. Hydrolyzed formulas-fed infants displayed higher urinary CML excretion if compared with those on non-hydrolyzed ones. Thus, MRPs from formulas are partially absorbed and rapidly excreted via urine (Šebeková et al., Annals N Y Acad Sci, 2008; 1126: 177). Mentioned differences observed in the toddlers (when milk is solitary source of nutrition) gradually diminish when milk is substituted by diversified mixed diet.

Breastfeeding is the optimal method of infant feeding. Benefits increase with the duration and exclusivity of breastfeeding up to 6 months (Turck D: Arch Pediatr. 2005;12S3:S145). Composition of

breast milk substantially differs from that of infant formulas: it is low in solute load, richer in carbohydrates, contains less but qualitatively superior proteins, highly bioavailable minerals, nucleotides, and constituents that can not be replicated in infant formulas, such as living cells, hormones, growth factors, cytokines, active enzymes and immunoglobulins. It is well known that some blood and urine chemistry parameters are tightly related to actual dietary regimen of the individual. In comparison with formula-fed, the breast-fed babies have higher bilirubin, cholesterol, and LDL-cholesterol concentrations, AST and ALT activities, lower, iron, urea and phosphate levels, urinary excretion of urea and sodium, urine osmolality, and are more insulin-sensitive (Owen et al.,. Am J Clin Nutr 2006;84:1043; Jørgensen et al.: J Pediatr Gastroenterol Nutr 2003; 37:559; el-Kholy et al., J Egypt Public Health Assoc 1992; 67:237; Gourley and Arend: Lancet 1986;1:644; Harit et al.: Eur J Clin Nutr 2008; 62:203; Owen et al.: Pediatrics, 2002; 110:597; Belton et al.: Arch Dis Child 1977; 52:167; Davies et al.: Arch Dis Child 1973;48:563; Schmidt et al.: Pediatr Nephrol 2004; 19:1137; Hoppe et al.: Pediatr Nephrol 1997; 11:687). Our data confirmed that the mentioned differences in blood and urine chemistry parameters occur in infants uniformly and concurrently. Some of them could be attributed to feeding of hydrolyzed formulas. Differences in some parameters persisted even when mother milk/formula represented only a supplement to diversified diet. In babies, dietary regimen is a main determinant of the mentioned biochemical variables. In older infants the impact of diet diminishes and that of the age increases. As suggested previously, early nutrition-associated changes might be of importance in manifestation of obesity, metabolic syndrome and/or type 2 diabetes, hypertension, cardiovascular and renal disease in the adulthood (Schmidt et al.: Pediatr Nephrol 2004; 19:1137; Owen et al.: Pediatrics 2005;115:1367; Rich-Edwards et al.: Epidemiology 2004;15:550; Wilson et al.: BMJ 1998;316:21; Lawlor and Smith: Curr Opin Nephrol Hypertens. 2005;14:259; Koletzko et al.: Adv Exp Med Biol 2009;646:15). Interestingly, some blood chemistry parameters showed tight relationship between mother-child pairs.

WP10: Pre normative issues

In order to fulfil all NFC pre-normative issues that is concerning at european level, have been developed for ICARE Project two european action scenarios:

1)NFC european official normalization; 2) NFC reduction european official guide-lines; 3)Future european official NFC analytical normalization and standardization.

1)NFC european official normalization

This scenario was divided in two parts:

Part A) Analytical methods developing intended NFC on foods

Is absolutely necessary to establish an european official trustable, precise and accurate analytical method, this mission falls just at CEN(Center of European Normalization) frame, that is the organism intended to elaborate official analytical methods and standards obligatory for European Union members food application.

As european member of CEN/TC275(T.Committee risponsible for horizontal foods normalization) belonging Spanish Mirror Group for Foodstuffs Standardization , I proposed the NFC Working Group creation to standardize NFC on foods.

The official creation of WG13(NFC) for NFC analytical normalization was made at july of 2008 at the CEN/TC275 Plenary Session, main important resolutions were the NFC definition, the european representatives that will become WG13 members and both the NFC priority and secondary list in order to decide the NFC analytical determination.

On the priority list will be placed two ICARE-NFC: Acrylamide and furanes, on the secondary list will be: Carboxymethyl-lisine and HMF among others NFC.

At the last CEN/TC275 Plenary Session Meeting 13-14.july,2009 were proposed the initial analytical methods for NFC, that become fully approved.

Part B) NTDS(NFC Technical Data Sheet)

A specific model was designed in order to summarize the main characteristics of NFC **,** for a better knowledge, use and possible application.
The chemical structure, origin, NFC formation mechanism, NFC range of values on foods, NFC analytical detection on foods(synoptic), toxicological data, NFC reducing substances, food legislation references.

2)NFC reduction european official guide-lines

This scenario was divided in three parts:

Part A) CIAA Toolbox

A global document was elaborated by CIAA, with a list of recommendations to both manage NFC on foods and how to reduce NFC on foods, specially that relates acrylamide generation on foods. In general the report is structured as GMP procedure to be adopted by food industry. The referred document has been sent to ICARE Consortium.

Part B) CODEX Guide-lines intended NFC reduction on foods

CODEX prepared a guide-lines for several NFC diminishion on foods, specially for acrylamide and PAH (that is out of the ICARE scope). The procedure is based on specific measures applied at: Bothanic origin, cropping, environmental conditions, storage, transport, NFC reductive substances, foods elaboration process(temperature, holding-time, vacuum application,etc.)

For ICARE using purpose, have been prepared a document that summarizes the main points and specifications.

3)Future european official NFC analytical normalization and standardization

Next november,2009 will be held in Brussels the second WG13/CEN/TC275 meeting intended to define analytical methods will be adopted for all identified NFC on priority list, on which acrylamide is the most important issue to be normalized.

I´ll attend as WG13/CEN/TC275 spanish representative member regarding to study and improve future analytical NFC methods. Resolutions and Draft Reports adopted will be informed to ICARE Consortium.

Training activities

WP12:

The specific objectives of the WP12 were to train the SMEs and IAGs of the ICARE consortium on NFC issues. Moreover, the IAGs were in charge of the dissemination of ICARE results to all their members (European food SMEs).

The goal was to let a maximum number of companies know more about NFCs, their forming, the existing tools to monitor their presence and the possible ways to anticipate and control their overcome.

SMEs of the core group have been systematically informed of ICARE's partners' work in progress. They have participated to all every six month ICARE's meeting. They could follow ICARE's researchers' presentations of their works and were able to bandy with them about specific points when necessary.

IAGs have participated to these regular meetings. More over as they were in charge of ICARE's results dissemination towards their members, they benefit of specific trainings performed by the ICARE's RTD.

These trainings to IAGs were focused on vulgarisation of the results (in order to make understandable by the SMEs), identification of interesting results for the companies (what will be of interest for a company).

Some training material has been developed by the IAGs with the help of the RTD performers. This material presents the definition of NFCs; the way they are formed; for each kind of

product studied within ICARE (fried products, cereals, dairies, malt) the list of typical NFCs found; the factors influencing the formation... It is written in English and has been translated in their national language by the IAGs and disseminated to their members. This material is also available to the public on ICARE's website.
This material has also been used by the IAGS to build some training programs to the SMEs. The trainings were product focused.

12 training sessions have been organised in 5 countries to update the companies from the different countries participating to the program about state of the art on NFCs. More than 200 companies have been trained through Europe.
IAGs have also been trained on the subject of implementation of improved processes and fluorimetric method.

A closing conference on ICARE has been held the 5th of June in Paris at SERI (European research and innovation congress). Inès Birlouez has presented the final results of the program to the companies visiting the show. The presentation made and the documents that were distributed to attendants will be available on the Icare website.

Use and dissemination and management activities

WP11: Dissemination activities

The specific objectives of the WP11 (Innovation related activities) were to disseminate the results of the project in the industrial agro-food sector and inside the scientific community, to manage the Intellectual Property Rights in accordance with the Consortium Agreement and in relation with the exploitation plan.

All along the project we have worked to do the contents of the web-site dedicated to ICARE: www.icare-project.eu/. The structure of the website is the following:

- Brief presentation about ICARE.
- Objectives: Main objectives of the ICARE project.
- Partners - List of all project partners.
- Coordinator – Project coordinator name, project scientific coordinator and project coordinator organisation name.
- News – This section contains the bulletins of the project.
- Events – Information about training session and general assemblies of ICARE.
- Work package activities - Brief description of each work package (main objectives).
- Publications – Brief abstract of publications of ICARE.
- Links to ENFFI and other relevant links on food project, as TRUEFOOD

We have worked to do the dissemination by leaflet and technical presentation in different meetings, congress and exhibitions. Info-sheet on ICARE results for each WP has been prepared and sitributed to the WP Leaders:

- **Needs / challenges:**

(A short description on the needs that should be addressed)

- **Possible solutions / Improvements through research activities (WP and task):** (A short description on the solutions produced by the WP. Description on what we've learned from the task that can be applied by SMEs)

- **Expected benefits/Impact of the results and possible application by SMEs:** (Description on the benefit for the company from an economic and qualitative point of view. Description on how to apply the results / tools to use)

- **Possible benefits for the consumers (if available)**

- **Other relevant information related to this research result (if available):** (i.e., information on trainings for SMEs, conferences, scientific publications, information on EC and national legislation relating to the results, etc.)

WP13: Management

The specific objectives of the WP13 (Project management) were to perform the administrative management activities in order to follow-up and monitor the program work progress and to assist the Management Team in achieving the project objectives and goals.

- ✓ Technical management

From a technical point of view, the work foreseen have been well performed. All the RTD performers managed to work with the SME's of their countries. Most of the deliverables have been delivered. Inès Birlouez coordinated the technical work by ensuring a good exchange of informations between the partners. All the meetings were organized with an agenda and a logistic document forwarded to the partners one calendar month before. Suitable templates for the reports were prepared and improved during the project. All the EC recommandations and deadlines were reminded to the partners during the meetings. The PUDK have been updated all along the project with the new events linked to ICARE project. Conference call with the technical coordinator in order to make regular update of the work performed and the progress toward the objectives.

- ✓ Financial management

The financial manangement wa ensured by ACTIA. A financial follow up was ensured all along the project. Indeed, as ICARE is a research collaborative project meaning that SME's as reimbursed only for their travel costs, it was really important to explain to the partenrs the rules of this type of project and especially for the SME's. A specific follow up for some of the SME's was performed in order to ensure the reimbursement of the RTD performers. Another aspect concerns the financial reports. Suitable templates for the costs follow up were sent to all the partners on calendar month before. All the form C were collected and checked before sending to EC. The procedure for the audit certficates was explained to the partners during the two last meetings. During the meeting at Bratislava specific meetings with some SME's were organized.

End results achieved and perspectives

The main results of the project can be assigned to three different topics, all part of a global problematic related to management of the risk associated to absorption of neoformed contaminants via heatprocessed food.

1- the clinical studies have evidenced for the first time under reaslistic conditions and with reliable analytical techniques that Maillard products present in regular diet are absorbed significantly in healthy humans. The level of Maillard products, including NFC, ingested through the diet conditions the inner exposure to those compounds, as revealed by significantly higher plasma ad urine concentrations. In turn, the modification in inner exposure to NFC consecutive to ingestion of heat-processed food, is associated to quantifiable metabolic changes in agreement with results obtained in animal studies. In infants, such as in young adults, the main changes evidenced concern the increase in oxidative stress, and the lower insulin sensitivity following ingestion of Maillard products through the diet. Such changes are rapid (1 month) and long lasting.

These important and new results demonstrate the primor importance to improve the quality of processed food regarding NFC levels, and the need for controlling the contamination levels in all the products concerned. Identification of the contribution of each food product to the total exposure to NFC gives indications on those products that will need to be surveyed in view to decrease the exposure rate to NFC: fried products, sevre-heat treated cereal products, including biscuits, toasted dough, and bread.
Regarding infant feeding, breastfeeding is recognized as the optimal method. Differences in blood and urine parameters of the breast- versus formula-fed infants may be explained by the high protein content of the formulas in comparison with breast milk as well as the level of Maillard products absorbed from the infant formulas. Most of them diminish when milk is only a supplement to diversified diet. Despite of this observation it is suggested that hypoallergenic hydrolyzed formulas should not be indicated just as general preventive approach to potential unsubstantiated allergies.

2- The levels of NFC in european food products are very variable. Part of this variability results from differences in ingredients and receipes, and part from different processing parameters. Depending on the food product concerned, controlling the ingredients or raw material is the best strategy (potato crisps), but for others decreasing the process temperature is easier (biscuits, bread crisps). Combination of the two strategies induces a synergic impact. Generally including a first drying process before grilling or frying can be of interest to decrease the final heat treatment severity. Limiting the final browning is another trend to develop progressively in order not to later the consumer acceptability for the product. These parameters could be considered as different aspectsof a global strategy for good manufacturing practices, without appealing to new additives such as asparagine, citric acid, leavening agents, etc … whose use compromise the final sensorial attributes of the food.

3- The development and validation of a new sensor allowing rapid, reliable, sensitive assessment of NFC in the food product at line in the industrial site will allow to provide SMEs with an innovative and easy tool for the control of NFC in the final product. This device will also help identifying the critical steps in the process, or evidencing the insufficient quality of some ingredients as part of a new quality control strategy. It will also be a useful

tool to assist receipe or process innovation allowing guaranteeing that the new product fulfill the possible future regulations regarding NFC.
The sensor will be further improved and commercialized by the spin off innovative SME SPECTRALYS Innovation in collaboration with Argonix and IAGd. Great attention will be paid on the validation of the analysis by using as reference method for the indirect assessment of NFC, analytical methods selected by the new WG13 of the CEN. IAGs will participate in promoting the sensor by widespread dissemination to their members.

2. Dissemination and use

The below table give some details on the already publishable exploitable results and non-confidential knowledge available from the project. For any inquiries (like for publications), please contact:

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Result no	Description of knowledge	WP	Contact partner	Other partners involved	Status *	Key code*
R1	Cartography of fluorescence fingerprints of the food products	WP02	LasalleBeauvais		C	SEC
R2	Calibration models for NFC prediction in Bread crisps	WP3	LasalleBeauvais		IP	SEC
R3	Calibration models for NFC prediction in cookies	WP3	LasalleBeauvais	CSIC / UNINA	IP	SEC
R3'	Calibration models for NFC prediction in potato crisps	WP3	LasalleBeauvais		IP	SEC
R4	Calibration models for NFC prediction in infant formulas	WP3	LasalleBeauvais		IP	SEC
R5	Calibration models for NFC prediction in malt	WP3	LasalleBeauvais		C	SEC
R6	Prototype for measuring fluorescence at selected wavelength For NFC prediction in food matrices	WP4	ARGONIX		IP	SEC
R7	Prototype for sterilizing small milk samples at the lab scale	WP5	PROGEOTECH		IP	SEC
R8	Kinetic data on NFC formation during malt roasting	WP5	IFBM	CSIC / Samples suppliers		

Result no	Description of knowledge	WP	Contact partner	Other partners involved	Status *	Key code*
R9	Model for the optimisation of microwaves roasting	WP6	LasalleBeauvais	CSIC / IFBM		