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The Impact of Fat Deterioration on Formation of Acrylamide in Fried Foods

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The current study investigates to what extent the reaction products of thermal degradation directly influence acrylamide formation in French fries. The frying tests at 170 and 180 °C are carried out with rapeseed oil for 32 h with 128 frying cycles. Acrylamide content in French fries is determined by LC-MS/MS. Oxidative and thermal degradation is followed by measuring total polar compounds (TPC), di- and polymerized triacylglycerols (DPTG), monomer oxidized triacylglycerols (MONOX), *p*-anisidine value (AnV), mono and di-acyl-glycerols (MAG and DAG), acid value (AV), epoxy fatty acids, iodine value (IV), saponification value, and fatty acid composition. During frying, the nature and degradation level of the frying medium have a direct impact on acrylamide formation. It can be shown that the pH-dependent reaction is strongly inhibited at acid values above 0.5 mg KOH g^{-1} oil. Acidity measured as AV or FFA is mainly caused by oxidation, and less so by hydrolysis of triacylglycerols (TAG) as assumed up to now. Obviously, acid functional groups formed by oxidation of unsaturated fatty acids bound in TAG can act not only as catalyst for dimerization of TAG but also interact with asparagine as most important precursor for acrylamide formation so that no reaction with carbonyl groups for the formation of acrylamide is necessary.

***Practical applications*: The same acidic functional groups that are known to catalyze the formation of dimeric TAG under frying conditions (160–190 °C, access of oxygen) in a nonradical mechanism apparently can also deactivate asparagine by protonization as a potential precursor for the formation of acrylamide. It is recommended not to reduce acidity of used frying oil by active filter aids below AV ≥ 0.5 as it helps to reduce acrylamide contamination of fried food.**

1. Introduction

Acrylamide is classified as potentially carcinogenic to humans (Group 2A) in animal studies due to its neurotoxic and genotoxic properties.[1] In April 2002, high concentrations of acrylamide were found in foods, particularly foods containing cereals and potatoes prepared at temperatures above 120 °C, due to the *Maillard* reaction between asparagine and reducing sugars such as glucose and fructose.[2]

Food safety studies[3] related to acrylamide mainly focus on the food material (potential source materials), storage and processing conditions (temperature, frying time) and analytical methods. French fries and other fried potato products are responsible for the largest share of total exposure in children and adolescents, up to 50%.[4] In 2017 COMMISSION REGULATION (EU) 2017/2158 of 20 November 2017[5] established mitigation measures and guidance values for the reduction of acrylamide content in food. A maximum temperature (175 °C) and a benchmark value for French fries (500 μg kg^{-1}) were set. In the presence of amino acids and reducing sugars in food, acrylamide formation can occur either via a general amino acid pathway or via an asparagine-specific pathway.[6] The di-carbonyl compounds subsequently react with asparagine via the Strecker degradation, leading to the formation of acrylamide.

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In many studies,[7] the acrylamide forming potential of potatoes and cereals including different varieties, storage conditions, plant breeding, and crop management were investigated. Asparagine is, among all free amino acids, the most relevant amino acid for acrylamide formation. There is a significant correlation between free asparagine concentration and acrylamide formation.[8]

Possible indirect pathways to acrylamide formation have also been identified. For example, lipid oxidation contributes to acrylamide formation in fried products.[9] Carbonyl groups, such as aldehydes and ketones, and other degradation products of lipid oxidation could react with asparagine to form acrylamide even though no reducing sugars are present.[10] This is consistent with a study by Kuek et al.,[11] which indicated that secondary lipid oxidation components have a positive influence on acrylamide.

Apart from the asparagine pathway, Weisshaar[12] suggested a shorter pathway via acrolein (2-propenal) as an alternative route for acrylamide formation.

Acrolein can be generated during heating of oils depending on the fatty acid composition, the heating time and the temperature.[13] Oils with a high content of unsaturated fatty acids, especially linolenic acid[14] (e.g., rapeseed oil and linseed oil), generated higher acrolein concentrations when heated than oils with a high content of saturated or monounsaturated fatty acids (e.g., coconut oil, olive oil). Maximum acrolein concentrations were formed at temperatures in the range of 140–180 °C for most oils, whereas a further temperature increase to 220–260 °C resulted in lower acrolein concentrations. Often, the formation of acrolein was suggested to be from the glycerol part of TAG, although a clear influence of the unsaturation of the fatty acid moiety was obvious in previous studies. However, in an isotope labeling study, Ewert et al.[15] demonstrated that the glycerol backbone of TAG is not a significant source of acrolein, indicating that hydrolytic reaction may not be the driving force for acrolein formation. Indeed, acrolein has been reported to be almost exclusively formed via the oxidation of linolenic acid, with relatively negligible amounts from oleic and linoleic acids.

At the beginning of the acrylamide story in 2002, many researchers only studied the influence of temperature on acrylamide formation. Gertz[9] et al. (2002) showed by practical tests which parameters of the frying process have to be changed to lower the acrylamide level in fried foods and set a maximum limit of 175 °C. In this study, it was also assumed that the level of frying oil degradation may have an impact on the acrylamide formation due to better heat transfer caused by the presence of polar oxidation products reducing the interfacial tension between food and frying oil.

Gökmen[16] suspected that both the degree of unsaturated fatty acids and the oxidation rate influence the acrylamide content. In a recent study,[17] consumers were advised to use hazelnut and olive oil, and avoid polyenic oils such as corn oil, according to acrylamide levels detected in French fries. Higher polyunsaturated fatty acid ratio of frying oil is thought to encourage acrylamide formation.

However, the different oils and fats do not behave during frying in the same way as expected. The mean acrylamide content of potatoes fried in sunflower oil (1477.07 ± 178.64 μg kg^{-1}) was higher than the one obtained when palm oil (1258.95 ± 121.01 μg kg^{-1}) was used.[18] Similar results were obtained with sunflower, rapeseed, and HOSO sunflower oil as well as palm olein and tallow.[19] Ahmad et al. (2021)[20] studied the effect of palm olein (PO), red palm olein (RPO), sunflower oil (SFO), and soybean oil (SBO) on acrylamide formation during the intermittent frying with 80 cycles of beef nuggets. Oil was collected at every 16th frying cycle and analyzed for peroxide value, AnV, FFA, TPC, and fatty acid composition. FFA and TPC continued to develop across the 80 frying cycles. The lowest acrylamide content in fried products was observed in PO, while the highest content was observed in RPO. Bivariate correlation analysis showed no significant correlation between oil quality attributes and acrylamide concentration. It is assumed that the oil type but not the frying cycle significantly affected the acrylamide concentration in beef nuggets.[21]

Other authors stated an increasing formation of acrylamide in French fries with increasing number of frying cycles.[11,22] Consequently, many trials were made to stabilize frying oils. Some studies[23] with TBHQ, ascorbyl palmitate, and various plant extracts (rosemary,[24] oregano[25]) claimed mitigation, while others showed no effect or even an increase.

Finally, in 2014 Matthäus et al.[26] and others stated that "there is no significant difference in the acrylamide concentration of French fries fried with different cooking oils." Considering these conflicting reports, the physical and chemical aspects concerning acrylamide formation during frying cannot be considered as "settled," thus, the purpose of the current study was to stimulate further discussions around this important topic.

2. Experimental Section

2.1. Samples

Prefried French fries and rapeseed oil coming from the same batch were purchased from a local supermarket. The oil from different bottles was mixed before use to achieve homogeneity.

2.1.1. Sample Preparation

The frying process with rapeseed oil was realized in a fryer set to 170 and 180 °C, respectively, and potato strips were fried for 4 min 30 s. Frying was realized in a temperature-controlled fryer (Gastrofrit AG, Rorschach, Switzerland) with two units of customized 7 L capacity stainless steel, in which the oils were gradually heated at 170 and 180 °C, respectively, and maintained at this temperature for 30 min before food was introduced. For each frying cycle a 1/15 potato/oil ratio w/w was used. During each cycle 400 g potatoes were fried. The frying cycle was repeated every 15 min.

The content of FFA is considerably higher in frying oils used in practise after only a few hours due to the more intensive use of the deep fryers. Therefore, in another experiment used frying oils from a restaurant and a snack food restaurant, respectively, were used as basis for further frying. In both cases these frying oils had a TPC content of about 10%. The used frying oils from both restaurants were collected separately and used in the laboratory fryer (deep fryer 4 L) for the preparation of French fries under controlled comparable conditions (180 °C, 3“”45’, 200 g).

In addition, the used frying oils from the restaurants were treated with 2% Magnesol (Dalles, Whitehouse, USA) before further frying to refresh the oil for a longer application time. Subsequently, the acrylamide content of the French fries and the degradation level of the frying fat were determined.

At the end of the frying cycles, the frying basket with the sample was removed from the fryer and shaken. French fries were allowed to cool on a plate and then homogenized in a laboratory mixer (Büchi, Labortechnik AG, Flawil, Switzerland). The homogenized samples were transferred to a 100 mL centrifuge tube and kept at −20 °C pending analyses. About 15 g of oil was collected after each frying cycle to analyze the different analytical parameters. The oil was kept at room temperature in amber vials until all parameters were determined using NIRS (**Table 1**).

2.1.2. Acrylamide Determination by LC-ESI-MS/MS

The acrylamide content of the final product was analyzed using the solid phase extraction (SPE) technique and LC-ESI-MS/MS according to L 00.00-159-2016-02 (DIN EN 16618).[27] For acrylamide quantification two calibration curves in the range of 0.05–3 mg L^{-1} were used, which had correlation coefficients higher than 0.999. The method recovery was between 85.64 and 109.22%. The limit of detection (LOD) and limit of quantification (LOQ) were 10.29 and 30.87 µg kg^{-1}, respectively.

2.1.3. FT-NIR Spectroscopy

Samples of oil were filled in 8 mm (6.6 mm ID) disposable glass vials and submitted to FT-NIR analysis. All samples were thermally equilibrated to 50 °C for 5 min before measuring and all spectra were recorded in triplicate. Spectra were obtained in transmission mode from 9000 to 4500 cm^{-1}. Each spectrum was time-averaged based on 32 scans at a resolution of 8 cm^{-1} using a Bruker MPA-FT-NIR spectrometer (Bruker Optik GmbH, Ettlingen, Germany), equipped with OPUS software version 7.8.

2.1.4. NIR Calibration

Generally, the development of the NIR methods is based on the calibration against the corresponding standard reference method. For this purpose, the different parameters for each sample of the calibration set were analyzed using the reference method and additionally the NIR spectrum of the sample was recorded. Subsequently, the data were combined and statistically evaluated by validated calibration software OPUS to develop the multivariate equations using partial least squares (PLS2) algorithm. The validation of the calibration was carried out using test set evaluation with samples independent from the samples used for calibration. In general, 50% of all samples were selected as test samples per random access. Wavelength range and data treatment (1st derivative, vector normalization or a combination of both) were optimized individually for each method. The samples for the validation of the NIR calibration were obtained from different heating experiments and frying tests. This calibration and validation process of the NIR was not part of this work.

Basic quality parameters analysing frying fats and oils.

NIRS combined with multivariate calibration models allows to develop NIR methods, which, in the analysis of fats and oils, provide almost identical values compared to the standard method because even differently structured components can be determined simultaneously in a single run. Many traditional analytical methods used in oil chemistry are old wet chemical methods, often nonspecific, describing a group of differently structured compounds with changing shares (i.e., *AnV, TPC* AV). For the NIR calibration only ISO standards were used. All methods were revalidated using external test sets, different NIR spectrometers of the same vendor (Bruker TANGO and Bruker MPA 2) and checking the accuracy and robustness of the methods by analyzing the samples of the DGF proficiency tests 2019–2021. The comparison of the repeatability and reproducibility data of conventional and NIRS data (Table 1) demonstrates the high performance and reproducibility of the NIRS measurements.

2.1.5. Statistical Analysis

For acrylamide determination, French fries samples were analyzed in duplicate, and results were expressed as mean. The analytical parameters to monitor the fat composition and fat degradation were analyzed by NIRS in triplicate, and results were expressed as mean ± standard deviation. XLSTAT software (version 2022-1311 Base Version),[28] was applied for the determination of significant differences by ANOVA and ANCOVA test, calculation of normal distribution, nonparametric test for comparison of two samples, distribution tests for outliers, descriptive statistics, and discriminant analysis (DA).

2.1.6. Ethical Statement

Ethics approval was not required for this research.

3. Results and Discussions

The aim of this study was to find out to what extent the thermal-oxidative changes of frying oil influence acrylamide formation in fried foods. This has been studied many times before with different results. However, in the studies before, the focus was always only on the type of oil and temperature. Further, most laboratory studies were limited to few frying cycles (usually 4/h), often for only 1–2 days even though the chemical and physical properties of deep-frying oil are constantly changing due to the nature of the oil and rate of decomposition. For a systematic approach to investigate the effect of oxidation products on acrylamide formation it is necessary to differentiate the type of degradation products and not only to monitor the total level of degradation as it is assumed that individual oxidation products such as acid functional groups or acids may play a significant role in acrylamide formation. It is well known that the *Maillard* reaction is pH-dependent. At lower pH, the α-amino group of asparagine will be protonated as precursors and are therefore not available for nucleophilic addition reactions with carbonyl sources in the initial stage of the *Maillard* reaction.[29,30,31] In 2003, Gertz added citric acid to frying oil

Table 1. NIR-methods—calibration and validation.

Property	Reference methods	Range	Calibration data	Validation data (external test set)	Measuring	Data pre-treatment	Validation				Correctness-Robustness (GF Proficiency Test (Median /NIR Value/z-Score))		
			N Spectra	N Spectra	Wave-number region cm^{-1}		Rank	R2	RMSEP	Bias	RPD	2019	2020	2021
AV [mg KOH g^{-1}]	DGF-C-V-2 /ISO 660/AOCS Cd 3d-63	0–7.8	709	552	7502.1–6800.1 5450.1–4566.8	1st deri.	14	97.3	0.179	−0.027	6,1	0.64/0.76/2.12	0.95/0.95/-0.02	0.48/0.51/ 0.75
AnV	DGF-C-VI 6e/ ISO 6885/ AOCS Cd18-90	1–175	712	686	8836.7–6098.1 5450.1–4566.8	1st deri.	14	98.2	7.37	0.093	13697	119/72/-2.55	45/46/0.21	48/46/ -0.27
TPC [%]	DGF C-III 3b/DGF C-III 3e Rapid method	0.3–44.2	1196	934	6879.1–6098.1 5450.1–4551.4	1st deri. and vectornorm	13	99.25	0.812	−0.0445	11.6	17.37/17.37/0.0	15.77/15.92/0.10	11.66/11.19/-0.32
DPTG [%]	DG F C-III 3d/ ISO 16931/ AOCS Cd 22	0–29.3	1065	759	8929.3–6291 5415.4–4536	1st deri. and vectornorm	14	98.86	0.59	0.0525	9.41	9.07/8.53/-1.10	6.02 /6.85/ 1.06	4.69/4.25/-1.68
MONOX [%]	Márquez-Ruiz et al. J. Amer. Oil Chem. Soc. 1995, 72, 1171–1176.	0–11	1536	828	8836.9–6098.3 5450.3-5215	1st deri. and vectornorm	14	98.89	0.137	−0.00492	9.52		No results available	
MG/DG [%]	No reference method available	0.7–10.0	276	285	8998.6-6098.1 5450.1-4597.7	1st deri. and vectornorm.	14	99.47	0.122	0.0123	13.8		No results available	
Lovibond Red (Units)	DGF C-IV 4b/ISO 27608/AOCS CC13e-92	1.3–34	890	616	7502.1–6098.1 5450.1–4520.5	1st deri. and vectornorm	14	97.83	0.614	0.0008	6.79	12.5/9.1/−0.48	10.0/9.0/−0.48	1.7/1.8/0.25
IV	DGF C-V 11d/ ISO 3961/AOCS Cd 1c-85 q	29.2–96.5	720	706	8959.3–7498.3	1st deri. and vectornorm	15	99.99	0.228	−0.00419	96.6	80.81/81.7/−0.08	79.80/80.15/0.22	82.01/ 81.8/ −0.14

(*Continued*)

Table 1. (Continued).

Property	Reference methods	Range	Calibration data		Validation data (external test set)	Measuring	Data pre-treatment	Validation					Correctness-Robustness (GF Proficiency Test (Median /NIR Value/z-Score))		
			N Spectra		N Spectra	Wave-number region cm^{-1}		Rank	R2	RMSEP	Bias	RPD	2019	2020	2021
C16:0 [%]	DGF C-VI 11d/ ISO 5509/ AOCS Ce 1h-05	0–40	1116		1131	9002.8–6734.27 5377–4470.5	1st deri.	13	99.09	0.857	−0.0754	10.4	8.38/8.99/2.44	14.9/13.36/−2.43	5.74/6.05/2.33
C16:1 [%]	DGF C-VI 11d/ ISO 5509/ AOCS Ce 1h-05	0–3.6	1132		427	5304.6–5292.11	vectornorm	13	98.66	0.0812	0.00543	8.65	0.26/0.21/−1.00	0/0.0/0. −2.60	0.27/0.28/0.20
C18:0 [%]	DGF C-VI 11d/ ISO 5509/ AOCS Ce 1h-05	0.5–8.9	363		378	9004–8252	1st deri. and vectornorm	15	99.73	0.092	−0.0034	19.3	2.93/2.94/0.12	3.589/3.5/−0.52	2.91/2.28/−2.42
C18:1 (9c) [%]	DGF C-VI 11d/ ISO 5509/ AOCS Ce 1h-05	0–79.5	1368		1133	8959.8–7636.8 7197.1–6753.6 5434.5–4551.2	1st deri. and vectornorm	15	99.67	1.11	−0.00304	17.5	44.38/42.3/−2.02	49.43/52.78/0.84	66.57/63.81/−2.46
C18:1 (11c) [%]	DGF C-VI 11d/ ISO 5509/ AOCS Ce 1h-05	0.6–3.5	755		754	8539.9–6314.3 5427.1–4536.1	1st deri. and vectornorm	14	97.52	0.118	0.00129	6.36	1.52/1.59/0.37	1.97/2.26/1.25	1.93/2.35/1.48
C18:2 (6c) [%]	DGF C-VI 11d/ ISO 5509/ AOCS Ce 1h-05	2.5–68.2	1176		1240	8050–7151.3 5809–4910.2	1st deri. and vectornorm	13	99.8	0.711	0.0246	22.6	37.9/38.17/0.56	19.13/18.27/−1.14	21.07/21.94/2.81
C18:3 (alpha) [%]	DGF C-VI 11d/ ISO 5509/ AOCS Ce 1h-05	0.04–10.7	997		721	8879–7957.5 7502.3–7039.4 6584.3–6121.4 5666.3–5203.4	1st deri.	15	98.1	0.275	0.012	7.27	1.86/1.74/−1.20	0.592/0.935/6.35	1.06/0.94/−2.26
Epoxy-fatty acids [g kg^{-1}]	Mubiru et al. J. Agri. Food Chem. 2014, 62, 2982	0–9	133		159	8983.2–8096.1 7656.4–6765.4 6325.7–5434.7 4995–4551.4	1st deri.	16	96.2	0.0673	−0.00687	5.4	No results available		

and was able to observe a reduction in the acrylamide content in French fries. Unfortunately, citric acid is unstable above 100 °C and decarboxylates quickly.[19] In an asparagine-glucose model system,[32] the impact of pH on the formation of acrylamide reactions was studied between 120 and 200 °C. By increasing acidity, the acrylamide formation could be significantly reduced at temperatures above 160 °C. Lowering the pH of the cut potatoes (e.g., with citric acid 0.5–1.0% < 20 min) has been shown to lower the levels of acrylamide formed.[31]

With increasing thermal stress, the indicators for oxidative and polymeric fat degradation change continuously. These oxidative and thermal reactions give rise to a large number of compounds that are usually described summarily by parameters such as AnV, TPC, thiobarbituric acid number, and others. Some parameters such as TPC, AnV, or MONOX even change their composition with increasing thermal load. The composition of oxidation products also changes with temperature. Gertz et al.[33] used this to identify an unpermitted thermal treatment (soft deodorisation or refining) of virgin olive oil. In TPC, the content of DPTG increases with temperature, while the proportion of oxidized components such as MONOX decreases. MONOX are relatively stable compounds that result from the breakdown or decomposition of the primary oxidation products, i.e., the hydroperoxides, and are characterized by the presence of extra oxygen in at least one of the fatty acyl chains of the molecule. Hydroxy, keto, and epoxy are the main functional groups identified in the acyl chains attached to the TAG molecule in used frying fats and oils.[34] The sum of the amounts of epoxy-, keto-, and hydroxy-fatty acids is between 39% and 52% of TPC.[35]

In our test with rapeseed oil (**Table 2**), the MONOX content in the polar fraction (TPC) increased to about 44% after 32 h. However, it is striking that the AV increases but, contrary to expectation, the content of the hydrolysis products MAG and DAG remained constant which confirms other observations.[19] Mechanistically, hydrolysis of TAGto FFA and MAG and DAG would occur in stoichiometric amounts. During frying, water evaporates from the food rapidly and is released into the oil. Up to now, it is believed that under frying conditions the hydrolysis of TAGis affected by the presence of water in the fried product to form DAG, MAG, FFA, and glycerol.[36] Chang et al.[37] observed that soybean or corn oil showed a higher FFA content when used for continuous heating without food than for the same length of time with food. In heating tests with vegetable oils without the addition of water or food significant increase in AV is always observed.[38]

Cuesta et al.[39] observed during 75 successive frying cycles with sunflower oil that TPC, MONOX and TAG polymers increased rapidly during frying, while DAG and FFA levels, related to hydrolytic alteration, did not increase with continued frying.

Generally, FFA or AV are characterized by the amount of free fatty acids supposedly released from the glycerol backbone.[40] Both, AV and the content of FFA are determined titrimetrically in the same way. Only the calculation of their results (FFA*2 = AV) is done differently. During titration the type of functional acid groups cannot be distinguished. Water has a protective role during frying.[41] The evaporating water bubbles from the food create a steam blanket above the oil surface that reduces the contact of the oil with air, whereas large bubbles formed often during the entry of food may cause more oxidation but no hydrolytic effect.

Predominantly, through the degradation of the 13-hydroperoxide group of linoleic acid and further oxidative reactions and from 8-hydroperoxide of oleic acid, oxidized fatty acid fragments with a chain length of 8 C and 9 C atoms are finally formed, which remained bound to glycerol as a backbone having a molecular mass corresponding to a DAG.[42] Thus, in gel permeation chromatography (GPC) separation of TAG in frying fats according to method DGF-C-III 3d (02),[43] the DAG and the fragmented oxidzed TAG are eluted together with the same retention time in the chromatogram, as they have the same molecular weight. These peaks are often identified as DAG. In other words, the GPC separation analytical method potentially over-estimates products of hydrolytic reactions, and by extension, the prevalence of hydrolysis during batch frying. While the hydrolysis of fats and oils releases compounds with a known structure, i.e., DAG and fatty acids, MONOX, characterized by the presence of oxygen in at least one of the fatty acyl groups of the molecule (hydroxy, peroxy, epoxy), are formed by oxidation.

With the exception of PO, frying fats used contain only very small amounts of DAG (1–1.6%). It is noticeable that the acrylamide content in the test (**Table 3**) does not increase steadily with the time, in contrast to the formation of TPC, whereas many authors have shown that the acrylamide content also increases with increasing TPC content.[44–45] However, Table 2 clearly shows the trend that after a certain period of use of the oil, at 170 °C as well as 180 °C, the acrylamide content suddenly drops by about 40%. This phenomenon has not yet been described in the literature. The reason may be that most frying oil tests had been executed in a lab study not for such a long time. A closer look at the values in Table 2 and **Figure 1** shows that the drop in the acrylamide level in all test series starts at an AV value of about 0.5 mg KOH g^{-1}. Obviously, the increasing AV value of the oil during frying is caused by the oxidation of unsaturated fatty acids (see decrease of linoleic level in Table 2), while the level of DAG is unchanged, as previously explained). In a recent paper,[46] it is demonstrated that carboxylic acids with a glycerol backbone contribute to an increase in AV and not in FFA. They were identified by ultrahigh performance liquid chromatography time-of-flight mass spectrometry (UPLC-Tof/MS). The quantification of the carboxylic acids was performed by gas chromatography-mass spectrometry (GC-MS). It is assumed that acidic functional groups bound in oxidized TAGs act as catalysing agents during deep-frying resulting in the formation of further degradation products. A similar catalytic effect is observed for the formation of dimerized TAGs under frying conditions at 160–190 °C.

Up to now, it is generally assumed that the deterioration of fats and oils during the frying process follows a radical mechanism. The formation of dimeric and polymeric TAGs requires a lot of energy. During refining of fats, dimeric TAGs are formed at about 220 °C in a system without oxygen.[47] This formation is based on a radical mechanism and Diels–Alder-reaction products could be found using NMR. In biodiesel, phenolic antioxidants such as TBHQ are effective stabilizers and the Rancimat test provides correct information on the stability of biodiesel used above 220 °C. The situation is different in the batch frying process, where phenolic antioxidants are ineffective and the heat stability of the oil cannot be determined with the Rancimat test. Under frying conditions (160–190 °C with access of oxygen) when also dimeric TAG are formed no evidence for Diels–Alder reaction products

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Table 2. Frying tests—rapeseed oil, 170 and -180 °C, respectively, 0–32 h.

	Heating temperature 170 °C						Heating temperature 180 °C					
Heating time [h]	1	4	11	18	25	32	0	4	11	18	25	32
AV [mg KOH] g^{-1}	0.40± 0.02	0.36± 0.01	0.39± 0.02	0.35± 0.02	0.46± 0.01	0.55± 0.01	0.39± 0.02	0.38± 0.01	0.39± 0.02	0.50± 0.01	0.48± 0.03	0.59± 0.01
Mono- and MAG/DAG [%]	0.6± 0.1	0.6± 0.0	0.6± 0.1	0.5± 0.1	0.6± 0.1	0.6± 0.1	0.6± 0.0	0.6 0.1	0.5± 0.1	0.4± 0.1	0.4± 0.1	0.5± 0.1
AnV	44.1± 0.3	53.8± 0. 5	53.4± 1.1	71.0± 0.8	91.2± 0.6	99.9± 1.1	44.8± 1.0	53.9± 1.0	73.6± 0.8	85.9± 0.4	96± 0.7	103 ± 0.6
TPC [%]	4.0± 0.1	5.9± 0.1	5.8 0.1	10.4± 0.1	17.0± 0.0	19.9± 0.0	4.0± 0.0	6. 2± 0.1	11.2± 0.1	15.2± 0.0	18.6± 0.1	21.5± 0.0
MONOX [%]	2.3± 0.1	3.2± 0.0	3.2± 0.0	5.1± 0.0	7.7± 0.0	8.9± 0.0	2.4± 0.0	3.3± 0.0	5.5± 0.0	7.1± 0.0	8.4± 0.0	9.4± 0.0
DPTG TAGs [%]	0.6± 0.1	1.7± 0.04	1.7± 0.1	4.6± 0.1	8.8± 0.04	10.6± 0.05	0.6 0.05	1.8± 0.1	5.0± 0.1	7.7± 0.03	9.8± 0.1	11.7± 0.02
LOVIBOND RED	5.7± 0.1	6.6± 0.2	6.4± 0.4	8.1± 0.3	10.1± 0.1	10.9± 0.3	5.8± 0.2	6.8± 0.4	8.9± 0.4	9.6± 0.1	10.7± 0.2	11.0± 0.2
Iodine value	108± 1.7	107± 0.1	107± 0.1	105± 0.1	101± 0.1	99.6± 0.1	108±0.1	107± 0.1	104± 0.0	102± 0.0	101± 0.1	98.9 1.7
Epoxy fatty acids [g kg^{-1}]	0.3± 0.1	0.4± 0.1	0.5± 0.1	1.0± 0.0	1.6± 0.1	1.9± 0.1	0.3± 0.1	0.5± 0.1	0.9± 0.1	1.4± 0.1	1.7± 0.1	2.3± 0.1
Trans fatty acids [%]	1.3± 0.02	1.2± 0.01	1.2± 0.02	1.1± 0.03	1.2± 0.03	1.2± 0.03	1.3± 0.01	1.2± 0.04	1.2± 0.02	1.1± 0.02	1.3± 0.05	1.3± 0.03
C16:0 [%]	5.0± 0.02	5.3± 0.05	5.3± 0.10	6.5± 0.06	8.03± 0.06	8.42± 0.05	4.8± 0.12	5.5± 0.10	6.7± 0.03	7.5± 0.07	8.1± 0.04	8.5± 0.03
C18:0 [%]	1.67± 0.05	1.67± 0.04	1.72± 0.05	1.74± 0.04	1.68± 0.03	1.66± 0.03	1.7± 0.02	1.7± 0.03	1.7± 0.05	1.6± 0.012	1.6± 0.05	1.7± 0.02
C18:1 9c [%]	64.8± 0.44	64.4± 0.45	65.3± 0.59	64.8± 0.30	63.5± 0.33	62.5± 0.45	65.0± 0.17	64.8± 0.52	64.1± 0.64	63.6± 0.36	62.5± 0.63	62.2± 0.32
C18:2[%]	18.2± 0.81	17.8± 0.82	19.3± 0.84	19.2± 0.46	16.9± 0.37	16.4± 0.67	18.7± 0.21	18.5± 1.290	17.8± 0.751	17.6± 0.36	16.3± 0.65	16.9± 0.35
C18:3 [%]	7.4± 0.05	7.0± 0.12	7.0± 0.05	6.4± 0.09	5.8± 0.04	5.6± 0.09	7.27± 0.03	6.8± 0.03	6.3± 0.10	5.8± 0.05	5.5± 0.08	5.4± 0.04
Dry matter [%] (French Fries)		53.8	57.1	54.4	59.9	55.2		53.1	55.4	56.6	55.4	57.6
Fat content [%] (French Fries)		11.9	11	11.2	13.1	10.5		10	10.4	11.5	10.9	11.4
Acrylamide [µg kg^{-1}] Dry matter		146± 58.4	214± 85.6	189± 75.6	253± 101.2	74± 29.6		592± 236.8	747± 298.8	227± 90.8	231± 92.4	230± 92
Acrylamide [µg kg^{-1}] French Fries		88± 35.2	141± 56.4	132± 52.8	252± 100.8	158± 6 3.2		517± 206.8	587± 234.8	184± 73.6	199± 79.6	215± 86

Table 3. Comparison of some analytical parameters after frying in a restaurant with sunflower oil and a fast food restaurant with rapeseed oil, respectively, with the use of magnesium silicate and filtration and without treatment after frying (final frying of French fries for investigation in a lab fryer).

	Test A	Test B
Location	Fast food	Restaurant
Frying oil	Rapeseed	Sunflower
Frying temperature	170–180 C	170–180 C
Use-life (to reach about 13% TPC)	10 h	6 h
TPC [%]	13.0	16.4
DPTG [%]	4.2	7.5
MONOX [%]	6.2	7.7
Acid Value [mg KOH g^{-1}]	0.8	0.4
Acrylamide (µg kg^{-1} dry matter)/French fries fried in the laboratory (3 min 45 s; 180 °C)	160 (± 96)	380 (± 96)
Treatment with 2% magnesium silicate and filtration		
TPC [%]	12.3	16.5
DPTG [%]	3.8	7.5
MONOX [%]	6.2	7.7
Acid Value [mg KOH g^{-1}]	0.3	0
Acrylamide (µg kg^{-1} dry matter) French fries fried in the Laboratory (3 min 45 s; 180 °C)	600 (± 96)	730 (± 183)

was found.[48] Many authors[49,50] demonstrated that dimerization of unsaturated fatty acids can also be initiated by a cationic mechanism without forming Diels–Alder products. In this case, the data in Table 2 indicate that a certain level of acidity (AV ≥ 0,5) results in a reduction of acrylamide formation.

The first tests were carried out in the laboratory under controlled conditions with rapeseed oil. Further tests were conducted with oil obtained from a restaurant and a fast food restaurant, respectively. These oils had TPC of about 10%. The use of fryers in both restaurants was very different because the number of frying cycles per hour was obviously much higher in the snack bar than in the restaurant. For comparable results the final frying experiments were carried out with lab fryers under standardized conditions using the above-mentioned oils. The acrylamide content and the thermal degradation products were also determined here (Table 3). The acrylamide content of French fries fried in oil with a AV > 0.5 mg KOH g^{-1} oil, obtained from the fast food restaurant was lower in comparison to French fries fried in oil from a restaurant with a AV below 0.5 mg KOH g^{-1} oil. This result from practise is in line with the above-mentioned theory that a certain level of acidity results in lower acrylamide contents in fried products.

In order to reduce the so-called FFA value, active filter aids based on magnesium silicates such as Magnesol, Britesorb, or Hybersorb are often used in fast food restaurants to reduce the "FFA"-value and thus extended the period of usability of the oils. To investigate the effect of such filter aids, 2% of Magnesol was added to oil from both restaurants before filtering the oils and using them for frying under standardized conditions in lab fryers. The TPC content is only slightly reduced because this adsorbent can only adsorptively bind about the same amount of polar substances as the adsorbent (Table 3). Strong acid groups in the TAG (not FFA), on the other hand, are obviously better bound by these strongly polar adsorbents such as magnesium silicate. A reduction of the "FFA" value only leads to an apparent improvement of the frying fat and thus only feigns a better than actual quality, since the TPC content as an objective measure is not significantly reduced.

4. Conclusion

Results from frying experiments in the literature on the formation of acrylamide are very contradictory with regard to the influence of both the type of frying oils and the specific nature of degradation products on acrylamide formation.

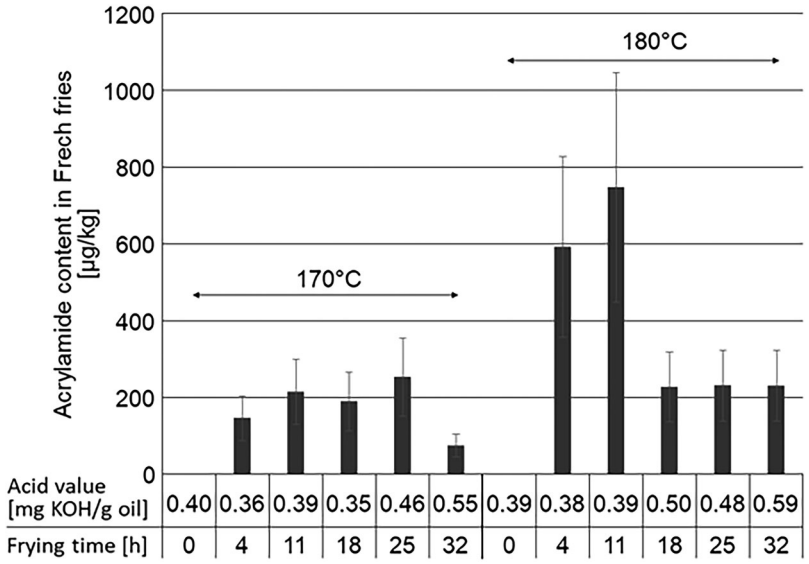

Figure 1. Acrylamide content in French fries fried at 170 and 180 °C, respectively, at different acid values.

A common misconception that FFA are formed by hydrolysis of TAGs during the frying process, and that these are determined as AV or FFA has led to a failure to recognize the interaction of acidic functional groups bound in MONOX. Since acrylamide formation is pH-dependent, it stands to reason that a change in pH through the removal of acidic functional group in the oil will significantly affect acrylamide formation. Although more data points are needed, it is clear from the current study that an AV value at or above 0.5 strongly inhibited acrylamide formation. The reduction of acidity by active filter aids binding acidic compounds in used frying oils to reduce the "FFA" below 0.5 will lead obviously to a higher level of acrylamide in fried food.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

C.G.: Conceptualization, Project administration (lead), Investigation (equal), Validation (equal), Writing—original, review, editing (equal); F.A.: Validation (equal), Writing—original, review, editing (equal); M.P.: Investigation (equal), Validation (equal), Writing—review, editing (equal); B.M.: Validation (equal), Writing—original, review, editing (equal).

Data Availability Statement

Research data are not shared.

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