Towards a Better Understanding in Acrylamide Formation, Degradation and Reduction in Model Systems (and Foodstuffs)

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Abstract: A new baking methodology to study acrylamide formation, based on a closed stainless steel tube reactor, was tested on its repeatability. The main advantage of this frying procedure includes the possibility to study the acrylamide formation mechanism in different artificial mixtures, eliminating some variable factors during frying, such as heat flux, degradation of the frying oil and water evaporation. As a first application of this optimized heating concept, the influence of fat oxidation and fat hydrolysis on acrylamide formation was tested during baking of French fries, as well as during heating in the tube reactor. In both cases, no differences in acrylamide formation could be found between fresh oil and oxidized or hydrolyzed heating oils.

Keywords: acrylamide formation; food; modelling; oil degradation; LC-MS/MS

INTRODUCTION

The discovery of surprisingly high levels of acrylamide in foods in April 2002 provoked extensive international research, which progresses rapidly. Fried, toasted, baked or grilled potato and cereal products are widely consumed. These processed foodstuffs showed to be extremely susceptible for acrylamide formation, mainly because of the abundant presence of the free amino acid asparagine and reducing sugars. Till now, these two compounds are believed to be the major precursors of this potential human carcinogen in food. However, additional acrylamide formation pathways may exist.

Apart from the chemical (Maillard) reaction mechanisms, also physical processes take place during food preparation. For example, during deep frying of French fries water evaporates from the food, and oil is absorbed [2]. Mechanical deformations (such as development of porosity and surface roughness) and physicochemical transformations (such as gelatinization and glass transition) may also occur and make the course of a frying experiment dramatically complex and constantly changing.

Frying oil undergoes oxidation and hydrolysis during use. The formed oil degradation products may on one hand function as acrylamide precursors [3]. On the other hand, these processes can influence the surface tension between the water containing food and the non-polar oil, and thus affect the oil to food heat transfer [1]. To remove this source of variable heat transfer during frying, baking experiments were carried out in a closed stainless steel reactor tube, placed in a thermostated deep fryer. In such a way, it is also possible to estimate more accurately the specific impact of these oil degradation products on acrylamide formation mechanisms.

Working with this closed tube reactor gives opportunities to re-examine the Maillard reaction, without observing other (side) phenomena. For instance, it is possible to conduct a baking process, without fluctuation in the water content of the food,

and thus keeping the water activity somewhat under control. In this study, it is furthermore the aim to determine possible synergistic and antagonistic interactions during frying between different major components in food matrices, such as (reducing) sugars, (free) amino acids, starch, fat and water. Making and baking a homogeneous mix of all these components was a challenging problem to be solved.

EXPERIMENTAL

Acrylamide analysis. Homogenized test portions of 1.00 g were weighed into 50 mL centrifuge tubes with cap, and spiked with 40 µl of 10 ng/µl of [D_3]acrylamide internal standard. To defat the samples, 10 ml hexane was added, followed by a 10 min shaking period. Subsequently the samples were centrifuged during 10 min at 4000 rpm (4°C). After removal of the hexane fraction, 10 ml of purified water (Milli-Q) was added, followed by a 20 min shaking period, to extract the acrylamide out of the samples. Next, the samples were centrifuged during 20 min at 4000 rpm (4°C), followed by an ultrafiltration with a 0.45 µm membrane filter. Further sample cleanup was performed transferring 2 ml of the filtrate on Oasis HLB columns (after conditioning the cartridges with 5 ml methanol and 5 ml water), and subsequently on Varian Bond Elut Accucat cartridges. Finally, the samples were analyzed on a Waters Alliance 2690 HPLC, with an Atlantis dC_{18} HPLC column (2.1 × 150 mm; 3 µm), coupled to a Micromass Quattro triple-quadrupole mass spectrometer (operating in ESI+). The mobile phase consisted of 92% water (containing 0.1% acetic acid) and 8% water/methanol (35/65, with 0.3% formic acid), with a flow of 0.15 ml/min. The MS/MS transitions monitored for acrylamide were 72 > 72 at 5 V and 72 > 55 at 10 V, those for the internal standard were 75 > 58 at 10 V, and 75 > 30 at 20 V. The quantification and calibration was based on the 72 > 55 and 75 > 58 transitions. Additionally this analysis was integrated within the scope of official accredited and validated analyses in the laboratory of Food Analysis.

Preparation of homogeneous artificial mixtures. Dried potato powder was sieved to obtain a regularly sized product. Solutions containing dissolved compounds (such as sugars and amino acids) were added and mixed in a mortar and mixer. In similar way, oils could be added.

Preparation of French fries. Raw potatoes (variety Spunta) were cut into pieces (1 cm × 1 cm × 5 cm) with a French fries-shaped cutter. Only the potato cuts from the central part of the potato were hold back and were washed five times (each during 1 min). To eliminate variance between potatoes of the same variety, cuts coming from different potatoes were thoroughly mixed before baking.

Frying experiments. All frying experiments were conducted in a semi professional thermostated deep fryer, equipped with a mixing mechanism to assure homogeneous temperature in the oil. The temperature was carefully monitored with a digital thermometer. French fries were heated during 5 min at 175°C (±1°C). The homogeneous mixtures were baked in a stainless steel reactor tube (internal diameter 1 cm, length 30 cm) at different time intervals.

Repeatability tests. To assess the repeatability of the baking procedure in the reactor tube, baking tests were performed using different dried potato powder mixtures and applying different baking durations. A mixture of 60% potato powder and 40% phosphate buffered solution (the approximate water content of baked French fries) was heated during 1, resp. 2 min (Figures 1A and B). To boost acrylamide formation, another 0.5% fructose was added, and the mixture was heated during 3 min (Figure 1C).

Table 1. Influence of oil oxidation on acrylamide formation in French fries and in the model

Hours of heating	Oil oxidation parameters		Acrylamide concentration (ppb)	
	p-anisidine value	peroxide value	French fries	Mix heated in tube reactor
0	1.3	3.1	380	544
2	149.3	2.5	357	ND
4	189.6	1.5	330	ND
6	320.2	2.6	305	ND
8	455.3	3.4	372	476

ND – not determined

Influence of oil oxidation. To test the impact of oil oxidation products, soy oil (prone to oxidation because of high levels of C18:3 and C18:2) was used as deep frying oil. This oil was kept at 175°C during eight hours and was constantly mixed. Each two hours, potatoes were fried in it and acrylamide formation was measured. Simultaneously, these oxidized oils were used as a compound in a homogeneous mix with water (38%) and potato powder (41%), and heated in the reactor tube. The composition of this mix approached the one of baked French fries. The degree of oil oxidation was measured on the basis of the *p*-anisidine value (AOCS Official Method Cd 18-90) and the peroxide value (AOCS Official Method Cd 8-90).

Influence of oil hydrolysis. To test the impact of oil hydrolysis products on acrylamide formation, different oil hydrolysis compounds were combined, as shown in Table 2. To eliminate the effect of oil oxidation, hydrogenated soy oil was used as major compound (triacylglycerols, TAG). The pure diacylglycerols (DAG) added were produced out of hydrogenated rapeseed oil, with the fatty acids on positions 1.2 and 1.3 (35%:65%). As monoacylglycerols (MAG) a glycerol monostearate powder (>85% purity) was used. Also a mixture with glycerol (GLY) was tested. The magnitude of these compounds was based on the composition of extremely hydrolyzed oils. The same baking experiments were carried out, as the ones described above to examine the influence of oil oxidation.

RESULTS AND DISCUSSION

Repeatability tests. Since a new baking methodology of food matrices was introduced, namely using a stainless steel reactor tube, the first objective was to test the repeatability of the baking procedure. This repeatability was assessed in three acrylamide concentration regions, namely the region around 20ppb, 300 ppb and 6000 ppb. The results for the 300 and 6000 ppb range are presented in Figures 1A and 1B. The error bars reflect the uncertainty of the acrylamide analysis (95% confidence level). Concentrations between 14 and 22 ppb were obtained in the lowest concentration range. In order to produce repeatable results, the potato powder used should have a homogeneous particle size. Obtaining a uniform blend, mixing together different powders, appeared to be very difficult. When heating these mixtures, no repeatable acrylamide concentrations could be found, especially in higher acrylamide concentrations, as shown in Figure 1C. It seams that acrylamide formation is extremely prone to heterogeneity in this heating model system. As shown in Figure 1B, this problem can be solved adding other components (like sugars) in dissolved form to the potato powder.

Influence of oil oxidation. As shown in Table 1, oil oxidation, as assessed by the para-anisidine value, had no effect on acrylamide formation during frying of potatoes in the oil. In order to evaluate only the influence of the chemical changes during

Table 2. Influence of oil hydrolysis on acrylamide formation in French fries and in the model

Oil composition (%)				Acrylamide concentration (ppb)	
TAG	DAG	MAG	GLY	French fries	Mix heated in tube reactor
100	–	–	–	317	436
85	15	–	–	315	318
98	–	2	–	372	408
99	–	–	1	261	335

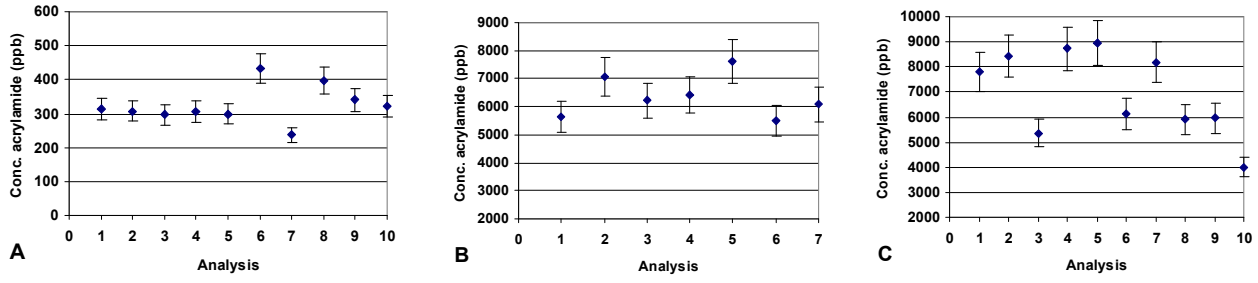

Figure 1. Repeatability tests of acrylamide formation during heating of an artificial mixture in a closed reactor tube

oxidation, the virgin and highly oxidized oil were applied respectively in the optimized tubular reactor model. As can be observed, no differences could be detected. The values in the table are average of two heating experiments.

Influence of oil hydrolysis. Yasuhara *et al*. (2003) postulated that acrylamide can be formed during hydrolysis of triolein, yielding glycerol, which further degrades to acrolein. This compound can finally produce acrylamide. According to the results, presented in Table 2, this proposition cannot be proven both in French fries and in the mixture, heated in the tube reactor. On the other hand, a lower acrylamide concentration was found in French fries, baked in oil containing 1% glycerol. The acrylamide formation in the tube reactor appeared not to depend on the contact of fat hydrolysis products in the oil.

Conclusions

Based on the results presented above, it seems that oil oxidation and oil hydrolysis do not have an impact on acrylamide formation. Moreover, a repeatable heating model is set up, which allows scientists to study the acrylamide formation mechanism eliminating some variable factors during frying**.**

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