Modelling

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

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

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

For the multi-response modelling of acrylamide and HMF formation in biscuits, data from the basic formulation (see Table 3) baked at 200°C were available. The biscuits from the basic formulation were baked for different times and samples were collected between 8 and 15 minutes. The baking experiments were performed in duplicate. Seven target compounds (herein called “responses”) were measured in the above mentioned samples: sucrose, fructose, glucose, asparagine, total amino acids (AA), acrylamide and HMF. Temperature profile and moisture loss were also measured.

Table 3. Composition of the biscuit recipe

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

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

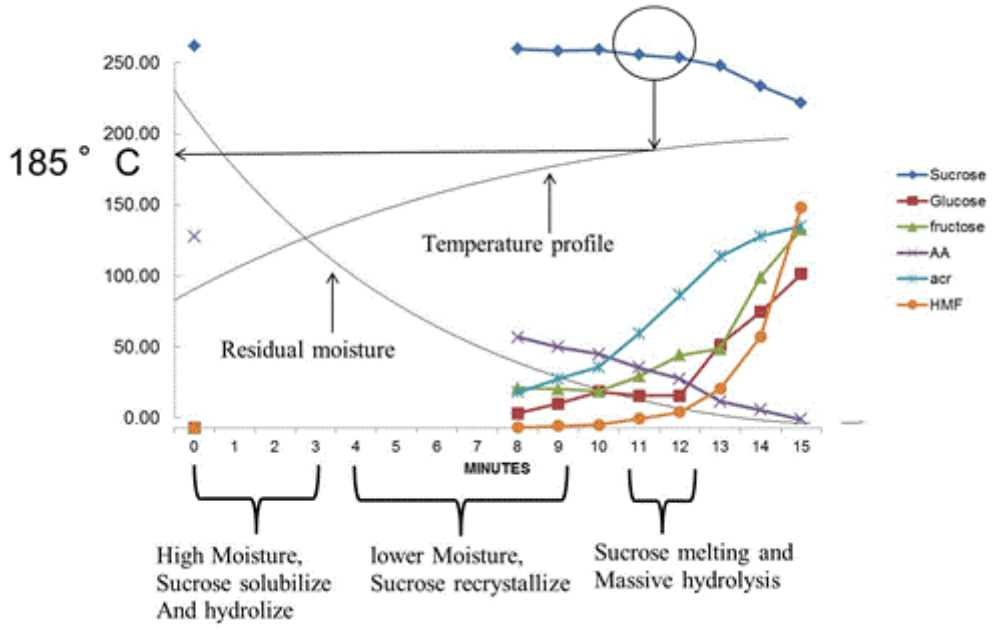

Figure 26. Evolution of the measured responses during biscuit baking at 200 ˚C, and the temperature profile of the biscuits (crust). The concentrations of each single compound are not to scale.

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

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

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

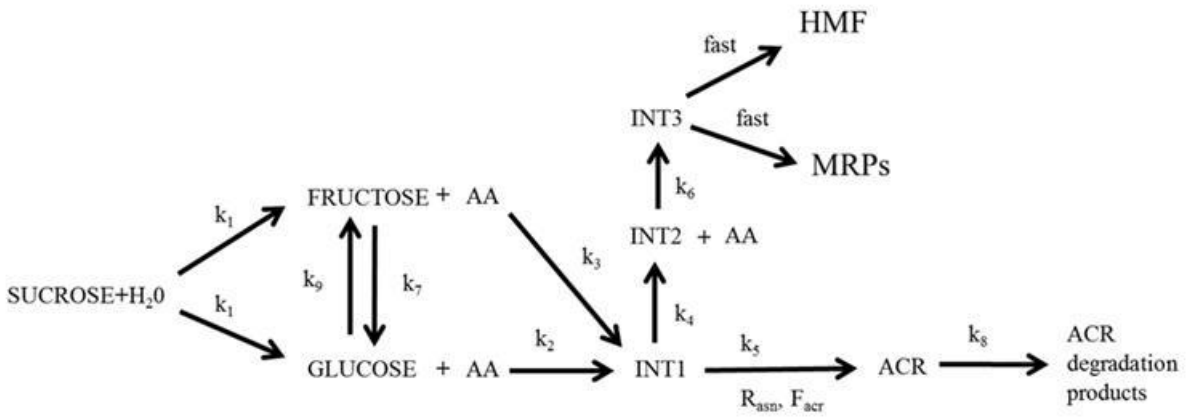

Figure 27. Simplified kinetic scheme selected by model discrimination analysis

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

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

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

Contact: H.J. van der Fels-Klerx, RIKILT Wageningen UR, the Netherlands, ine.vanderfels(at)wur.nl