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Influence of Oil Type on the Amounts of Acrylamide Generated in a Model System and in French Fries

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Acrylamide formation was studied by use of a new heating methodology, based on a closed stainless steel tubular reactor. Different artificial potato powder mixtures were homogenized and subsequently heated in the reactor. This procedure was first tested for its repeatability. By use of this experimental setup, it was possible to study the acrylamide formation mechanism in the different mixtures, eliminating some variable physical and chemical factors during the frying process, such as heat flux and water evaporation from and oil ingress into the food. As a first application of this optimized heating concept, the influence on acrylamide formation of the type of deep-frying oil was investigated. The results obtained from the experiments with the tubular reactor were compared with standardized French fry preparation tests. In both cases, no significant difference in acrylamide formation could be found between the various heating oils applied. Consequently, the origin of the deep-frying vegetable oils did not seem to affect the acrylamide formation in potatoes during frying. Surprisingly however, when artificial mixtures did not contain vegetable oil, significantly lower concentrations of acrylamide were detected, compared to oil-containing mixtures.

KEYWORDS: Acrylamide formation; food; modeling; oil type; LC-MS/MS

INTRODUCTION

The detection of surprisingly high levels of acrylamide in fried or toasted potato and cereal products in April 2002 provoked extensive international research, which progressed rapidly. These processed foodstuffs are widely consumed and shown to be extremely susceptible to acrylamide formation by the Maillard reaction, mainly due to the abundant presence of the free amino acid asparagine and of reducing sugars (*1*). Till now, these two compounds are believed to be the major precursors of acrylamide, a potential human carcinogen.

The possible role of different factors influencing acrylamide formation has been extensively investigated. In this context, the eventual role of the deep-frying oil type was discussed on several occasions. It is believed that the type of oil affects acrylamide formation, due to the differing ability to transfer heat into foods (*2*−*5*). Specifically, it was suggested that different quantities of substances such as mono- and diacylglycerols and short- and medium-chain fatty acids cause different surface tensions between the nonpolar oil and water-containing food, giving rise to different oil to food heat transfers. Therefore, much higher concentrations of acrylamide were found in foods heated in palm olein (*3*). Moreover, the formation of acrylamide appeared to be more elevated in olive oil compared to corn oil (*4*). It should be noted, however, that these findings could not be confirmed by others (*5*).

Apart from chemical reactions such as the Maillard reaction and acrylamide formation, physical transformations take place as well during deep-frying. For instance, water evaporates from the food and oil is absorbed (*6*). Mechanical deformations, such as development of porosity and surface roughness, and physicochemical transformations, such as gelatinization, may also occur. These processes gradually change the physical environment in which chemical reactions occur. Furthermore, the heating medium progressively degrades as well, due to oxidative and hydrolytic processes, which may in their turn influence the heat transfer (*3*, *7*). All these events make the course of a frying experimental setup dramatically complex and constantly changing. Moreover, not only is the foodstuff to be fried complex, but a food product (e.g., potato) is also extremely variable between and within species (*6*, *8*, *9*). To cope with this complex situation, care should be taken during selection of raw materials, during sample preparation, and at the frying stage. All these precautions are essential in order to avoid variability in acrylamide formation, occurring within the framework of a complex high-temperature environment. Possibly this inherent

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variability could be the cause of the conflicting data on acrylamide formation as mentioned above.

To simplify the complex frying process, and thus to remove possible abovementioned sources of variability, heating experiments were performed in an optimized model system. Hereby, several artificial mixtures were heated in a closed stainless steel tubular reactor, placed in a thermostated deep-fryer. Working with these artificial mixtures gives the opportunity to perfectly control the composition of the raw material, which is less achievable when working with potato tubers. Moreover, this tubular reactor simplifies to some extent the variable heat and mass transfer. For instance, it is possible to conduct a heating process without fluctuation in the water or oil content of the food. In such a way, the heat transfer from the heating oil toward the food remains more constant. Moreover, the use of the tubular reactor prevents oil exchange between the artificial mixture and the progressively changing heating medium. So it is possible to estimate more accurately the specific impact of the type of frying oil on acrylamide formation mechanisms, without observing other above-mentioned and constantly altering physical processes. As there is no direct contact between the deep-frying oil and the food, it is additionally possible to determine the influence of the presence or absence of the deep-frying oil in artificial mixtures.

The aim of this study was initially to test the repeatability of both the model system and the French fry preparation procedure. Subsequently, the influence of the oil type was tested by use of these two experimental preparation tools. Specifically, it was our objective to further clarify the role of the oil type on acrylamide formation in foodstuffs. To evaluate the reliability of the model system, some results obtained from the model system were compared with parallel running French fry preparation tests. Finally, the effect of the presence or the absence of oil in the artificial mixtures was investigated.

MATERIALS AND METHODS

Reagents and Chemicals. Phosphate-buffered saline (PBS) (pH 7.4) consisted of 0.135 M NaCl, 1.5 mM KH_2PO_4, 8 mM $NaH_2PO_4 \cdot 12H_2O$, and 2.7 mM KCl. These reagents were supplied by Chem-Lab, Belgium. Fructose, asparagine, and *p*-anisidine were from Acros Organics, Belgium. [2,3,3-D_3]Acrylamide (Polymer Source Inc., Dorval, Canada) and acrylamide (Sigma−Aldrich, Belgium) were used as standards. Acetic acid, formic acid, methanol, isooctane, and *n*-hexane (BDH Laboratory Supplies) were supplied by VWR, Belgium. Deionized water (Milli-Q; Millipore Corp.) was used throughout. All these reagents were of analytical grade.

Preparation of Homogeneous Artificial Mixtures. Two dried potato powders (A and B) were used to prepare the mixtures. The dried potato powders were sieved to obtain a product with a fine and homogeneous powder size distribution. The fraction between 90 and 160 μm was retained. Solutions containing dissolved compounds, such as reducing sugars or buffer solutions, could subsequently be added. The components were thoroughly mixed in a mortar to obtain a homogeneous blend. Similarly, oils could be added as well. In such a way, mixtures were prepared with a composition similar to that of finally prepared French fries, that is, 38% PBS, 21% fat or oil, and 41% potato powder, with a dry matter content of 58%, unless otherwise mentioned.

Potato Washing and Cutting. Depending on the commercial availability at the time of research, two potato varieties (*Solanum tuberosum* L., var. Spunta and Frieslander, harvest 2003) were used. The acrylamide results within each table or figure were generated from the same batch of potatoes. Tubers were cut into pieces (1 cm × 1 cm × 3 cm) with a French fry-shaped cutter. To obtain potato cuts with the same dimensions and thus the same contact area, parts that were in contact with the outer peel of the tuber were rejected for frying. Consequently, only the potato cuts originating from the central part of the potato were washed five times (each for 1 min), followed each time by a 5 min rest under tap water. For this, approximately 7 L of water was used for 1 kg of potato cuts. To eliminate variance between potatoes of the same variety, cuts coming from different potatoes were thoroughly mixed together. Prior to frying, the potato cuts were kept under distilled water at 4 °C for a period of maximum 4 h. Just before frying, the potato bars were patted dry with absorbing paper.

French Fries Preparation Experiments. All frying experiments were conducted in a 5 L semiprofessional thermostated deep-fryer (Fritel 2505, Belgium), equipped with a stirring mechanism to ensure a homogeneous temperature in the oil bath. The temperature was carefully monitored with a digital thermometer (Testo 925 with waterproof needle probe for measurements between −60 and 250 °C). Ten French fries (total weight ca. 33 g) were heated for 5 min at 175 °C (±1 °C) in a heating basket, which was large enough to enable free movement of the fries in the frying oil. Potato-to-oil weight ratio was deliberately maintained low in order to stabilize the frying temperature (±1 °C). Directly after frying, the French fries were cooled on an absorbing paper. Finally, all fries were thoroughly mixed together before acrylamide analysis.

Heating Experiments with Model System. One gram of the homogenized mixture was introduced as a cylinder (diameter 1 cm) into the middle of a cylindrical stainless steel tubular reactor (internal diameter 1 cm, length 30 cm). The mixture was kept in place by two stainless steel supporting bars (diameter 1 cm), which were introduced at both sides of the stainless steel tube. Subsequently, the tubular reactor was sealed hermetically and was heated at selected temperatures and time intervals in the deep-fryer. The same heating and temperature measuring equipment was used as for the French fries. Immediately after heating, the tubular reactor was transferred to an ice bath (for 2 min), to enable a quick cooling of the artificial mixture. Subsequently, the reactor was opened and the 1-g mixture was analyzed for its acrylamide content.

Acrylamide Analysis. The analysis method was based on a previously described FDA method (*10*), with some modifications. A homogenized test portion of 1 g was weighed into a 50 mL centrifuge tube with cap and spiked with 40 μL of 10 ng/μL [2,3,3-D_3]acrylamide internal standard. To defat the sample, 10 mL of *n*-hexane was added, followed by a 10 min shaking period. Subsequently the sample was centrifuged for 10 min at 4000*g* (4 °C). After the hexane fraction was discarded, 10 mL of deionized water was added, followed by a 20 min shaking period, to extract the acrylamide from the food matrix. Next, the sample was centrifuged for 20 min at 4000*g* (4 °C), followed by ultrafiltration through a 0.45 μm membrane filter. Further sample cleanup was performed on two solid-phase extraction columns. The Oasis HLB (6 mL, 200 mg; Waters, Milford, MA) column was conditioned by passing 5 mL of methanol followed by 5 mL of water through the column. The Bond Elut-Accucat column (200 mg mixed-mode packing: C_8, SAX, and SCX) (Varian, Harbor City, CA) was conditioned with 3 mL of methanol followed by 3 mL of water. The filtrate (2 mL) was transferred onto the Oasis column and was allowed to pass first through the Oasis and subsequently through the Accucat column. Finally, 20 μL of the purified extract or of a reference standard solution was injected into a Waters Alliance 2690 HPLC system, equipped with an Atlantis dC_{18} HPLC column (2.1 × 150 mm; 3 μm) (Waters, Brussels, Belgium). The detection of acrylamide was performed on a Quattro LC (Micromass, Manchester, U.K.) triple-quadrupole mass spectrometer, operating in positive electrospray ionization (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 an isocratic flow of 0.15 mL/min. The capillary voltage was 2.8 kV, the cone voltage being at 22 V. The source block temperature was 120 °C and the desolvation gas temperature was 350 °C. The desolvation gas flow was 500 L of nitrogen/h. The argon collision gas pressure was adjusted to 1.8×10^{-3} mbar for MS/MS. The collision energy was varied for each monitored transition in multiple reaction monitoring mode (MRM). The MS/MS transitions monitored for acrylamide were 72 → 72 at 5 eV and 72 → 55 at 10 eV collision energy, those for the internal standard were 75 → 58 at 10 eV, and 75 → 30 at 20 eV. The dwell time for each monitored transition

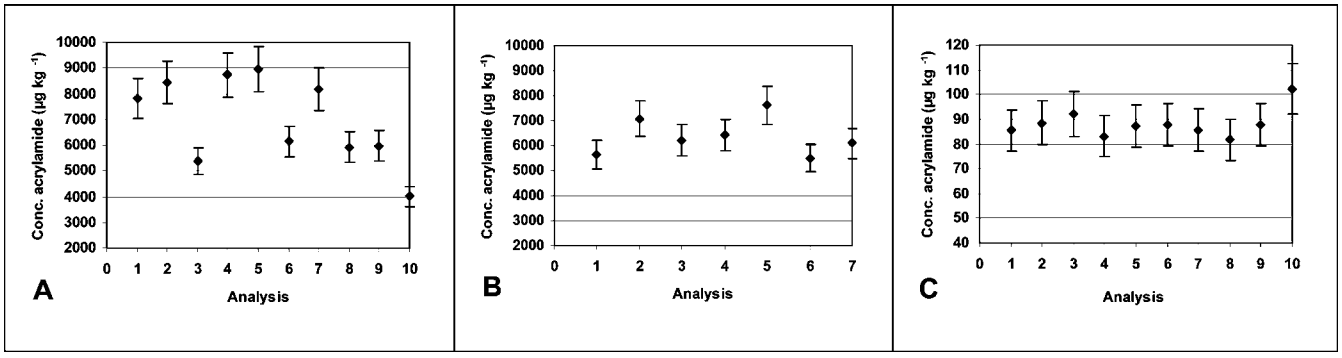

Figure 1. Repeatability tests of acrylamide formation during heating of the artificial mixture in a closed tubular reactor. The error bars reflect 95% confidence intervals. (**A**) Mixture with 6.1% fructose, 6.1% asparagine, 48.4% potato powder A, and 39.4% phosphate-buffered saline (PBS) (w/w), heated for 2 min at 175 °C. (**B**) Mixture with 0.6% fructose, 60.0% potato powder A, and 39.4% PBS (w/w), heated for 3 min at 175 °C. (**C**) Mixture with 41.0% potato powder A, 21.0% rapeseed oil, and 38.0% PBS (w/w), heated for 2 min at 175 °C.

was 0.2 s. The quantification and calibration was based on the 72 → 55 and 75 → 58 transitions.

The analyses were integrated within the scope of an accredited laboratory controlled by the official Belgian organization for accreditation (BELAC). The method was validated in-house for linearity, specificity, limit of detection, limit of quantification, repeatability, and recovery. Furthermore, the accuracy of the method was demonstrated during participation in three interlaboratory proficiency tests. The results of the first European interlaboratory comparison study on the determination of acrylamide in butter cookies and crispbread [Institute for Reference Materials and Methods (IRMM), Geel, Belgium, 2003] yielded *Z*-scores between −0.14 and −1.20. In the beginning of 2004, IRMM organized another proficiency test, again on the determination in crispbread samples, yielding *Z*-scores between 0.37 and 1.17.

Reference standard solutions of acrylamide were prepared in water from a stock solution and were stored at 4 °C for a maximum of 3 months. An external calibration curve was established in the concentration range between 0 and 10 000 μg kg^{-1}, with a correlation coefficient > 0.999. Data interpretation was performed by use of Quanlynx integration software (Micromass, Manchester, U.K.).

Confirmation of the identity of the response was based on four criteria (*11*). First, the relative retention times of the acrylamide ions (compared to the internal standard ions) in the sample were within a 2.5% margin of the relative retention times of the ions in the reference standard solutions. Second, the relative abundances of the ions recorded (compared to the intensity of the most specific ion for quantification) corresponded with those of the ions in the reference standard solutions within fixed margins. Third, the signal-to-noise ratio of each ion was larger than 3. Finally, the signals of two daughter ions and one mother ion were followed to reach the four required identification points. For this, one identification point is attributed to each mother ion and 1.5 points to each daughter ion.

The limit of detection (LOD), defined as the mean value of the matrix blank readings plus 3 standard deviations (expressed in analyte concentration) was 12.5 μg kg^{-1}. The limit of quantification (LOQ), being the mean value of the matrix blank readings plus 6 standard deviations, was 25 μg kg^{-1}. Bread crumbs were used in order to validate the method. These crumbs were used as a blank food matrix, as this inner part of bread did not contain acrylamide, as was determined before.

To assess the repeatability of the analysis method, bread crumbs were spiked with 1, 1.5, and 2 times the limit of detection concentration. These analyses were performed six times, yielding a relative standard deviation (RSD) of 10%. The recovery was evaluated by spiking bread crumbs with 1, 1.5, and 2 times the limit of detection concentration. Instead of adding the internal standard at the beginning of the sample cleanup, it was added just before the LC-MS/MS analysis. Again, the analyses were performed six times. The absolute recovery was calculated according to the EU Commission Decision 2002/657/EC and was about 65% for all concentration levels. To compensate this loss of analyte during sample cleanup, deuterated acrylamide was added as internal standard in the first step of the analysis method.

Dry Matter Content. Determination of the dry matter content was based on an official AOAC method (*12*).

Repeatability Tests of Frying Experiments. To assess the repeatability of the heating procedure in the tubular reactor, mixtures with various compositions, including potato powder and PBS, were heated at 175 °C for 2 or 3 min. To boost acrylamide formation, fructose or asparagine was supplementarily added for some experiments, as these compounds are reported acrylamide precursors.

Influence of Heating Medium. Nine different types of lipids, including olive, rapeseed, corn, sunflower, grapeseed, and soybean oil, palm fat, and a highly hydrogenated soybean fat were purchased from retail sources. The oxidative status (p-anisidine value, PAV, *13*) and the fatty acid profile (*14*) were determined for each lipid. As an alternative heat transfer medium, the influence on acrylamide formation of paraffin oil, with a melting section between 54 and 56 °C, was investigated. The amounts of acrylamide generated by use of paraffin oil were compared to experiments with soybean oil and palm fat as heating media. The palm fat was a commercial mixture of 80% palm oil and 20% palm stearine (Vandemoortele, Belgium). The potato cuts were fried in the different heating media for 5 min at 175 °C, to evaluate acrylamide formation. For every experiment, two batches of potato cuts were fried simultaneously, so the reported acrylamide levels are the average of two batches. As explained previously, each oil was mixed with PBS and potato powder B to obtain a homogeneous mixture. These mixtures were heated for 2 min at 175 °C. Some model experiments were repeated at more severe heating conditions; that is, at 170 °C for 5 min. All heating experiments with the model system were performed in duplicate, so the reported acrylamide levels are the average of two experiments.

RESULTS AND DISCUSSION

Repeatability Tests of Heating and Frying Experiments. In this investigation different potato powder mixtures were heated in a stainless steel tubular reactor. Since this is a new heating approach to study acrylamide formation, the first objective was to test the repeatability of this procedure. During the first series of experiments, the used potato powder (powder A) was not sieved. Fructose and asparagine were added to the potato powder as a solid (finely crushed crystals), without previous dissolution in the aqueous phase. The mixture had a final composition of 6.1% fructose, 6.1% asparagine, 48.4% potato powder, and 39.4% PBS (w/w). It was heated for 2 min at 175 °C, yielding an average acrylamide concentration of 7053 μg kg^{-1}, with a RSD of 24% (n = 10) (**Figure 1A**). In a second series of experiments, a lower amount of fructose (0.6% in total sample) was initially dissolved in the aqueous phase. Subsequently the aqueous solution was added to the potato powder (powder A). The asparagine was omitted, since it is not the limiting reaction partner in acrylamide formation in potatoes

(*16*). The final mixture composition was 0.6% fructose, 60% potato powder, and 39.4% PBS (w/w). These experiments ($n = 10$, results not shown) yielded an average of 851 μg kg^{-1} acrylamide after 2 min of heating at 175 °C, with a lower RSD of 18%. A mixture with an identical composition was heated for 3 min at 175 °C in a third series of experiments (**Figure 1B**). However, in this third set of experiments the potato powder (powder A) was sieved before the aqueous fructose solution was added. On average, the acrylamide concentration was 6359 μg kg^{-1}, with a RSD of only 12% ($n = 7$). Presumably, the repeatability of the heating experiments can be increased significantly if the potato powder used is sieved and thus a more homogeneous particle size distribution is obtained. Finally, the repeatability was assessed with omission of fructose from the mixture (**Figure 1C**). Here the mixture consisted of 41% potato powder B, 38% PBS, and 21% rapeseed oil (w/w). It was heated for 2 min at 175 °C. A lower average acrylamide concentration level was obtained (88 μg kg^{-1}), but the heating experiments proved again to be very repeatable (RSD = 6%, $n = 10$). The error bars in **Figure 1** reflect the 95% confidence intervals of the acrylamide analysis.

From these preliminary experiments, it was found that the heated mixture should be very homogeneous in order to obtain repeatable results. Upon simply mixing different powders, no repeatable acrylamide concentrations could be generated. Therefore, it was essential to prepare homogeneous blends prior to heating. It seemed that acrylamide formation was particularly dependent upon heterogeneity in the model system. This problem could be solved by adding the other constituents to the potato powder in the form of aqueous solutions. Moreover, it appeared that the potato powder used should have a homogeneous particle size. Sieving the powder before mixing together made the heating procedure even more repeatable (Figures 1B and 2C).

In addition, the repeatability of the French fry preparation method was assessed. The French fries used for each set of experiments originated from the same batch of potato cuts. The test was repeated 10 times. An average acrylamide concentration of 729 μg kg^{-1} was obtained with a RSD of 15% ($n = 10$) (results not shown). Comparison of this value with the RSD of the acrylamide analysis, which was 10%, makes it clear that the frying procedure of French fries generates only very limited extra variability in the total procedure of acrylamide generation and analysis.

Furthermore, the final dry matter content of the French fries was determined each time in duplicate. All values fluctuated around 60% ± 1% ($n = 20$), again indicating the good repeatability of the applied frying procedure. So, although the model system eliminated some variable factors occurring during deep-frying, such as oil and water transfer, it was demonstrated that preparation of French fries could also generate acrylamide in a repeatable way. It should be stressed, however, that these results can only be produced if sufficient attention is paid to sample preparation, such as potato cutting and washing, and to the frying procedure, that is, temperature control during the frying experiment.

Influence of Heating Medium. To test the impact of the heating medium on acrylamide formation, experiments were performed with different vegetable oils. The fatty acid composition (results not shown) was in agreement with average fatty acid profiles for such vegetable oils (*16*). To ensure the freshness of the oils, the p-anisidine value (PAV) was determined (*13*). The oils showed similar low PAV (below 15), except for palm fat (between 30 and 50). Rapeseed, olive, sunflower, soybean, corn, and grapeseed oil were subsequently added as components in the model system, which was heated in the tubular reactor at 175 °C for 2 min to assess acrylamide formation (**Figure 2**). Additionally, palm fat, olive oil, sunflower oil, soybean oil, saturated soybean fat, and corn oil were separately added in the model system as well. These mixtures were heated at 170 °C for 5 min (**Figure 3**). Within each of these two figures, it can be observed that the acrylamide concentrations were not exactly the same for the different heating oils used in the artificial mixture. On the other hand, no significant differences could be demonstrated between the vegetable oils.

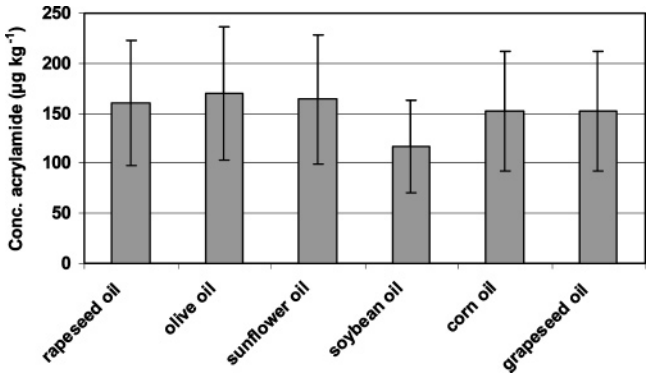

Figure 2. Influence of different oil types on acrylamide formation in the mixture, heated in the tubular reactor at 175 °C for 2 min. The error bars reflect 95% confidence intervals of the frying procedure and acrylamide analysis.

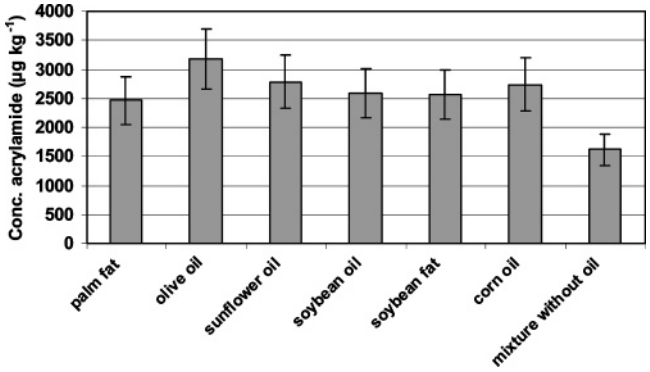

Figure 3. Influence of different oil types on acrylamide formation in the mixture, heated in the tubular reactor at 170 °C for 5 min. The error bars reflect 95% confidence intervals of the frying procedure and acrylamide analysis.

During a second series of experiments, French fries were prepared for 5 min at 175 °C as shown in **Figure 4**. The acrylamide concentrations did not significantly differ between the different deep-frying oils, confirming the results obtained with the model system.

In addition, paraffin oil was applied both in the model system and in French fry preparation experiments. The amounts of acrylamide generated by use of paraffin oil were compared to experiments with palm fat and soybean oil. Acrylamide concentrations are shown in **Table 1**. The acrylamide concentrations of the French fries in **Table 1** cannot be compared with those in **Figure 4** since different potato varieties were used for the two experiments. In **Table 1**, no significant differences in acrylamide formation were found between paraffin and the vegetable oils. Paraffin is chemically inert. Consequently, no oxidation reactions can occur and paraffin acts only as a heat transfer medium. Moreover, this oil is devoid of triacylglycerols, so acrolein, an oil degradation product and at the same time a

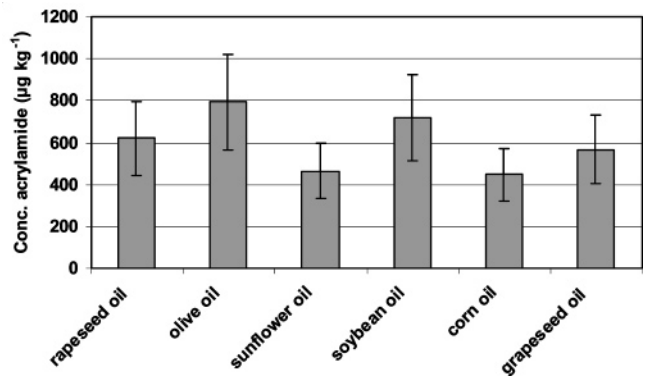

Figure 4. Influence of different oil types on acrylamide formation in French fries, prepared at 175 °C for 5 min. The error bars reflect 95% confidence intervals of the frying procedure and acrylamide analysis.

Table 1. Influence of Different Heating Media on Acrylamide Formation in French Fries,[a] and in the Tubular Reactor[b]

	acrylamide concn[c] (μg kg^{-1}) ($n = 2$)	
	French fries	mixture heated in tubular reactor
palm fat	436 (±88)	103 (±17)
soybean oil	335 (±68)	109 (±18)
paraffin	360 (±73)	102 (±17)

[a] Prepared at 175 °C for 5 min. [b] Heated at 175 °C for 2 min. [c] Corresponding 95% confidence intervals are given in parentheses.

suspected acrylamide precursor (*2*, *4*), cannot be formed starting from these compounds. From these results, another indication is presented that the heat medium as such did not influence the acrylamide formation.

Previously, Gertz and Klostermann (*2*) postulated that palm oil exhibits much higher acrylamide formation in French fries, compared to other deep-frying oils. In addition, Becalski et al. (*4*) found that olive oil induced higher formation compared to corn oil. On the other hand, Matthäus et al. (*5*) and Williams (*17*) could not find any significant effect of the oil type. Obviously, there is still some confusion about the influence of the heating medium on acrylamide formation. The results shown in **Table 1** and Figures 2−4 confirm the findings of Matthäus et al. (*5*) and Williams (*17*). Hence any significant influence of the heating medium could be demonstrated in the above-mentioned experimental setup.

Influence of Oil Presence in the Model System. In a final experiment, an artificial mixture without oil was heated in the tubular reactor. This mixture had a composition of 48% PBS and 52% potato powder B (w/w). When the acrylamide formation of oil containing artificial mixtures was compared to the mixture without oil (**Figure 3**), significantly higher concentrations were found in the oil-containing ones. Even in mixtures containing paraffin oil, higher concentrations were found. Consequently, it can be stated that the heat transfer in the mixture was changed when oil was added to the mixture. The different texture of the oil-containing mixtures, compared to those devoid of oil, could be a possible explanation for this phenomenon. It is plausible that the oil-containing mixture had better contact with the inner wall of the stainless steel tubular reactor. Consequently, the heat transfer may be facilitated. Moreover, it could be possible that heat is better distributed in more oily mixtures, due to oil convection flows in the foodstuff, and thus again resulting in a better heat transfer.

From these results, it could be concluded that the tested frying media did influence the acrylamide formation during frying to the same extent. In the next paper, more details on the influence of oil oxidation and hydrolysis on acrylamide formation during frying will be presented.

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