The ICARE project

ICARE Project – COLL-CT-2005-516415

Impeding neoformed Contaminants Accumulation to Reduce their health Effects

- Reason of being of the project :

Possible health impact of severely heat-treated foods due to NFC formation

- Strategy adopted

Evaluate NFC distribution in european food products
Understand the impact of processing on NFC accumulation
Develop a rapid, simple and low cost method for NFC monitoring
Propose strategies to improve the final NFC content in foods

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The ICARE project

ICARE Project – COLL-CT-2005-516415

A collective research targeting Food Industry National Associations
ANIA – France
FIAB- Spain
FIPA – Portugal
FEDERALIMENTARE – Italy
FFDI – Czech Republic

5 research centers and 3 technical centers
Agroparistech, LaSalle beauvais – France
ITERG, CTCPA, IFBM, ACTIA centers – France
UNINA – Italy
DFA – Germany
CSIC – Spain
SMUIPCM - Slovaquia

12 SMEs in potato crisps, bread crisps, biscuits, malt and infant formulas

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The objectives

Technological Devt

Reactions Modelling

Impact of processing

Alternative technologies

Analytical Control

Development
and validation
of an analytical tool
for online monitoring
of food NFC

Clinical studies

•Health impact of grilled versus steam diet in young healthy adults

•Health impact of infant formula versus Breast milk in infants

Validation at industrial scale
Of food safety improvement

Certification procedures
Analytical methods and process

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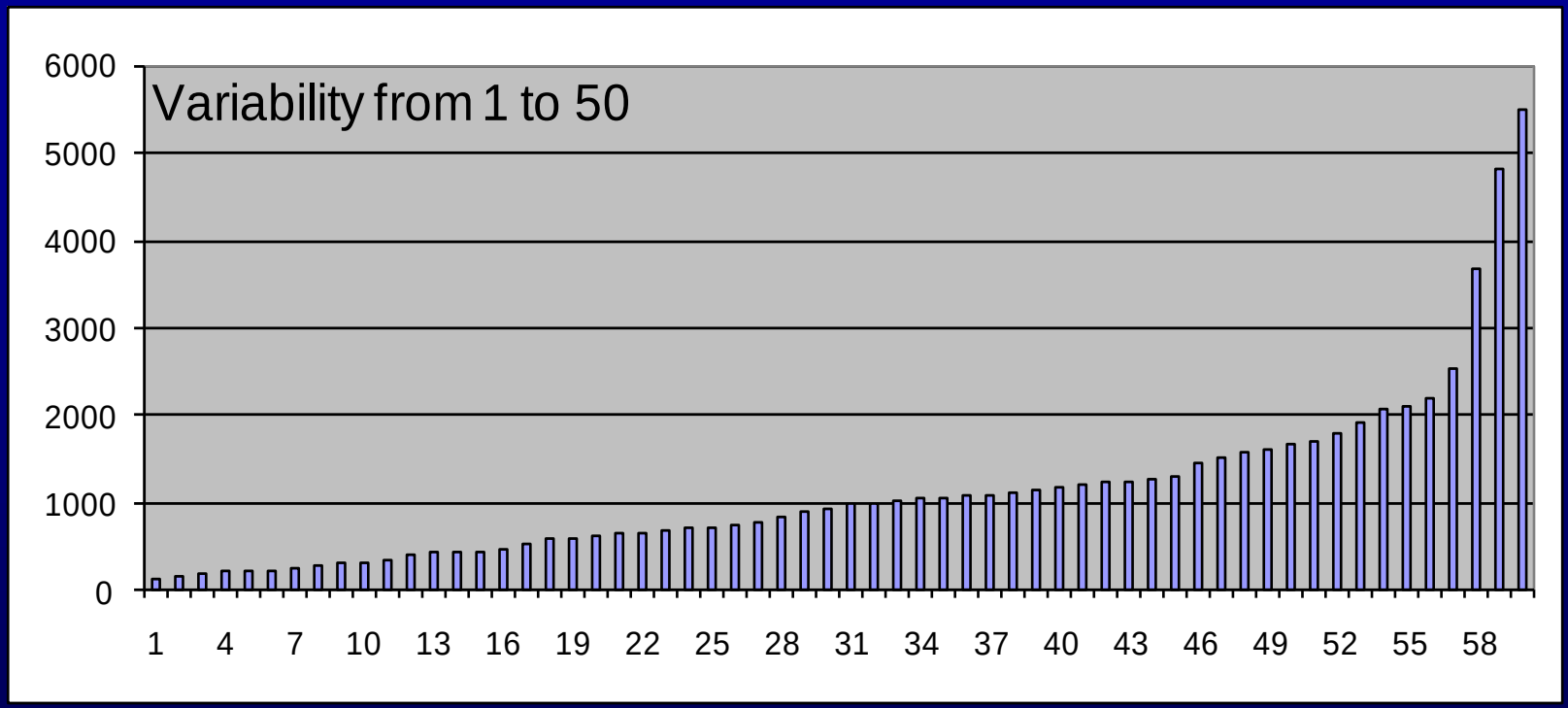
Distribution of acrylamide level (µg/kg) in manufactured chips



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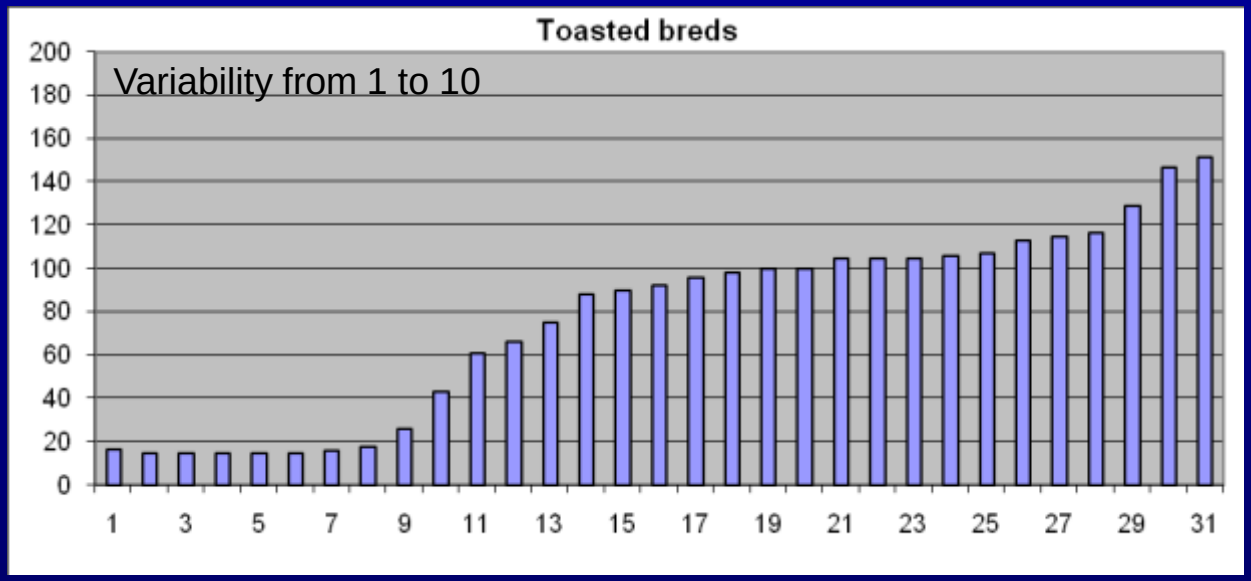
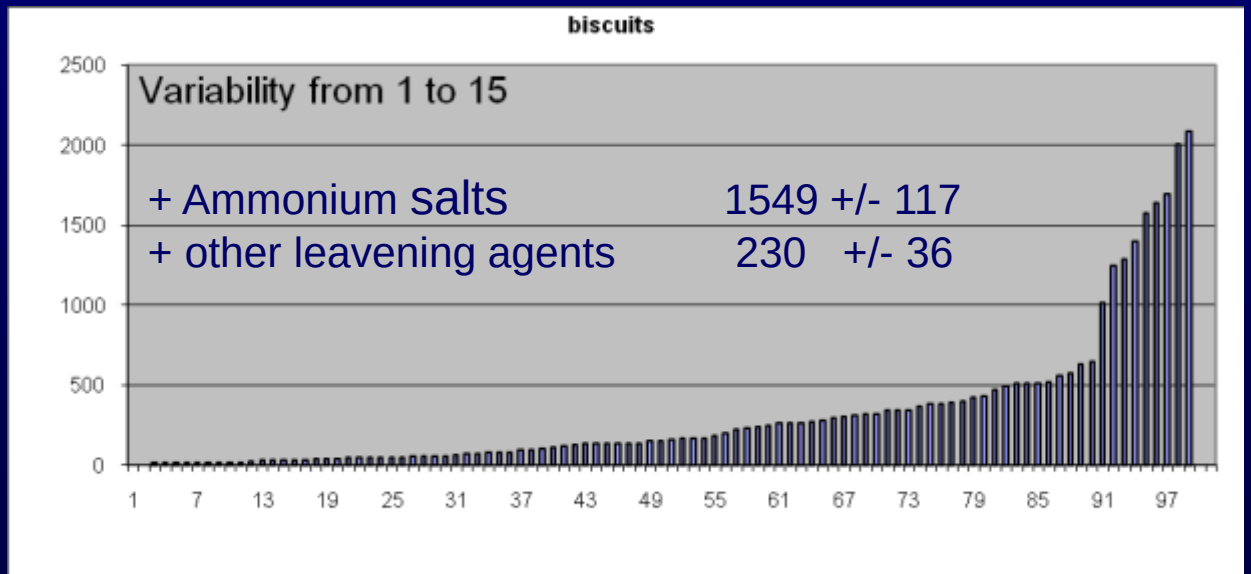
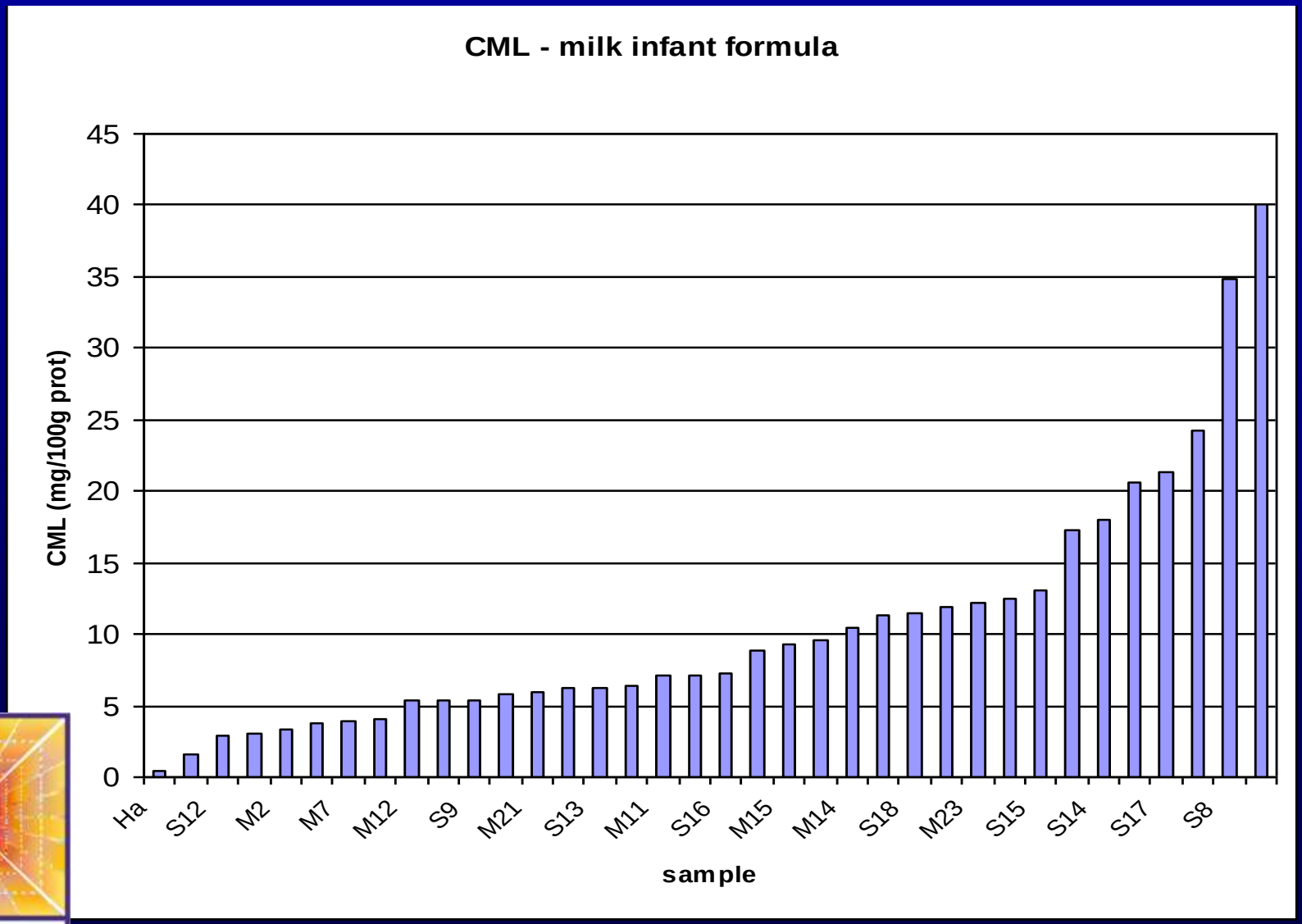
Distribution of acrylamide in cereals products (µg/kg)





A high variability in the level of Maillard products of foods

Carboxymethyllysine in infant formulas



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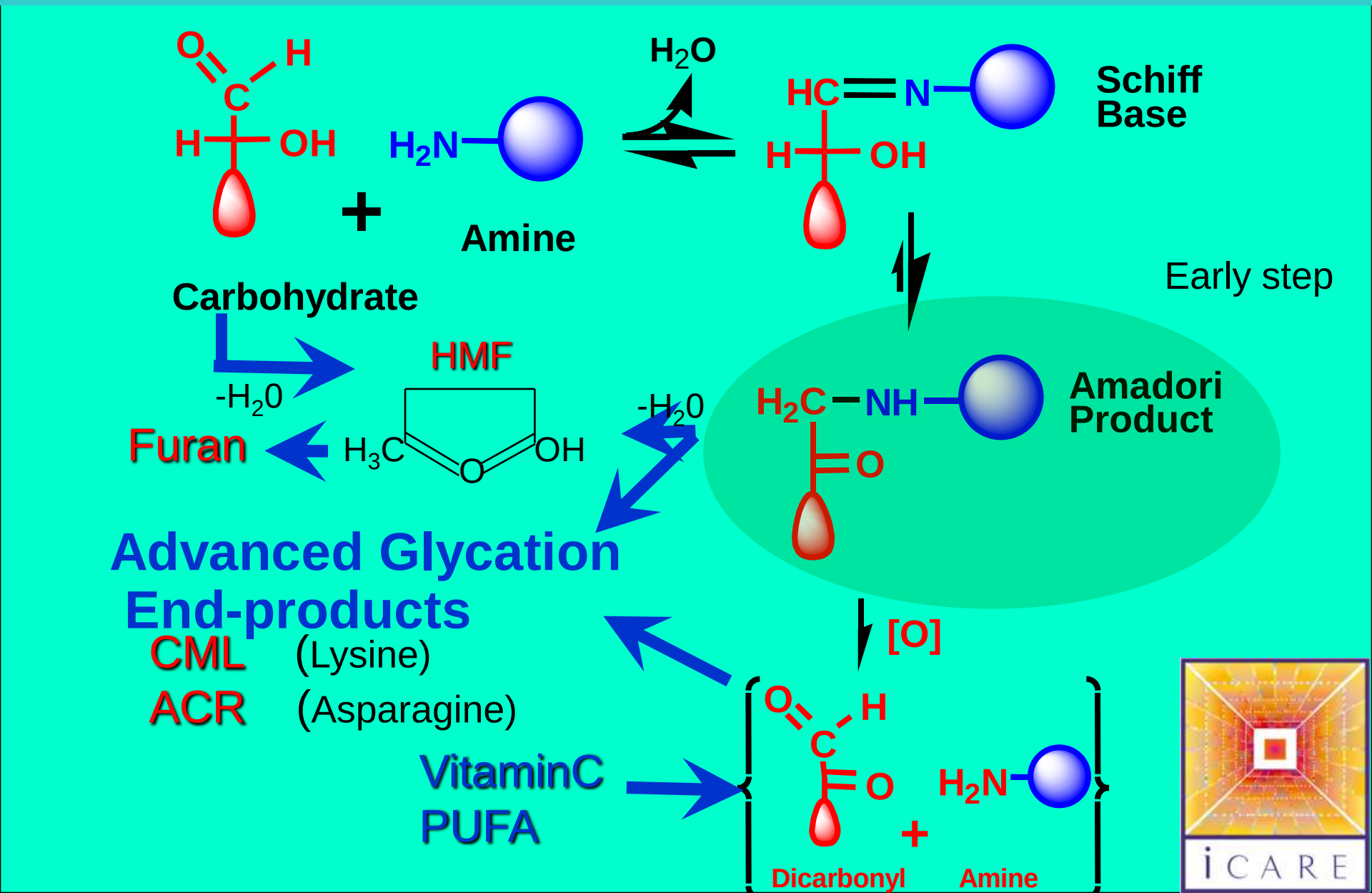
General background of the ICARE clinical study

Some arguments for a role of heat-treated foods

- Mutagenic and carcinogenic NFC in heat-treated foods
  Hydrocarbon polycyclic hydrocarbons; Heterocyclic amines; Nitrosamines;acrylamide;furane; cyclic monomers...
- Pro-inflammatory and cytotoxic Maillard products
  Advanced Maillard products; carboxymethyllysine, oxysterols
- **Increased consumption of severely heat-treated foods**
  Extrudated, oven baked and/or fried cereal/potatoe products especially consumed by children and young adults
- **Complexification of the food formulas**
  Fortification with labile nutrients, source of NFC: infant formulas

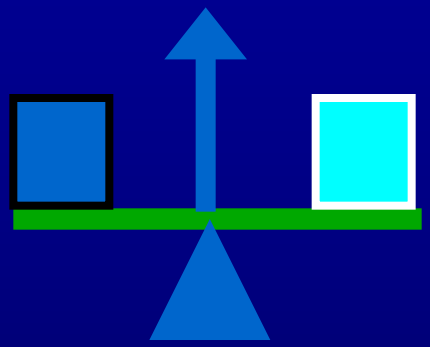
The Maillard reaction in food also gives rise to undesirable compounds



The clinical trial on adults was performed in LaSalle Beauvais
In 2006-2007

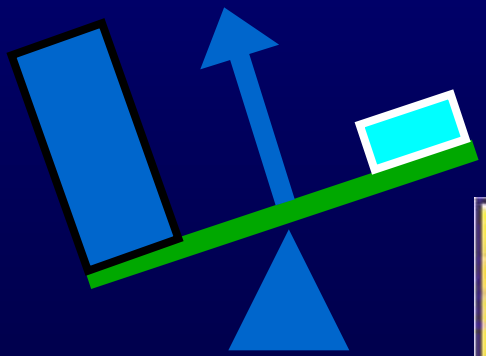
Responsible person for logistic aspects : Philippe POUILLART (LaSalle Beauvais)
Scientific coordinator : Inès BIRLOUEZ-ARAGON (AgroParisTech)

Comparing two similar, nutritionally equilibrated diets
One based on minimal cooking (steam),
the other using conventional cooking techniques (grilling, frying…)



Similar content in

calories
proteins
carbohydrates
fats
vitamins



But differing by 3 to 5-fold
in NFC content

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Methodology : Producing similar diets with two MRPs levels

Food → *Heat treatment* → *Standard Steam-cooked* **+/- Water loss** **+/- Nutrient Degradation** → *+ proteins and amines* → **+/- Maillard reaction Pts**

Need for Corrections :

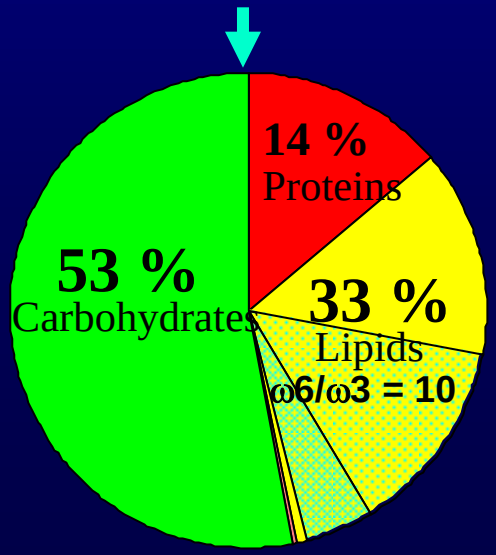
Compensating the **higher caloric density** of the STD diet by a **lower amount of food** proposed

Replacing **cooking fat** by seasoning **raw fat** in STEAM diet

Increase the **amount of cooked vegetables** to compensate for the **vitamin loss** in the STD diet

Decreasing the level of toxic MPs by a *steam-cooked diet*

- **HMF OH-methylfurfural (550µg) : 46**
- **CML carboxymethyllysine (6 mg) : 2.6**
- **Acrylamide (30 µg) : 5.4**
- **Fluorescent MRPs : 1.8**
- **Trans FA (1.5 g) : 1.6**

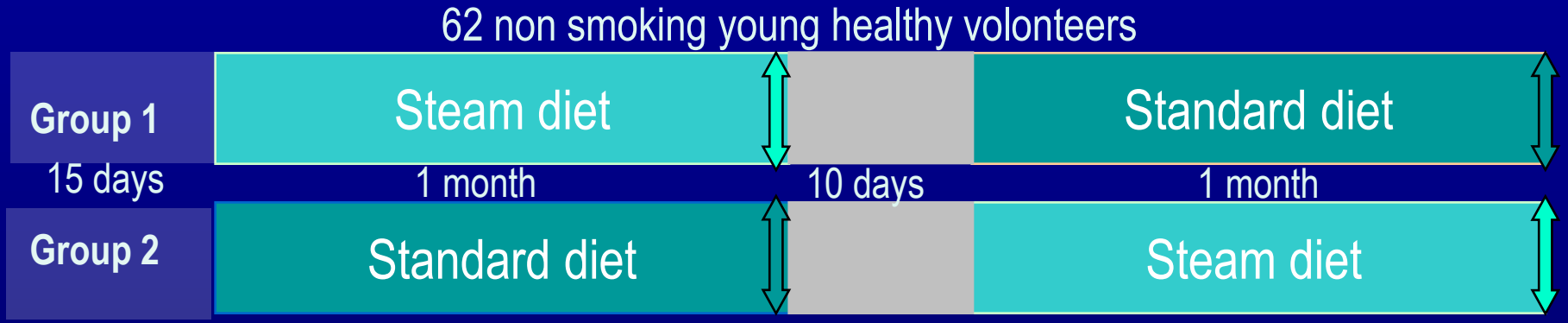


Not significantly different nutritional composition Between the two diets

Energy equilibrated diet

French RDAs reached

Methodology : A Cross-over design



Method

Statistical evaluation of the specific diet impact by only comparing the data at the end of the controlled diets and correcting for the sequence effect and the Visit (time) effect

Group 1 and group 2 on Std diet versus Group 2 and group 1 on steam diet

Impact of lowering MRPs level in foods on plasma antioxidants

Parameter	Steam diet	Standard diet		
Plasma VitC (µM)	69.6 ± 1.73	65.3 ± 1.73	+ 7%	p = 0.015
Plasma VitE (µM/mgCL)	3.25 ± 0.05	3.05 ± 0.05	+ 8%	p < 0.0001
CoQ10 total (nM)	710 ± 28	773 ± 28	- 8%	p<0.025
CoQ10H2 (nM)	616 ± 25	685 ± 25	-10%	p<0.025

Effect of sparing the hepatic CoQ10 synthesis ?
Resulting in no change in lipid peroxidation level
(no change in plasma MDA or global antioxidant power and urine isoprostane)

Metabolic impact of lowering MRPs in food

Steam diet	standard diet		
• Fasting Insulinemia mU/l			
6.52 ± 0.4	7.63 ± 0.4	- 15%	p=0.0099
• HOMA (µM*mUI)			
1.12 ± 0.06	1.35 ± 0.06	- 17%	p=0.0022
• Plasma Cholesterol HDL, mmol/l			
1.40 ± 0.04	1.56 ± 0.04	- 10%	p<0.0001
• Plasma total Cholesterol, mmol/l			
3.83 ± 0.08	4.05 ± 0.08	- 5%	p=0.0005
• Plasma triglycerides, mmol/l			
0.72 ± 0.04	0.79 ± 0.04	- 9%	p=0.0096

Impact of lowering MRP level in foods on plasma fatty acid profile

Parameter	Steam diet	Standard diet		
Total w3 FA (%)	4.39 ± 0,09	3.90 ± 0,09	+ 13%	p = 0.0001
C18:3 (n-3) (%)	0.39 ± 0.01	0,38 ± 0.01		p < 0.301
C20:5 (n-3)(%)	0.67 ± 0.03	0,54 ± 0.03	+ 24%	p< 0.0001
C22:6 (n-3)(%)	2.80 ± 0.07	2.52 ± 0.07	+ 11%	p< 0.0001
C18:2 (n-6) (%)	23.5 ± 0.3	24.7 ± 0.3	- 5%	p< 0.0003
C20:4 (n-6) (%)	7.11 ± 0.13	6.62 ± 0.13	+ 8%	p< 0.0003

BUT no impact on inflammation markers

Cooking derived MP : risk factors for accelerating ageing

	Steam diet	Std diet	Young CI	Aged CI
Insulin (UI/L)	6.5 ± 0.4	7.6 ± 0.4	6.1[a]	12[b]
HOMA index	1.12 ± 0.06	1.35 ± 0.06	1	1.01[c]
VitE/tot Lip(nM/mg)	7.47 ± 0.14	7.01 ± 0.14	10.3 ± 4.8	7.78 ± 2.0
Tot CL (mg/dL)	153 ± 3	162 ± 3	170 ± 30	190 ± 22
TG (mg/dL)	62 ± 3	68 ± 3	75 ± 34	99 ± 38
CoQ10H2	0.62 ± 0.03	0.69 ± 0.03	0.66 ± 0.41	0.77 ± 0.35

From Clin. Chim. Acta, 2004;344:173-179

a- Ann. Biol. Clin. 39 (1), 2001

b- Arterioscl ThrombosisVasc Biol. 1995;15:2213-2221

c- Wisniacki et al. Heart.2005; 91: 32-37

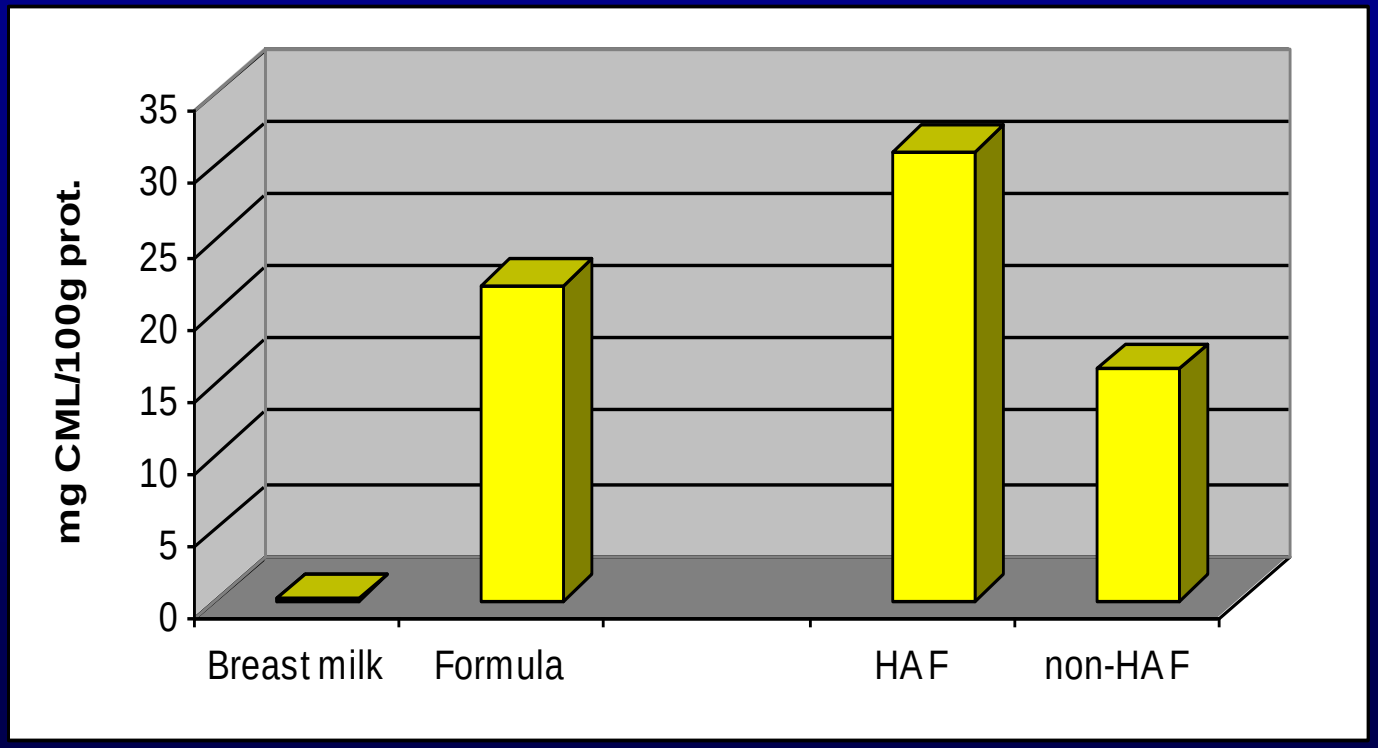
The clinical trial on infants was performed in Bratislava medical University In 2006-2008

coordinator : Katka Sebekova

Characteristics of investigated toddlers

	Breast fed (n=55)	Formula fed (n=47)
Gestational age (weeks)	38.4±2.9	37.5±2.9
Age at investigation (months)	6.0±1.0	5.7±1.1
Weight at birth (kg)	3.4±0.7	2.8±0.6**
Actual weight (6 m) (kg)	7.6±1.0	6.8±0.9**
Daily weight gain (g)	23.3±4.4	23.1±4.7

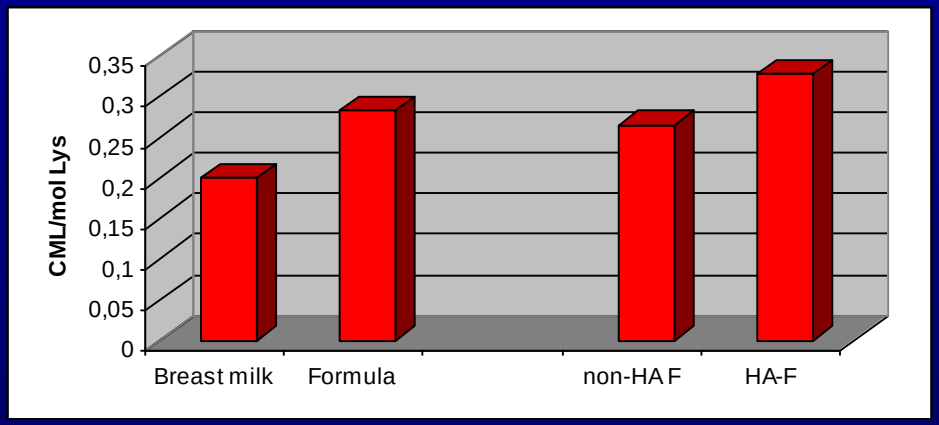
Only trace amounts of MRPs in human milk
Strong impact of protein hydrolysis



Protein hydroysis strongly enhances the formation of CML

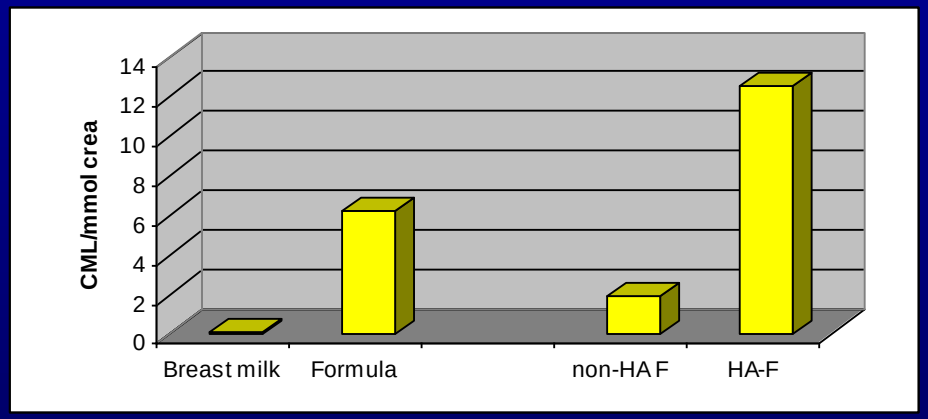
CML levels in 6 months infants plasma and urine

Plasma



CML is present in plasma of breast-fed infants

Urine



CML is not present in urines of breast-fed infants

Similarly to what was observed in adults,

formulas MRPs are absorbed into circulation,

and rapidly excreted into urine

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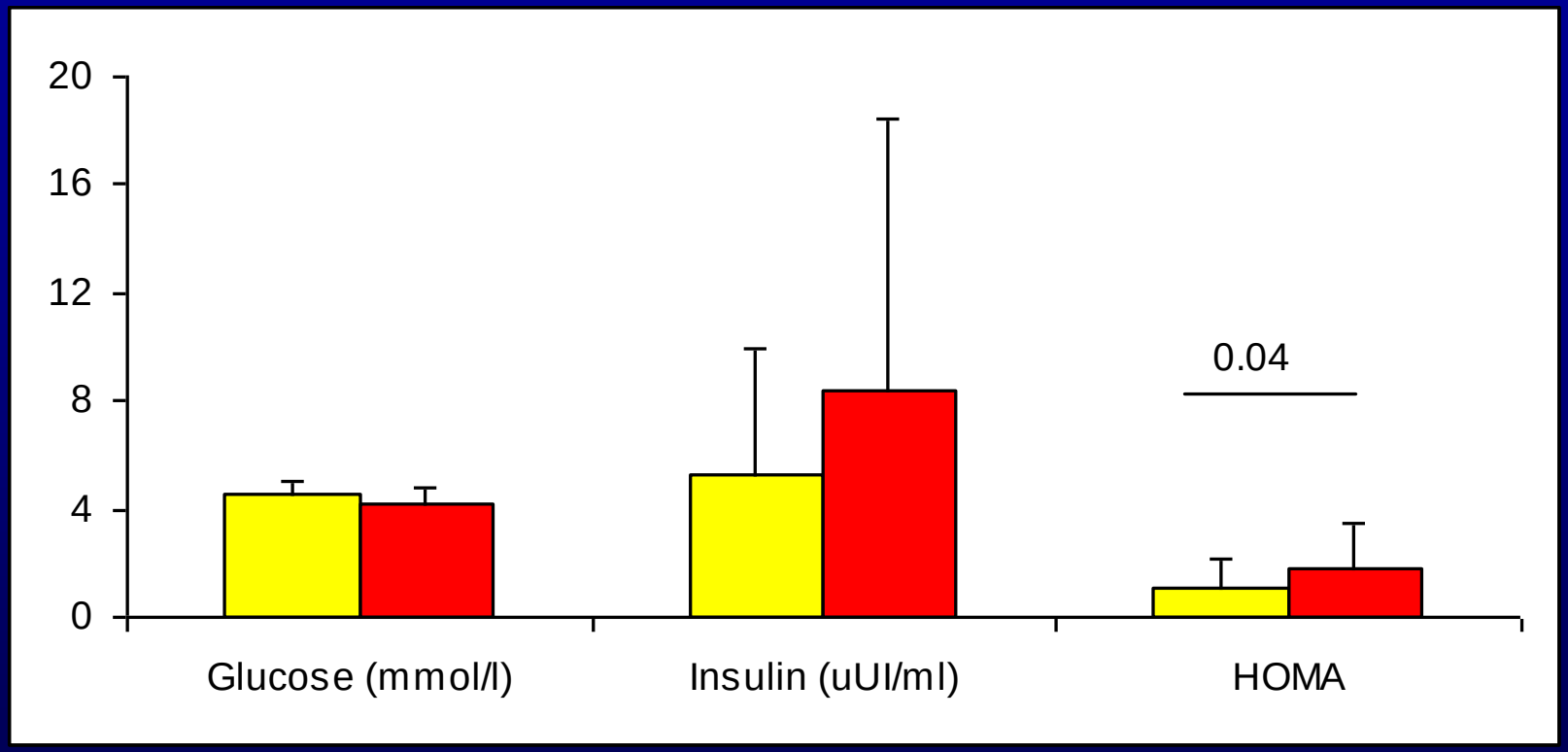
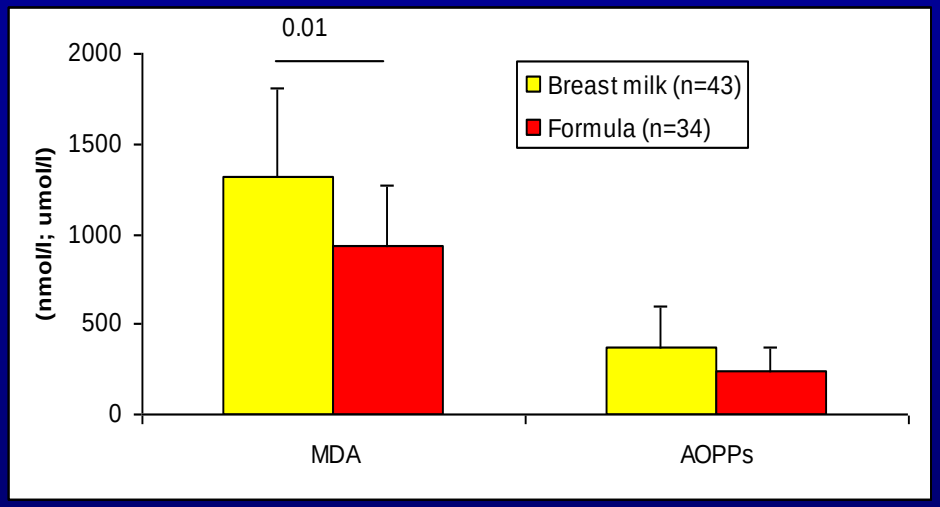
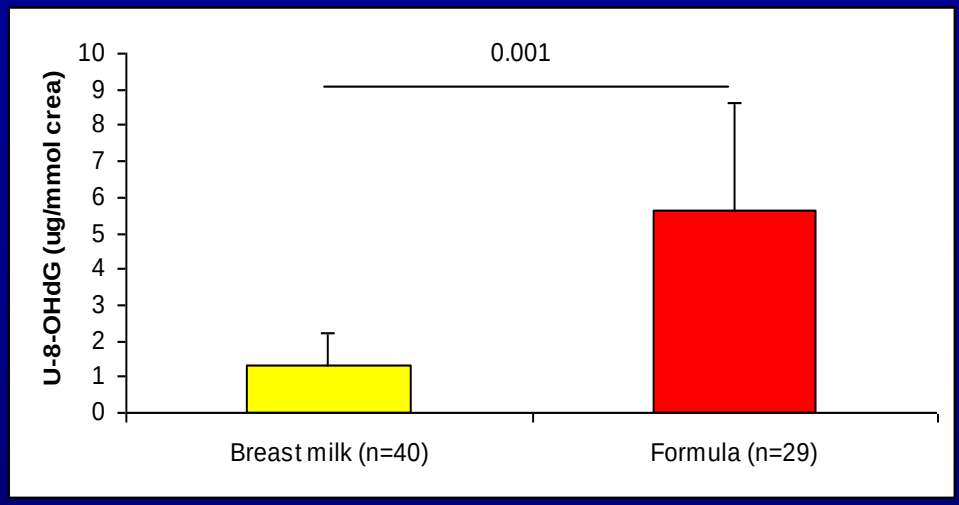
Glucose metabolism

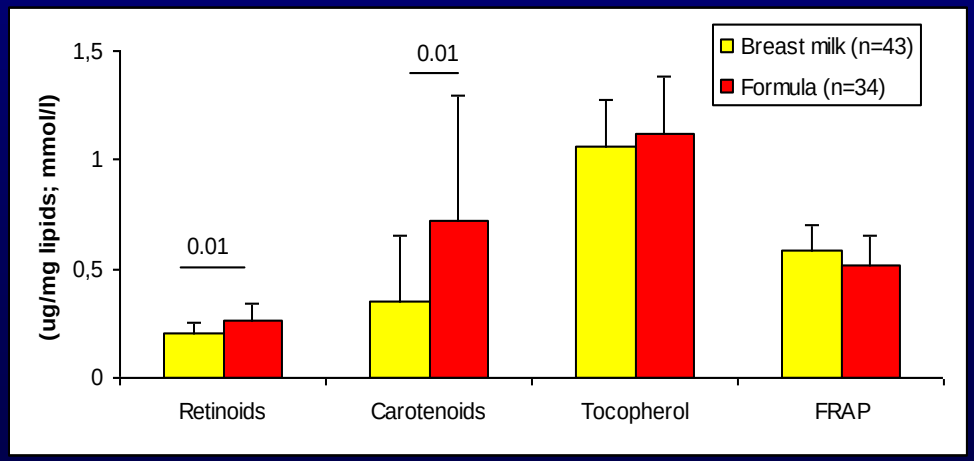


- Breast-fed infants are more insulin-sensitive

Oxidative damage to lipids, proteins, DNA







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Understanding the effect of formulation and processing steps on NFC formation at laboratory and pilot scale

Objectives

3 different food products were studied: ***infant formulas, cereal products including bread crisps, biscuits and roasted malt, and potato crisps*.**

For these different food products, the objectives were

- To define and develop **simple food models**

- To analyze the **influence of the heat treatment**, the type of technology and the ingredients used on NFC formation for each food model

- To **model the effect of process using kinetic data** and compare the efficiency of alternative technologies to conventional ones regarding NFC formation

Infant formulas

Partners : AgroParisTech, Laiterie Montaigu, Laiterie UCLV, Emmepiemme

A new microfiltrated formula was developed which was demonstrated to be stable for 3 weeks at 4°C and to contain up to 50 times less *CML* than the mean commercial liquid formulas.

VitaminC levels were also found to be stable, although 25% was lost during homogeneisation.

Infant formulas

Regarding powdered infant formulas, which represent 80% of the commercial IF, the following steps were evidenced as critical for the final quality of the product regarding furosine and CML

- **Ingredient quality** : milk powder or whey protein isolate
  Levels of furosine varying by a factor 5
- **The pasteurization process** before concentration
  depending on the temperature-time applied, 10-25% furosine and CML formed
- **The dry spraying process** :
  Most furosine and 50% CML formed during this last step.

In addition, powder storage was evidenced to increase significantly the furosine and CML content of the product between 3 and 6 months at ambient temperature.

Bred crisps

Partners : UNINA, CSIC, Bageta,

Bread crisps manufacturing

•The main ingredients are flour, water, salt, yeast whereas additional ingredients such as starch, sugars, fats can be used as well.

•Mixing of ingredients and kneading, baking of dough, cutting of bread in slices or pieces, toasting, cooling and packaging.

•The activities carried out within the project allow us to point out some suggestions in order to lower NFC concentration as much as possible. They can be summarized as follows:

Suggestions and recommendations

Free asparagine content of flour is the main factor affecting acrylamide formation whereas free amino acids content can affect HMF formation in bread crisps. Since free amino acids occur mainly in the outer layer of the grain, **wholemeal flours** are proner to form acrylamide and HMF than more refined ones.

The concentration of **sodium chloride** does not significantly affect NFC content. Acrylamide content is slightly lower when sodium chloride concentration increases. However, the amount of NaCL necessary to get a significant decreasing is too high to be used in practice

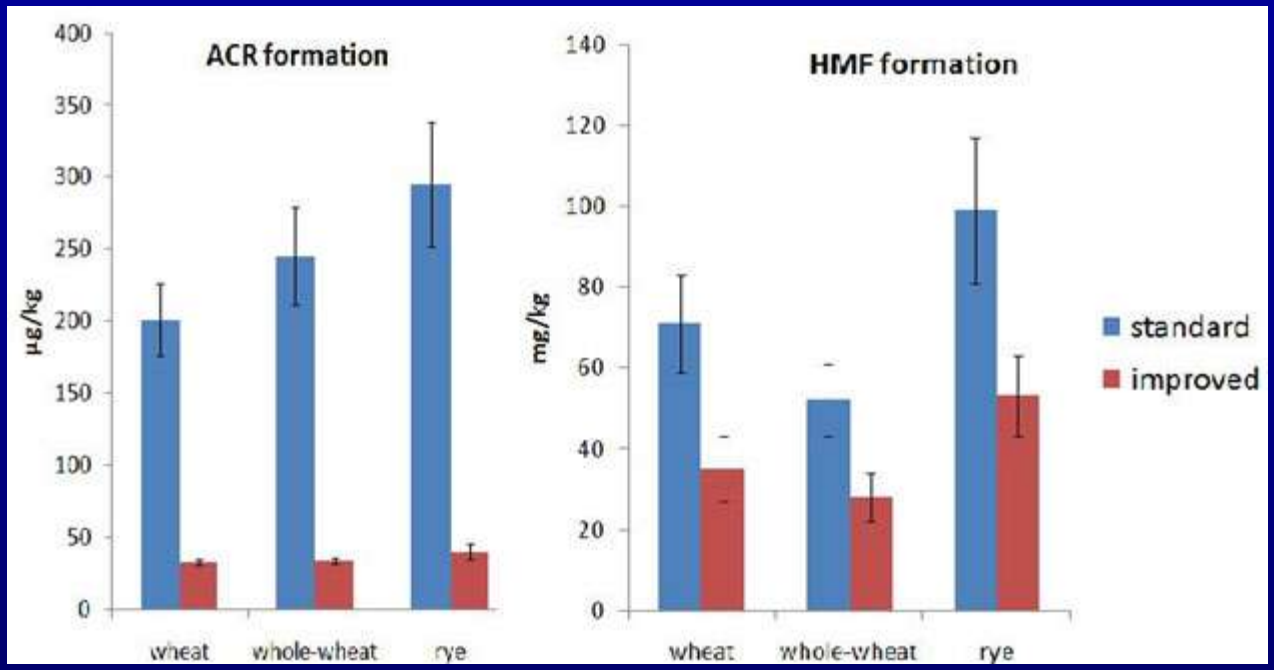
Malt extract and **malted flours** slightly increased HMF formation because of the reducing sugars provided by those ingredients. The effect on acrylamide is negligible because in bread crisps free asparagine is the limiting factor affecting the final content of this compound whereas sugars concentration has no effect. An adequate amount of those additives should be used only weather no alternative tools to improve browning development can be used.

Asparaginase addition proved to be the most effective tool to keep acrylamide levels under control. Asparaginase is an enzyme able to hydrolise free asparagine to aspartic acid and ammonia. Addition of moderate amount of asparaginase (1000 U/kg of flour) reduces acrylamide formation by up to 50%, whereas an amount of 2000 U/kg of flour reduces formation by up to 75%. Higher asparaginase concentration results in a further but limited decreasing in acrylamide formation. Due to relatively high cost of the enzyme, the addition of moderate amount of asparaginase (1000-2000U/kg of flour) should be recommended.

Suggestions and recommandations

During **leavening** of dough, yeast consumes free asparagine for their metabolism. This reduces acrylamide content when a prolonged leavening step is applied

introduction of a **drying step** prior to toasting at high temperature resulted in a reduction in both acrylamide and HMF content . 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. 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.



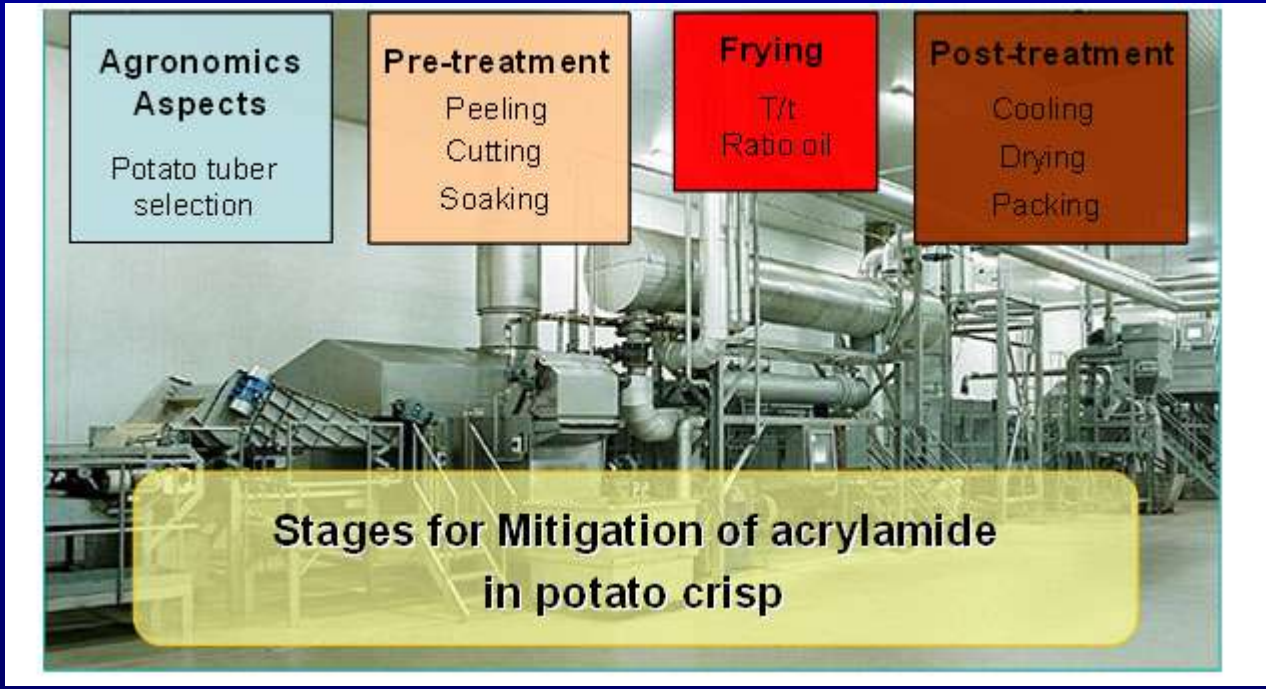
- Red bars represent the NFC content in bread crisps when the proposed strategies are implemented.
- The mitigating effect varies according to NFC and formulation but effectiveness is clearly demonstrated.

Potato food model

Partners: CSIC-IF, ITERG, APERITIVOS MEDINA, HUILERIE CAUVIN, CHIPS LEBON, FIAB

Different steps has been identified for quality inspection:

- ***raw materials*** (storage conditions and levels of reducing sugars)
- ***pre-treatment*** (soaking)
- ***frying conditions*** (type of oil, frying temperature and time, ratio oil/potato)



The different stages for potential mitigation

Some conclusions at industrial level:

❖**Quality of the raw material** is 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.

❖**Excessive soaking time** will not reflect a net reduction of acrylamide in the potato crisp and there is a risk of softness apart of loss of micronutrients.

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.

Effects of formulation and baking conditions on neo-formed contaminants in model cookies

The objective of this work was to analyse the influence of the ingredients and of heat treatment, on NFC formation in cookies type 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)

Main significant results in model cookies

❖ NFC production is dependent on ***thermal input***, either ***baking temperature*** for HMF and ACR or ***baking time*** for CML and FUR,

❖***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,

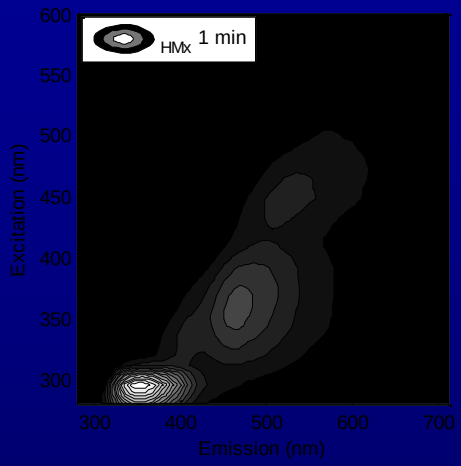
❖***The use of a sugar alcohol*** allows limiting HMF production. Surprisingly, HMF appeared to be affected by the presence of ammonium carbonate whereas no significant effect was obtained on ACR

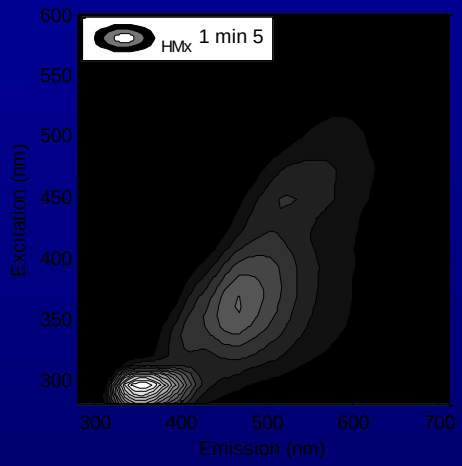
A new technology

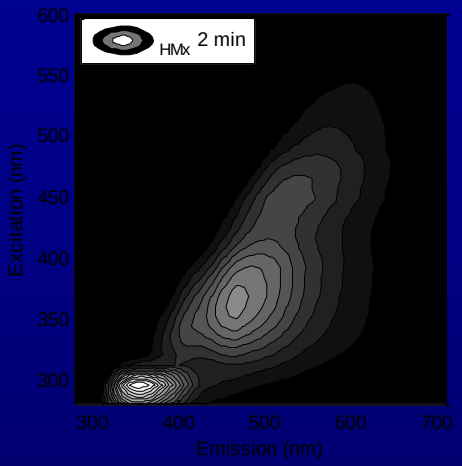
A sensor based on fluorescence analysis to measure in real time NFC composition in processed food

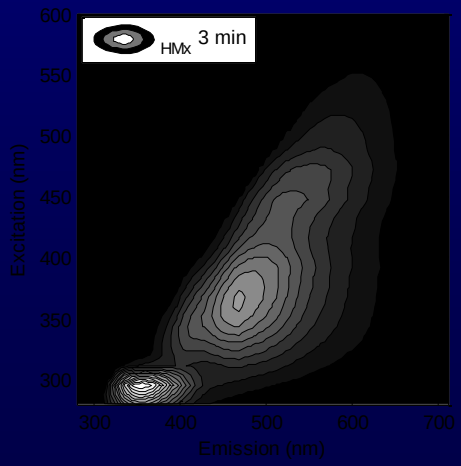
European project ICARE

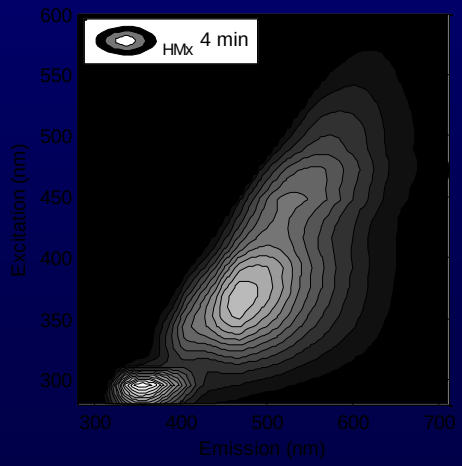
Fluorescence : a very sensitive technique of analysis to evaluate thermic treatment

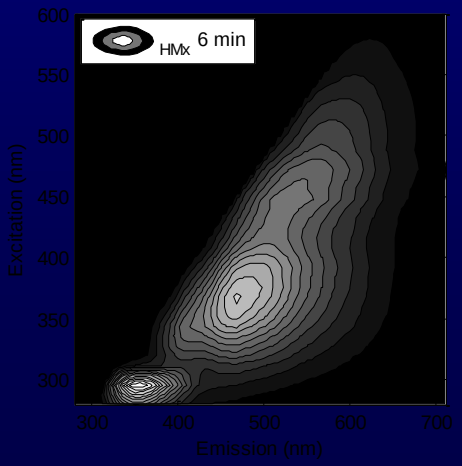












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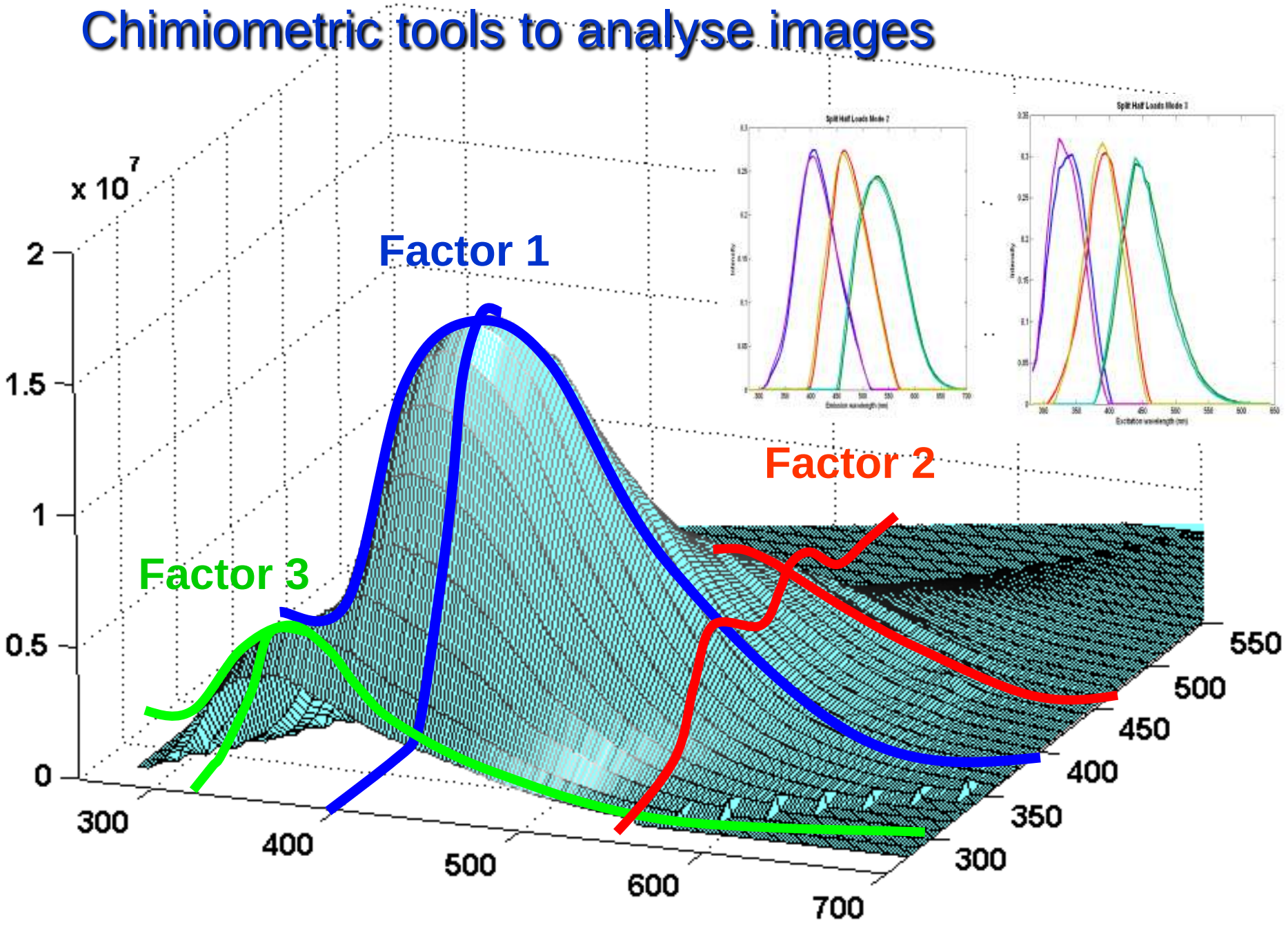
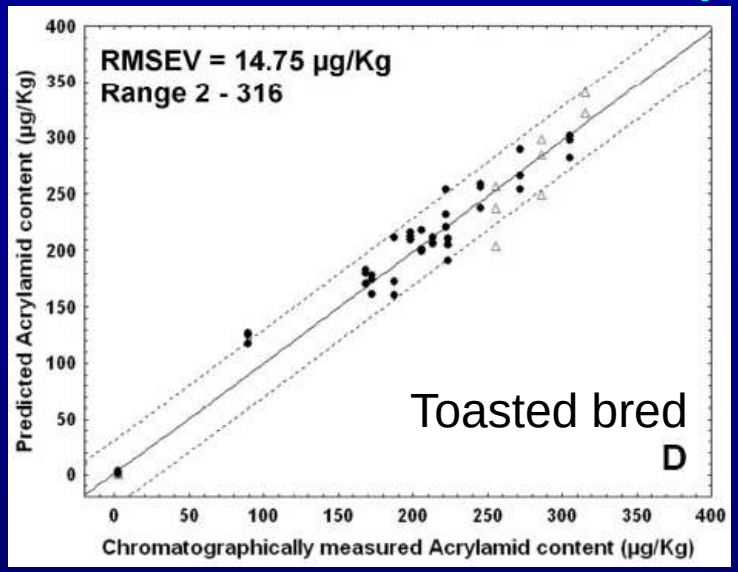
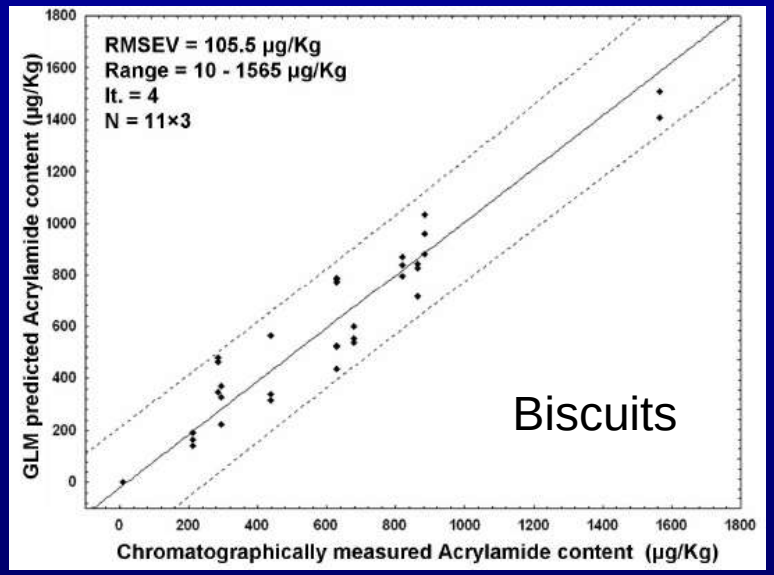
Toasted bred

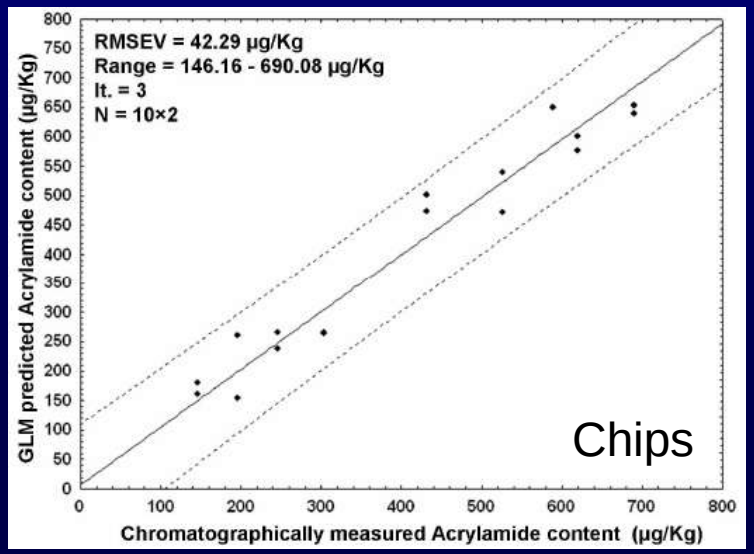
Chimiometric tools to analyse images



Predictive models for prediction of NFC during process:
example of acrylamide







ICARE Results

Spectralys Innovation

Activity

Impact of processing food on health

www.spectralys.fr
contact@spectralys.fr

Studies, diagnostic and advice

To improve quality of food products

Physicochimical analysis

Identify critical steps of processing

Evaluate performance of new technologies

GEA

SEB

Bonduelle

Quick

Main conclusions (1)

- **Clinical studies performed with adults and toddlers demonstrate that some inflammatory markers are more present in a context of diet rich in NFCs**
- **Use of alternative technologies like homic heating and micro- waves – alone or associated with traditional technologies allows reduction of NFCs (biscuits, French fries, infant formulas…)**

Main conclusions (2)

- **Raw materials (potatoes varieties), formulation (leavening agents for biscuits, citric acid…) have a significant effect on NFCs formation.**
- **A prototype based on fluorescence measurement has been set up for industrial use to measure on line NFCs production in a qualitative and quantitative way**
- **Creation of the spin off “SPECTRALYS”**

Programmes for the future

KBBE-2010-2-4-02: Identification of the effect of processing on food contaminants

Thank you for your attention !