MAILLARD REACTION PRODUCTS: OCCURRENCE, MITIGATION STRATEGIES AND THEIR PHYSIOLOGICAL RELEVANCE

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1 INTRODUCTION

After more than 100 years from the first paper describing the reaction between sugar and amino group leading to browning formation and later on indicated as Maillard Reaction is still at the very centre of the interest of scientists of different disciplines. Among food scientists Maillard Reaction (MR) is important because of colour and flavour formation in an enormous variety of processed foods. The quality of food, from the nutritional, microbial safety point of view and sensory aspects depend on a range of variables from farm to fork, including the processing techniques. The main purpose of industrial food processing is to provide safe and high quality food as demanded by the consumer. The conduction of thermal processing in an appropriate way is the key to obtain safe food and in many cases also with enhanced nutritional functionality respect to the starting raw material. Thermal processes are frequently used in food manufacturing to obtain safe products with a prolonged shelf-life and may have a strong impact on the final quality of foods.

Beside the beneficial effect thermal treatment also has some negative effects on food healthy properties: the major concern arising from heating processes come from the formation of hazardous compounds, such as heterocyclic ammines, nitrosamines and polycyclic aromatic hydrocarbons. Recently different food-borne toxicants have gained much interest because of their high toxicological potential and their wide occurrence in foods: acrylamide, carboxymethyl-lysine (CML) and hydroxymethylfurfural (HMF).

2 OBJECTIVES

Many consumers considered thermally treated foods as potentially harmful and after discovery of acrylamide formation in many heated, protein- and carbohydrate-rich foods this idea was further straightened.

In this framework, food companies need to be pro-active and many efforts were directed towards the reduction of acrylamide concentration in industrial products. As many strategies to reduce the acrylamide presence are possible the mitigation of its presence in different thermally processed foods is still sparking a growing body of interest worldwide.

Unfortunately many of the mitigation measure implied a modification also of the other MR products having often significant sensorial implications.

The objective of this research was to investigate the formation of Maillard reaction products and particularly acrylamide, carboxymethyl-lysine (CML) and hydroxymethyl furfural (HMF) in foods and model systems using various processing technology.

The food chain approach was widely used, identifying at the different step of the single food production chain some key elements that can influence the formation of these potentially harmful compounds.

On one hand the effect of formulation with the effect of antioxidants such as curcumin or the use of new ingredients such as soybean okara will be investigated, on the other the potentiality of new mild processing such as radiofrequency heating will be assessed.

Last but not least the use of encapsulation technology by reducing the amount of some key catalyzers of the Maillard reaction will be tested.

Moreover, as the fast and reliable detection of acrylamide still is a bottleneck for routine analysis a fast method using a High Resolution Mass Spectrometry will be also proposed.

The overall aim of the research is to address the current knowledge of compounds that may be formed during the cooking process that may affect health.

3 RESULTS

Okara is a protein and dietary fibre rich products obtained from soybean after extraction of the water-soluble component. It is considered a healthy ingredient and from the nutritional point of view this is true. However the data of the first paper (Palermo, *et al.,* 2012) demonstrated that the formation of MRPs can be enhanced by the use of ingredients containing their precursors. In particular acrylamide and CML are present at significantly higher concentration in soybean products than in the conventional ones in almost all commercial products. Results of this paper confirmed those previously obtained by our group using four rye flours with different extraction rates. Data of this paper clearly indicated that the amount of acrylamide formed during biscuits cooking is related to the amount of precursor (asparagine) present in the rye which is higher in not refined grain.

However this evidence about MRPs formation does not imply that the intake of not-refined cereal should be discouraged. It is plenty of evidence that a diet rich of whole cereal is beneficial for human health and all dietary recommendation suggest increasing their consumption. They advocate for the achievement of the right trade-off between appropriate formulation and processing technology.

In the second paper, developed in the framework of the EU project Prometheus, aimed at investigating the potentiality of encapsulation to limit the formation of potentially harmful Maillard Reaction products in different products (Fiore, *et al.*, 2012).

Data demonstrated that in a cookies model system the encapsulation of NaCl by three different hydrophobic coatings prevented HMF formation. The key point establishing the efficacy of the encapsulation was the thermal resistance of the wall material: the higher the melting point the more pronounced the reduction of HMF formation. Interestingly, this procedure does not modify the cookies sensorial properties: in fact, modulating the type of coating it is possible to block sodium chloride during the reaction time releasing it close to the end of the cooking process.

The third paper **(**Hamzalıoğlu, *et al.,* 2013) tackled the entangled issue of the role of antioxidant compounds on MR development. There is a general belief that antioxidants can inhibit MR development. However there are many aspects to control such as the nature of antioxidant compounds, its concentration and finally the target MR products that is considered: while heterocyclic amines (HA) are generally inhibited by the presence of antioxidants, HMF is not and many different results have been obtained with acrylamide.

Curcumin is very well known for its antioxidative properties, however data of this study clearly demonstrated that from the MR point of view curcumin has a carbonyl function which is able to catalyze the conversion of asparagine into acrylamide. So the addition of curcumin increased the presence of acrylamide in food

In the paper the possibility to detect intermediates of the reaction between curcumin and acrylamide by High-resolution mass spectrometry was also shown for the first time, introducing the possibility of using this new mass spectrometry tool in the investigation of Maillard Reaction products.

In the fourth paper (Fiore, *et al.,* 2013) the possibility to use radiofrequency (RF) for domestic food processing was investigated with the aim to verify if using this technology it is possible to reduce the formation of potentially harmful Maillard Reaction product such as acrylamide and prevent the degradation of some vitamins.

The heat transfer using RF is similar to that achievable with microwave frequency, but while in the latter case the energy is

transferred to the water present in the food, the RF device used in this work allowed a selective heating of different food component.

As expected the measure of acrylamide concentration on the surface of potato cubes cooked in the RF oven using a power of 2350 KJ is less than the half of those measured in the conventionally cooked potatoes. This paper paved the way to the marketing of RF oven in the domestic cooking as a device able to favour healthy cooking behaviour.

Since the discovery of the acrylamide presence in foods in 2002 there was a continuous fine tuning and optimization to develop low cost robust and reliable methodology.

Looking at the wide literature available on the topic and at our own laboratory experience it is clear that the main bottleneck for routinely acrylamide analysis is not the detection system but the extraction procedure.

In the fifth study of the thesis (Troise et al., 2014) the High Resolution Mass Spectrometry (HRMS) coupled with an Orbitrap apparatus was used with the aim to take advantage by the enormous discrimination capacity of this mass spectrometry detection to avoid any extraction and purification step of the sample. Being the instrument able to discriminate up to the fifth digit after the coma, most of the potential interference is eliminated and it is possible to analyse directly raw watery extracts from the different matrices. Results obtained on potato products, biscuits and coffee were well in line with those obtained with LC-MS-MS, moreover the procedure is so stable and robust that also avoid the addition of internal standard results remain satisfactory at least for screening purposes.

4 NOVEL SCIENTIFIC RESULTS

1. It has been demonstrated, in a cookies model system, that the presence in bakery products of potentially healthy ingredients such ad bran fraction, protein-rich by product such as okara, or antioxidant like curcumin can lead to an increase of acrylamide concentration.

2. It has been proved, by the example of sodium chloride and HMF, that the use of encapsulation can be a smart strategy to reduce the available reactants and to modulate the concentration of Maillard Reaction Products.

3. It has been concluded that radiofrequency heating providing a controlled heat transfer can result in food cooking with less acrylamide amount.

4. It was the first time to use the high resolution mass spectrometry for acrylamide quantification. This new method was proved to be a powerful tool allowing minimizing the extraction steps and the use of deuterated internal standard in the investigation of Maillard Reaction products.

5 CONCLUSION AND SUGGESTIONS

The results published in the research papers collected in the Appendix of this thesis contributed to elucidate some key aspects of acrylamide, HMF and CML formation in different foods.

Although each of the paper reported in this thesis dealt with very specific aspects in different foods the overall take home message is that the development of Maillard reaction could be modulated according the specific requirement of the specific food of interest.

A clear understanding of the mechanisms underlying the biological effect is essential for obtaining the intended beneficial effect in the food product. If such knowledge becomes available, new technologies may provide new opportunities to deliver health, quality and safety in food systems.

The translation of consumer perceptions (particularly flavour, texture, the presence of health promoting components) into manageable industrial scale technologies is a major challenge for the food industry and it is a limitation of the state of the current science underpinning modern food processing technology. Systematic studies are required to provide a balanced optimization for the thermal processes that are accepted and widely used by the food industry in terms of food safety, providing acceptable risk and the desired benefits that are satisfactory to both to the consumers and food safety risk managers (i.e. nutritional and organoleptic quality, release of bioactive or functional compounds formed during food processing).

Further work is required to find or synthesize pure standard compounds to enable the conduct of more accurate mechanistic studies and to further identify other bioactive or functional compounds, thus providing stronger evidence of the beneficial effects of food processing.

6 PUBLICATION

Palermo M, Fiore A, **Fogliano V**. Okara Promoted Acrylamide and Carboxymethyl-lysine Formation in Bakery Products. J Agric Food Chem. 2012,60; 10141–10146.

Fiore A, Troise AD, Ataç Mogol B, Roullier V, Gourdon A, El Mafadi Jian S, Hamzalıoğlu BA, Gökmen V, **Fogliano V**. Controlling the Maillard Reaction by Reactant Encapsulation: Sodium Chloride in Cookies. J Agric Food Chem. 2012 60, 10808-10814

Hamzalıoğlu A, Mogol BA, Lumaga RB, **Fogliano V**, Gökmen V Role of curcumin in the conversion of asparagine into acrylamide during heating. Amino Acids. 2013, 44, 1419-1426

Fiore A, Di Monaco R., Cavella S., Visconti A., Karnieli O., Bernhardt S., **Fogliano V**. Chemical profile and sensory properties of different foods cooked by a new radiofrequency oven, Food Chemistry, 2013, 139, 515–520

Quantitation of Acrylamide in Foods by High-Resolution Mass Spectrometry. Troise AD, Fiore A, **Fogliano V**. J Agric Food Chem. 2014, 62, 74-79