The health and technological implications of a better control of neoformed contaminants by the food industry

Les enjeux santé et technologiques d'une meilleure maîtrise des contaminants néoformés par les industriels du secteur alimentaire

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ABSTRACT

The recent discovery of the presence of variable amounts of the carcinogenic compound acrylamide in a wide range of severely heat-treated food products, such as fried potatoes, biscuits, bread and coffee or malt, as a result of the heat process, has induced an important research in the area of the Maillard reaction in food. The interaction between a specific food composition and the heat process applied results in the development of complex oxidation and glycation reactions, which give rise to a mixture of flavoured compounds and possible neoformed contaminants (NFC). Recommendations by the European Commission aim at monitoring the content of major NFC, such as acrylamide and furan, in a list of food products commercialized in Europe. On the other hand, the Commission for European Normalization (CEN) has created recently a new workgroup (WG13) responsible for normalization of analytical method for NFC assessment. The European collective research ICARE was carried out to identify the possible health consequences of the ingestion of heat-treated products, characterize the reaction kinetics leading to NFC and evaluate some mitigation procedures proposed by the CIAA toolbox, and finally develop a simple, rapid and non destructive control method based on fluorescence acquisition on the crushed food products and chemometric analysis of the spectral information. This paper summarizes the objectives and essential results obtained in the scope of the project, highlighting the need for evaluating the distribution of NFC in food products commercialized in Europe, as well as the impact of the food formula/recipe and process on Maillard derived NFC food levels. The potential of the Fluoralys sensor regarding its ability to control food contamination with NFC is presented. A decrease in NFC concentration of heat processed food should allow significantly limiting the exposure of populations to NFC and consequently the potential related health risk.

RéSUMÉ

La découverte récente de la présence de concentrations variables du composé cancérogène acrylamide dans une large gamme de produits alimentaires soumis à de forts traitements thermiques, tels que les pommes de terre frites, les biscuits, le pain et le café ou le malt, a favorisé une recherche importante dans le domaine de la réaction de Maillard dans les aliments. L’interaction entre la composition spécifique d’un produit alimentaire et le traitement thermique qu’il subit induit le développement de réactions d’oxydation et de glycation qui aboutissent à la formation de molécules aromatiques mais aussi de contaminants néoformés (CNF). La Commission européenne de normalisation (CEN) a créé récemment un groupe de travail (WG13) pour normaliser les méthodes d’analyse des CNF. Le projet de recherche collective ICARE a été construit pour identifier les répercussions sur la santé de l’ingestion de produits soumis à de forts traitements thermiques, caractériser les cinétiques de réactions conduisant à la formation des CNF et évaluer l’intérêt de quelques

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1. Introduction

Neofomed contaminants (NFC) are supposed to be essentially formed at high temperatures during the Maillard reaction (MR) from reactive amino groups of proteins or free amino acids and carbonylated derivatives of sugars [1]. In addition, a wide range of food ingredients (lipids and vitamin C) may generate upon heating, especially in the presence of iron, carbonyl compounds which are highly reactive in the MR [2–4] and produce namely the carcinogenic compound furan [5]. In the case of lipids, besides the aldehydic compounds entering the MR, a number of thermo-xidized products with possible adverse effects are formed during frying. Amino acids can also be degraded via the Strecker reaction in particular conditions (little water content and basic pH), and further react with Maillard products (MP) and creatinine in meat/fish products to form heterocyclic amines (HA) [6]. or with carbonyl compounds to form acrylamide (ACR) when the amino acid is asparagine [7]. The high level of free asparagine in potatoes, cereal grains and coffee, associated to the severe heat treatment applied during frying, cooking and roasting explains why such processed food products are the most concentrated in ACR.

In milk and cereal products where reducing sugars are present in high concentration (lactose in milk) or produced during the heat treatment (heat-derived hydrolysis of added sugars and starch in cereal products), a wide range of MP are formed during sterilization and baking respectively. Among those MP, lactulosyl- or fructosyl-lysine, pyrraline and carboxymethyllysine (CML) revealed to be the most concentrated [8,9]. In addition in severely heat-treated starch and sugar rich food products with low moisture, hydroxymethylfurfural (HMF) is formed in an exponential way [10].

The effects of any technological operation at high temperature cumulate with that of storage to induce NFC formation in the ready-to-eat foods. The observed variations in NFC levels within different food products as well as different brands for a given food result from the amount of precursors (free asparagine, ammonium salts, unsaturated fatty acids or reducing sugars), and variations in processing conditions.

In general, the NFC amount increases with increasing temperature and time during industrial processing or home cooking. For instance, levels of ACR in fried potato products ranged from the limit of quantification (20–50 µg/kg) up to 15,000 µg/kg [11] depending on the frying temperature. Regarding infant formulas, the heat sterilisation technology strongly influences the level of lactulosyllysine, HMF and CML in milk and infant formulas [12].

These various MR derived undesirable compounds have not been precisely evaluated regarding their potential health effects. However, epidemiological studies have identified possible relations between some health parameters and the ingestion of foods processed at high temperatures, in particular an association between the consumption of meat rich in mutagenic HA and cancer [13]. More recently, the presence of ACR, classified as probably carcinogenic (EC, 2002), was detected in numerous foods cooked at high temperatures [11]. Besides some antimutagenic and antioxidant activity evidenced in vitro for certain MP of melanoidin type [14], a global increase in oxidative stress and inflammation was evidenced in diabetic patients ingesting high versus low NFC-containing diets [15]. More recently, the levels of circulating and urinary MP were correlated with the content of NFC ingested with foods in healthy elderly subjects [16]. The high plasma and urinary CML evidenced in infants and toddlers fed infant formula with high CML levels compared to mother milk [17] confirms the absorption and excretion of ingested MP.

Despite an active urinary excretion [18], some absorbed MP could accumulate in some tissues as already evidenced in animals [19] and exert biological effects through the RAGE receptor [20]. Urribarri et al. [16] found significantly higher insulin and CRP levels in subjects exposed to higher dietary NFC levels, indicating a possible role in insulin resistance and inflammation in healthy volunteers. Lipid degradation products, formed during frying and grilling, have been associated to increased risk of cardiovascular disease [21] and hypertension [22].

Evaluating the health effects of dietary MP is a particularly difficult issue because of the lack of knowledge on their chemistry, bioavailability and toxicity [23]. Two clinical studies were performed within the ICARE project whose objective was to identify the global health impact of severe heat treatment as compared to mild or absence of heat treatment. Although this approach does not allow characterizing the molecules and doses at which biological changes may occur, an objective view of the global effect of any MP ingested from the diet due to the heat treatment, as well as their interaction, is obtained. The main effects observed in young healthy subjects by comparing two one-month diets similar in macro- and micronutrients but differing by two to five fold in the content of NFC [24] were the evidence of a significant decrease in the plasma oxidative stress, as well as insulin, triglyceride and cholesterol levels after the diet low in MP compared to the high MP diet, while long chain omega3 fatty acids were significantly higher (not published). ACR exposure in the diet with high MP levels was similar to the mean exposure determined in the European population (0.8 µg/kg per day and 1 µg/kg per day, respectively).

In this paper, we report the NFC distribution in a wide range of food products commercialized in Europe, and focus on the food ingredients which seem more prone to produce NFC in biscuits and infant formulas, two food products majorly contributing to the exposure of infant and children to NFC. Furthermore, we present the rapid and simple alternative method developed in the ICARE project to allow Public Authorities and the food industry to better control NFC in the food products.

2. Monitoring NFC concentration in foodstuffs: evidence of a great variability among European food products

Despite no reference methods have been selected yet for NFC assessment in food products, some of them have proved their...
reliability in proficiency tests. The ring tests organized by the European Confederation of Food Industry (CIAA) or within the European network COST 927 (health impact of heat-treated food) allowed selecting LC and GC-MS as the most specific, sensitive and reliable techniques for ACR, CML and furan analysis.

More than 200 analyses have been carried out in commercial samples suspected to contain high amounts of undesirable MP: bread crisps, biscuits, potato chips and infant formulas. Analytical methods were previously standardized and validated based on an internal ring test and a larger one developed in the frame of the COST 927.

ACR was quantified in potato chips, biscuits and bread crisps according to Rufian-Henares et al. [25]. CML was quantified in infant formulas and biscuits according to Charissou et al. [26] modified for quantification by tandem mass spectrometry instead of single ion monitoring. HMF was assessed in biscuits and bread crisps according to García-Villanova et al. [27].

Figs. 1 and 2 evidence the large variability (one to 100 times differences) of NFC among a range of food products. Variability was higher for ACR than for CML, probably because the temperature dependence is stronger for ACR than for CML, the latter being more influenced by the process time and sugar content of the product.

This strong variability evidences the need for a better control of the heat processes and the insufficient knowledge on the influence of some ingredients incorporated in the food products. Furthermore, no regulation has been proposed up to date regarding undesirable MP, except a recommendation for ACR and furan surveys in a list of food products (2007/196/CE and 2007/331/CE). Most existing recommendations regarding NFC concern lipid thermodegradation products, such as trans fatty acids whose concentration limit in food products is 2% of total fatty acids. The European Federation of Fats and Oils Industry (FEDIOL) recommends a maximum level of 25 ppb for total polycyclic aromatic hydrocarbons (PAH) which are mainly formed during seed drying before oil extraction, and less than 5 ppb of heavy PAH including less than 1 ppb of benzo(a)pyrene in vegetable oils and fats.

Although many countries are discussing about the need for national regulations on ACR, especially Germany, Norway and Sweden, no decision is taken presently. The only existing regulations concern drinking water where ACR is a decomposition product of poly-ACR added for treatment purposes.

3. Role of some ingredients and recipes in the formation of undesirable Maillard products in food?

Among the various reasons possibly explaining the high variability in NFC levels in European products, the formula or ingredients used in the recipes probably play an important role. A systematic analysis was carried out in the ICARE project trying to identify the sample parameters significantly influencing the NFC content.

Regarding powdered infant formulas, Table 1 indicates that, as expected and already evidenced in other studies [28], the lactose concentration is an important factor. Maltodextrins are often added to replace lactose in order to decrease the reducing power of the sugar. Actually, formulas with less than 100% lactose had lower furosine and CML levels than 100% lactose formulas. Furthermore, the protein profile strongly varies between formulas for digestibility reasons. We evidenced that the higher the whey-to-casein ratio above the normal level in cow’s milk (20%), the higher lactulosyllysine and CML content (Table 1). Finally, some formulas contain hydrolyzed proteins to circumvent allergies to cow’s milk proteins. These hydrolysates contain various sizes of peptides including free amino acids. A much higher lactulosyllysine and CML content was observed in hydrolyzed formulas confirming previous results [12]. This higher MR is very probably consecutive of a greater number of reactive lysine in the sample, liberated during hydrolysis.
Concerning biscuits, the main observation was the already known influence of ammonium-based leavening agents on ACR formation [29]. A concentration of 1549 ± 117 μg/kg ACR was evidenced in ammonium carbonate containing biscuits instead of 230 ± 36 μg/kg in the others. The reason for this observation is that ammonium salts is an active substrate of the MR and should accelerate asparagine decarboxylation prior to ACR formation.

Finally, in bread crisps, the important role played by the type of flour on ACR formation was confirmed: rye was much more reactive than wheat because of the higher asparagine content compared to wheat [30] (Fig. 3). Similarly, asparagine being more concentrated in the grains envelops, whole wheat bread formed more ACR than white bread [31]. Addition of asparaginase and glycine were confirmed to significantly decrease ACR formation in bread crisps thanks to asparagine degradation and competitive reactions respectively (Fig. 3).

4. Alternative technologies for food heat processing

On the technological point of view, it is necessary to better understand the impact of the various heating operations on the formation of MP, in order to favour the positive reactions giving rise to organoleptic compounds while minimizing the formation of NFC. Microwave and ohmic heating appear to be promising alternative technologies especially for milk heat treatment, despite their poor use in agro-food industry, mainly due to a lack of technological transfer. Both technologies allow a very rapid increase of the temperature in the core of the food. Some experiments were carried out to evaluate the potential of this alternative technology, with controversial results [32,33]. Ohmic heating is an emerging volumetric thermal technology allowing to homogeneously cook/sterilize without significant browning development [34,35]. However, some technical limits must be overcome such as adherence of food deposits to electrode surfaces, a

**Table 1**

CML content in infant formulas depending on the recipe.

<table>
<thead>
<tr>
<th>CML (mg/kgDM)</th>
<th>80% caseins</th>
<th>&lt; 80%&lt;sup&gt;a&lt;/sup&gt; caseins</th>
<th>Non hydrolysed</th>
<th>Hydrolysed</th>
<th>100% lactose</th>
<th>&lt; 100%&lt;sup&gt;b&lt;/sup&gt; lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>13.50</td>
<td>18.34</td>
<td>16.12</td>
<td>27.09</td>
<td>18.36</td>
<td>14.82</td>
</tr>
<tr>
<td>Std</td>
<td>8.89</td>
<td>12.25</td>
<td>10.89</td>
<td>23.08</td>
<td>8.88</td>
<td>11.83</td>
</tr>
</tbody>
</table>

<sup>a</sup> More than 20% whey is present due to enrichment with whey proteins.

<sup>b</sup> Less than 100% lactose is present due to replacement with maltodextrins.
phenomenon which can induce an electrical resistance leading to formation of some MP [36,37]. Compared to conventional UHT techniques, ohmic heating offers the great advantage of avoiding thermal gradient inside the product. The temperature is produced by the circulation of an electric current through the milk, and is proportional to the electric field and to the electrical conductivity of the product. Both heating and cooling can be achieved fast thanks to the very rapid transformation of electrical energy into heat energy in highly commonly conductive media. Fig. 4 shows the formation of CML in a typical infant formula during heating with a laboratory-scale static reactor of 100 mL capacity [38]. Different thermal treatments comprising successively heating, holding and cooling stages were applied to the formula and the formation of furosin and CML were analyzed by GC-MS [26]. I. Birlouez-Aragon et al. / Pathologie Biologie 58 (2010) 232–238

Regarding cookies and bread crisps, no alternative process could be proposed, but an optimization of the process currently used by the SMEs. A low baking temperature of 120 °C allowed limiting the formation of ACR and HMF in cookies, while a short baking time was more advantageous regarding furosin and CML formation (data not published). Concerning bread crisps, the best mitigation proposal was to add a drying step before toasting, as the toasting duration was consequently considerably decreased, resulting in minimized ACR and HMF formation (data not published).

5. An alternative analytical method to assess NFC: the Fluoralys sensor

The existing analytical methods currently used to analyze NFC content in foods are based on sophisticated, time-consuming techniques of high cost (HPLC- and GC-MS) which optimally need the use of expensive isotopic labelled external standards. HPLC coupled with tandem mass spectrometry and electrospray ionization has been applied for analysis of a wide range of NFC in foods. Such methods are consequently not adapted to a widespread use by SMEs, so that a great effort is now focused on the development of less expensive and easy analytical techniques.

Spectral methods are very simple analytical methods well adapted to industrial needs due to their rapidity, easy automation and low cost. Reflectance analysis was evidenced to be a rapid and accurate method to monitor non enzymatic browning in cereal products [39,40], and was highly correlated to the heat charge applied to the product during baking, so that this easy measure could be used to monitor efficiently the baking process.

Fluorescence spectroscopy is particularly sensitive and suitable to monitor trace amounts of contaminants [41]. It allows evidencing the heat degradation of naturally fluorescent nutrients in food products, such as vitamin E in vegetable oils [42,43] or tryptophan in infant formula models [44]. Moreover, as a wide range of neo-formed products are fluorescent, including advanced MP and thermoxidized lipids, fluorescence was shown to fit well with lysine degradation products [45] and with the polar fraction accumulating in oil during frying [42,43].

Therefore, the fluorescent fingerprint of a food product allows identifying the native and neoformed fluorophores whose concentration and front face fluorescence emission deeply change during the process [41]. In addition, multiway data analysis may advantageously be applied to the excitation-emission data obtained from fluorescence analysis of the food product to construct calibration models over NFC. Such models can be used to indirectly measure the concentration of process influenced molecules in the food. An example is given (Fig. 5A) on how the excitation-emission auto-fluorescence of roasted malt can be decomposed using PARAFAC, a 3D data analysis tool based on the extraction of excitation and emission profiles for all significant fluorescence present in the image and evolving linearly between samples. The intensity of the fluorescence profiles are then calculated for each malt sample obtained with different roasting parameters, and are further modelled over the NFC, ACR, furan and furfurals. The validated calibration curves obtained for a well chosen range of samples (Fig. 5B) are further used to predict the NFC concentration of new samples produced with similar roasting processes. Such an approach offers many advantages, such as the sensitivity, often higher than that of the reference method, the low cost destructivity (no sample preparation except crushing), the low cost formulas. To prolong further the shelf life and reinforce the safety of the product, an additional mild sterilization process is needed. Steam infusion or ohmic treatment could therefore be advantageously coupled to microfiltration.

Finally, microfiltration removes milk bacteria at 60 °C by retention on porous ceramic membranes, allowing the milk to be stable at 4 °C for more than three weeks. First assays were carried out a typical infant formula demonstrating the industrial feasibility of the technology and confirming its considerable efficiency regarding preservation of milk quality parameters. Furosin and CML concentrations as low as 10–20 and 0.8–1.2 mg/100 g proteins respectively were obtained in microfiltrated infant formulas. To prolong further the shelf life and reinforce the safety of the product, an additional mild sterilization process is needed. Steam infusion or ohmic treatment could therefore be advantageously coupled to microfiltration.

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and the possibility illustrated in Fig. 5 to simultaneously assess various NFC, such as ACR, furan, HMF and furfurals.

A sensor prototype, named Fluoralys, was constructed to reduce the size and cost of the equipment and to allow automation of the quantification thanks to implementation of a software executing the prediction models. Consequently, a very rapid analysis almost in real time is possible. A prediction error between 5 and 10% is obtained. The Fluoralys prototype is being validated at the industrial scale for at line assessment of ACR in biscuits as well as furosine and CML in infant formulas. The method is also useful for quantification of ACR in fried potatoes.

6. Conclusion

The ICARE project aimed at studying the influence of food processing on the MR, focusing on the undesirable compounds, also called NFC. Three types of food products were investigated, infant formulas, cereal products including bread crisps, biscuits and roasted malt, and potato crisps. The first result of this project is to evidence the wide discrepancy (up to 100 fold magnitude) among NFC concentration in specific European food products, revealing the lack of control of the heat process impact on food safety. Of course recipes and processes used for producing specific products may vary considerably between countries and partly explain, together with different food habits, the variable population exposure in different European regions. The mean margin of exposure of ACR was estimated between 75 and 300. By taking into account such variability in food ACR concentration, a wider margin of exposure is expected. However, the efforts by CIAA and the European Commission to inform food companies on this new issue were fruitful as a 30% mean reduction was observed in many food products since three to four years.
Moreover, a limiting point for the food industry is the cost and time needed to perform NFC assessment in their food products. By performing a large number of analysis in a short time, the new tool created with the Fluoralys sensor should help food companies and SMEs to better understand which process parameters and food ingredient may influence the formation of NFC, as a basis for improving the final food quality. This rapid and cost effective tool should therefore contribute to significantly decrease the global NFC level of European food products and therefore to lower the time needed to perform NFC assessment in their food products. By

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