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Phenylacrylic acids addition to potato and sweet potato showed no impact on acrylamide concentration *via* oxa-Michael-addition during frying

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ABSTRACT

Three phenolic acids, *p*-coumaric, ferulic and caffeic acid as well as cinnamic acid were added to raw potatoes and sweet potatoes before frying. A distinct mitigation of acrylamide was not detected. Fried samples were analysed for postulated adducts of a direct reaction between acrylamide and these phenolic acids using LC-MS. In a model system with pure compounds (phenylacrylic acid and acrylamide) heated on 10% hydrated silica gel one specific adduct (respective m/z for M + H$^+$) was formed in each reaction. MS/MS-data suggested an oxa-Michael formation of 3-amino-3-oxopropyl-phenylacrylates, which was confirmed by *de novo* syntheses along an S$_N$2 substitution of 3-chloropropanamide. Exemplarily, the structure of the ester was confirmed for *p*-coumaric acid by NMR-data. Standard addition revealed that 3-amino-(3-oxopropyl-phenyl)-acrylates occurred neither in fried potato nor in sweet potato, while a formation was shown in phenylacrylic acid plus acrylamide supplemented potatoes and sweet potatoes.

1. Introduction

In 2002, acrylamide was identified as a product of the Maillard reaction of free L-asparagine and a carbonyl source in heated carbohydrate-rich food (Mottram et al., 2002; Tareke et al., 2002). Acrylamide was classified as potentially carcinogenic by the International Agency for Research on Cancer (IARC, 1994). Due to the omnipresence of acrylamide in heated foods, a complete prevention of acrylamide intake is nearly impossible. The World Health Organisation (WHO) estimated the average daily intake of dietary acrylamide from 0.3 to 2.0 µg/kg body mass for the general population (Besaratinia and Pfeifer, 2007). Nevertheless, the margin of exposure is considered to be low for a genotoxic and carcinogenic substance (JEFCA, 2005; Matoso et al., 2019).

Since the first publication in 2002, the reaction mechanism of acrylamide formation in food was thoroughly examined (Zyzak et al., 2003), and much research was devoted to a deeper understanding the impact factors of its formation (Rydberg et al., 2003). Online monitoring of heated model systems using APCI-MS showed a constant acrylamide increase over 15 min and confirmed the established chemical pathways leading to acrylamide (Channell et al., 2008). A first review on mitigation strategies appeared in 2007 (Zhang and Zhang, 2007). Besides changing the process conditions towards shorter frying and baking times or lower

frying and baking temperatures, the lowest acrylamide concentrations were obtained by adding competing amino acids (Koutsidis et al., 2009; Vinci et al., 2012), or by the enzymatic conversion of the precursor L-asparagine using suitable asparaginases (Xu et al., 2016; Rottmann et al., 2020).

While for these minimization measures the chemical mechanisms are evident, the use of antioxidants led to contradictory results, and a plausible reaction mechanism for a mitigation has not yet been established so far. One reason for this might be the high structural inhomogeneity of antioxidants (Jin et al., 2013).

Phenolic compounds, typically derivatives of cinnamic acid, are among the most common natural antioxidants (Bassama et al., 2010) and are widely distributed in fruits and vegetables (Lafay and Gil-Izquierdo, 2008). A statistical analysis correlated naturally occurring phenolic compounds (e.g. chlorogenic acid, caffeic acid, tyrosine) in potato powder inversely with the acrylamide concentration in the fried products (more phenolics, less acrylamide) (Zhu et al., 2010). A study on potatoes with different flesh colour suggested a mitigating effect of chlorogenic acid and total phenolic acids on the acrylamide yield (Kalita et al., 2013). Direct addition of phenolic compounds showed no significant reduction of acrylamide (Bassama et al., 2010), but the opposite was found in an emulsion system with a direct addition of a mixture of phenolics to the

frying oil (Kotsiou et al., 2011). Natural and synthetic antioxidants slowed the deterioration of sunflower oil, which indirectly decreased acrylamide formation (Urbančič et al., 2014). A similar study added lipophilic grape seed proanthonycanidins to the frying oil and attributed the mitigation to an interception effect on acrolein (Yu et al., 2020).

Rosemary extracts decreased acrylamide in a non-dose-dependent manner in wheat buns, dittany (*Origanum dictamnus*) increased the acrylamide concentration (Hedegaard et al., 2008).

Many studies attempted an explanation of the described effects, but only a few enlightened the actual reaction mechanism. In two exemplary studies, one using *p*-coumaric acid and another one using rosmarinic acid, possible mitigation mechanisms were identified. The addition of *p*-coumaric acid led to significantly lower acrylamide level in both model reaction systems and potato crisps. Reaction of 3-oxopropanamide, a presumed side product of the acrylamide pathway, with *p*-coumaric acid in a low-moisture model system was supposed to yield a 2-(propenamide) coumaric acid adduct (Xu and An, 2016). Rosmarinic acid was demonstrated to reduce the acrylamide content significantly. A direct reaction of the vinyl group of acrylamide with one or more hydroxy groups of rosmarinic acid *via* Michael-addition was observed (Yuan et al., 2019). This was in agreement with earlier experiments in model systems, in which various nucleophiles with amino-, thiol-, and hydroxyl groups added onto acrylamide in a Michael-type reaction (Adams et al., 2010).

While some publications reported a mitigation, others did not find a significant effect, and a dose-dependency indicating a true correlation was not always described (Hedegaard et al., 2008; Bassama et al., 2010). These conflicting results, many of them deduced from model systems (Jin et al., 2013), call for a clarification, if phenylacrylic acids are capable of mitigating acrylamide formation in foods. This study investigated possible adduct formation reactions of acrylamide in fried potato and sweet potato using hydroxyphenylacrylic acids (*p*-coumaric, caffeic, ferulic acid), and cinnamic acid, a non-phenolic phenylacrylic acid as control. Basically, two different reactions were supposed, either a nucleophilic attack of a phenolic hydroxy group of the 3 hydroxyphenylacrylic acids directly at the α,β-unsaturated (Michael-)system of acrylamide (Yuan et al., 2019), or an electrophilic substitution at the phenolic ring of all 4 phenylacrylic acids by 3-oxopropanamide, a side product of acrylamide formation during the Maillard reaction (Xu and An, 2016). To prove the two hypothesis, the pure chemicals were reacted, the products analysed *via* UPLC-QToF-MS/MS, and the structures confirmed by spectral data. The reaction was then repeated in food to assess its relevance under realistic conditions.

2. Material & methods

2.1. Samples, chemicals, and materials

Potato tubers (variety *Linda*) and sweet potato roots were purchased from a local supermarket (Rewe, Hannover, Germany) and directly processed. Tubers and roots were peeled and cut into 512 mm^3 (8 × 8x8 mm) cubes (French fries cutter, Küchenprofi, Solingen, Germany). Frying palm oil was purchased from Cargill (Schiphol, The Netherlands). The fatty acid composition of the frying oil was C16:0, 39.5%; C18:0, 3.5%; C18:1, 36%; C18:2, 7.5%. Purity grades and distributors of chemicals used were: Acetonitrile (ROTISOLV® LC-MS grade), water with 0.1% formic acid (ROTISOLV® LC-MS grade), methanol (ROTISOLV® LC-MS grade), hexane, ethanol (ROTISOLV® HPLC gradient grade (high UV transmission), *p*-coumaric acid, caffeic acid (≥98%), ferulic acid (≥98%), magnesium sulfate (≥99%, per analysis (p.a.), water free), sodium chloride (99.8%), acrylamide (99.9%, p. a.), hydrogen chloride (37%, European Pharmacopoeia quality (Ph.Eur. quality), potassium hydroxide (≥85%, Ph. Eur. quality), and potassium iodide (≥99%, Ph. Eur. quality) purchased from Carl Roth (Karlsruhe, Germany). Cinnamic acid (97%), 3-chloropropanamide (98%), $^{13}C_3$- acrylamide (99 atom % ^{13}C, 98%), Silica gel (diameter: 1–3 mm, technical grade), and glass beads (diameter 5 mm, soda-lime glass) purchased from Sigma-Aldrich (MO, USA). Filter

paper (frit filter, 0.45 μm, cellulose acetate) purchased from Sartorius (Göttingen, Germany). MeOD (99.96 atom % D) was purchased from MSD isotopes (Merck Frosst, Montreal, Canada).

2.2. Adduct synthesis with acrylamide in a model system

Equal portions of 0.7 mmol of the respective phenylacrylic acid and acrylamide were mixed with 9 g silica gel and dispersed in 1 mL water, simulating the water activity of the frying process. The mixture was heated in an oven for 20 min at 180 °C (H2661–1 B, Miele, Gütersloh, Germany). The experiment was carried out once for each phenylacrylic acid.

2.3. Adduct synthesis with 3-chloropropanamide

Firstly, 6.1 mmol of the respective hydroxycinnamic acid or cinnamic acid (summarized as phenylacrylic acids) were mixed with 6.7 μmol potassium iodide in 2.437 mL ethanol in a 100 mL three-necked round bottom flask with dropping funnel (Labor-und Analysen-Technik (LAT), Garbsen, Germany). Under nitrogen atmosphere 12.7 mmol potassium hydroxide in 2.437 mL water were added within 10 min (0.25 mL/min). The solution was heated to 40 °C and 6.7 mmol 3-chloropropanamide was added within 10 min (72 mg/min). Under reflux (reflux condenser, (LAT), Garbsen, Germany), the solution was mixed at 76 °C overnight to complete precipitation. Water and concentrated hydrochloric acid (12 mol/L), each 2.843 mL were added sequentially under ice cooling and the suspension was filtered with a frit under vacuum. The filter cake was washed twice with 10 mL water and re-suspended in 10 mL acetone. The product was evaporated at 50 °C to dryness (rotary evaporator, Laborota 4000, Heidolph, Schwabach, Germany) ((Liu et al., 2011; Kondo et al., 2012) with modifications). The four reaction products were analysed by means of LC-MS/MS and UPLC-QToF-MS/MS and used as references. The experiment was carried out once for each phenylacrylic acid.

2.4. Adduct synthesis of p-coumaric acid with 3-chloropropanamide for NMR analysis

To a solution of 5 g of *p*-coumaric acid in 60 mL of ethanol in a 250 mL three-necked round bottom flask 60 mg of potassium iodide were added. A solution of 3.5 g potassium hydroxide in 12.5 mL H$_2$O was filled into a 50 mL dropping funnel with ventilation and the entire apparatus (LAT, Garbsen, Germany) was pre-flushed with N$_2$ for 2 min. After that, the dropping funnel was connected with the three necked round bottom flask and the KOH solution was added dropwise to the synthesis reaction mixture. The reaction mixture was heated to 45 °C until a clear solution was obtained. Then 6.5 g of 3-chloropropanamide were added and the approach was heated for 24 h at 76 °C. After cooling to room temperature (RT) white crystals precipitated and 30 mL H$_2$O were added. The suspension was extracted three times with diethyl ether and combined organic phases were evaporated with a rotary evaporator (Laborota 4000, Heidolph, Schwabach, Germany) and the solid was re-suspended in 30 mL H$_2$O. Concentrated HCl (1 mL) was added. The resulting precipitate was filtered and washed with ice cold water. Hot water (100 mL, 100 °C) were added so that the white crystals were dissolved. A few brown substances remained undissolved. The solution was kept hot (100 °C) and was filtered. The filter cake was washed twice with 20 mL ice-cooled water. After cooling to RT white crystals precipitated again. The solid was filtered and re-suspended in 50 mL H$_2$O. The pH was adjusted to 11.0 with KOH until a clear yellow solution was formed. Finally, the pH was adjusted to 6.0 with 2 mol/L HCl, which resulted in the precipitation of the product. After cooling at 6 °C in the fridge (Liebherr Profi Line FKU, 1800; Liebherr, Bulle, Switzerland) over night the product was filtered and dried. The purified adduct of 3-chloropropanamide and *p*-coumaric acid was used for NMR. The experiment was carried out once.

2.5. Pre-treatments and frying

To clarify the presumed mitigating effect of phenylacrylic acids, potato and sweet potato cubes (each sample 10 g) were dipped into water or in 0.1 mol/L each of *p*-coumaric, caffeic, ferulic, and cinnamic acid (each solution 50 mL).

To examine whether generation of acrylamide adducts with phenylacrylic acids during frying is feasible at all under the specific conditions in a potato or sweet potato matrix, cubes were supplemented additionally with acrylamide or 3-chloropropanamide and shaken for 10 min. Afterwards, the potato and sweet potato cubes were fried for 6 min at 180 °C (Deep fryer, F28.311. W1, DeLonghi, Treviso, Italy). Raw potato and sweet potato cubes were used for comparison. The samples were homogenized with a lab-mixer (Pulverisette 11, Fritsch, Idar-Oberstein, Germany) and stored at −20 °C in a freezer (Liebhherr Premium 1213, Liebherr, Bulle, Switzerland) until analysed. All experiments were performed in duplicate for each test series. For the determination of the mitigation effect four test series (Potato 1, Potato 2, Potato 3, and Sweet potato) were carried out.

2.6. Extraction of acrylamide and acrylamide adducts

2.6.1. Potato and sweet potato samples

The homogenized sample (1 g) was weighed into a 50 mL tube and 5 glass beads were added. For extraction 5 mL of acetonitrile was added and the mixture was shaken vigorously for 1 min. Afterwards, 5 mL of ultra-pure water and 5 mL hexane were added and mixed at 2000 rpm for 5 min (Multi Vortex Mixer, Heathrow Scientific, IL, USA). After the addition of 2.5 g QuEChERS mix (MgSO4, NaCl (4:1) (Anastassiades et al., 2003) tubes were shaken vigorously for another 1 min. After centrifugation at 4667×g for 7 min, 1.7 mL of acetonitrile layer (supernatant) was transferred without filtration to a screw neck vial (N9, VWR, Radnor, PA, USA).

2.6.2. Model system and syntheses

The synthesis products (10 mg) were mixed with 1 mL methanol and extracted at 2000 rpm for 5 min (Multi Vortex Mixer, Heathrow Scientific, IL, USA). The extract was centrifuged at 4667×g for 6 min. The clear supernatant was used for the analyses. For that reason it was transferred without filtration to a screw neck vial (N9, VWR, Radnor, PA, USA).

2.7. Determination of the acrylamide concentration via tandem mass spectrometry

Acrylamide concentrations in the fried potato and sweet potato cubes were determined as described previously (Rottmann et al., 2020).

2.8. Enzymatically determination of the reducing sugars d-glucose and d-fructose

Reducing sugars were converted enzymatically and measured photometrically according to manufacturer instructions (K-SUFRG, Megazyme, Bray, Ireland).

2.9. Analysis of extracts via tandem mass spectrometry

Extracts were analysed by means of LC-MS and extracted ion chromatograms were explicitly checked for the expected m/z values of acrylamide adducts of the four phenylacrylic acids according to Yuan et al. (2019) and Xu and An (2016). Adduct analyses were performed on a Varian LC-MS/MS system (Pump 212 and MS 320 with vESI housing; Varian) and a HILIC-Column (Nucleodur HILIC 125/4 mm, 5 μm, Macherey-Nagel, Düren, Germany). Sample injection volume was 20 μL. Flow rate was 200 μL/min and isocratic elution (20:80) with water 0.1% formic acid and acetonitrile 0.1% formic acid was performed at a flow rate of 300 μL/min. Mass spectrometry parameters in the positive mode
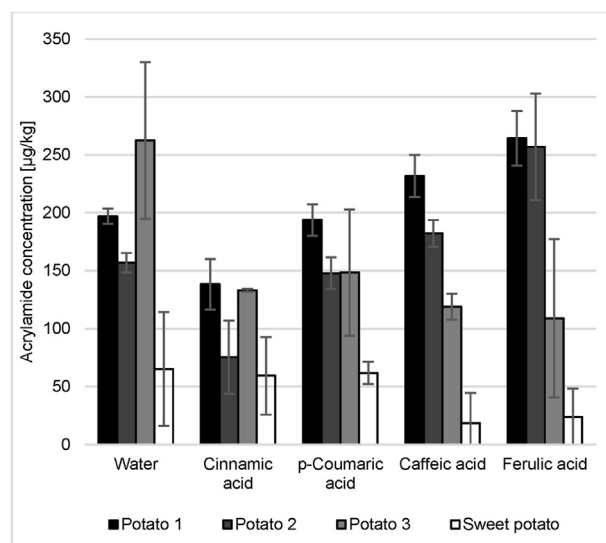

Fig. 1. Acrylamide concentrations [μg/kg] of potato and sweet potato cubes, which were dipped for 10 min in different 0.1 mol/L solutions (water = blank, cinnamic acid, *p*-coumaric acid, caffeic acid, and ferulic acid) and deep-fried for 6 min at 180 °C. Data are mean (n = 2) ± standard deviation.

were as follows: ESI housing 50 °C, drying gas 200 °C, capillary 30 V, collision gas pressure 200 mPa, collision energy 10 V, detector 1500 V. Multiple reactions monitoring (MRM) was carried out: $234 > 50$–234; $236 > 50$–236 for *p*-coumaric acid adducts, $264 > 50$–264; $266 > 50$–266 for ferulic acid adducts, $250 > 50$–250; $252 > 50$–252; $322 > 50$–322 for caffeic acid, and $218 > 50$–218; $220 > 50$–220 for cinnamic acid adducts.

2.10. Determination of the exact mass via UPLC-QToF-MS/MS

Determinations of exact mass were performed on a UPLC system (1290 Infinity II, Agilent, Santa Clara, CA, USA) connected to qToF mass spectrometer (MaXis Impact, Bruker, Billerica, MA, USA). Separation was done with RP-column (Zorbax RRHD SB-C18, 1.8 μm, 2.1 × 100mm, Agilent, Santa Clara, CA, USA). Flow rate was 200 μL/min and the gradient elution of the UPLC with (A) ultra-pure water 0.1% formic acid (B) and acetonitrile was as follows: 0 min: 95% A; 5 min: 95% A; 11 min: 85% A; 22 min: 50% A; 25 min: 20% A; 30 min: 20% A; 35 min: 95% A; 40 min: 95% A. Mass spectrometry parameters in the positive mode were as follows: housing 25 °C, nebulising gas 180 °C, capillary 4500 V. Single ion monitoring (SIM) scanned from 50 to 700 m/z. MS/MS collision energy was 10 eV. Online calibration was done using sodium formate clusters injected at the beginning of each run for 30 s.

2.11. Determination of molecule structure via NMR

The adduct P-3CPA-*p*CA (20.3 mg) was dissolved in 0.8 mL MeOD Spectra were recorded on a 600 MHz spectrometer equipped with Avance Neo console, DCH cryo probe and SampleCase sample changer from Bruker Biospin (Billerica, MA, USA).

2.12. Statistical evaluation

Statistical evaluations were done *via* DataLab 4.0 (Epina Software Labs, Retz, Austria). For test series, which yielded a sufficient number of data points, a full statistical evaluation was conducted. Analysis of significant differences were done *via* the Welch-Test. Furthermore, the three potato series were pooled and analysed for normal distribution (Shapiro-Wilk-test) and significance (Analysis of variance (ANOVA) and two sample-*t*-test).
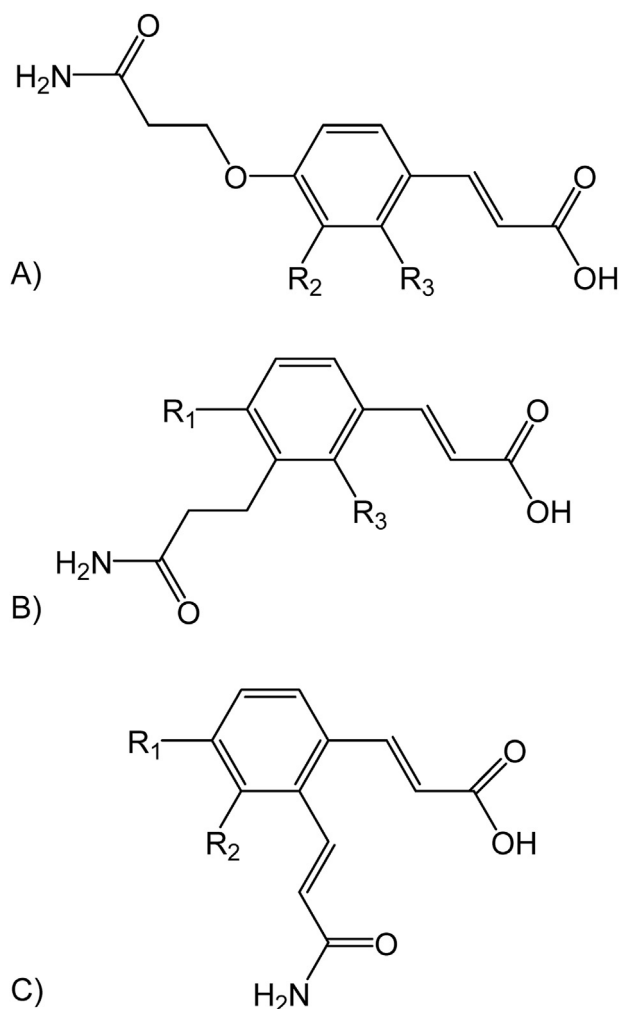
Fig. 2. Structure of postulated Michael-adducts of acrylamide/3-oxopropenamide with phenylacrylic acids (R1 and R2 according to (Yuan et al. (2019) R3 according to Xu and An (2016). A) shows the postulated adduct in *para*-position for *p*-coumaric- caffeic-, and ferulic acid. B) shows the postulated adduct in *meta*-position for cinnamic-, *p*-coumaric-, caffeic-, and ferulic acid. C) shows the postulated adduct in *ortho*-position for cinnamic-, *p*-coumaric-, caffeic-, and ferulic acid. The substituents of the different phenylacrylic acids, which are not a part of the adduct formation reactions are shown as R1, R2, and R3. All substituents are described in more detail in Table 1.

3. Theory

If phenylacrylic acids were able to mitigate the acrylamide yield, indicative reaction products should be formed accordingly. Therefore, this study searched for hypothetical acrylamide adducts. Raw potato and sweet potato cubes were treated with a selection of phenylacrylic acids and deep-fried. Acrylamide concentrations were quantified (Fig. 1), but the data set obtained was inconclusive, as were previous publications.

The treated samples were analysed *via* liquid chromatography coupled to mass spectrometry to substantiate the adduct formation as described in earlier literature. To confirm the theoretical reaction pathways, supposed educts were mixed and reacted in a model system. Product identification was derived from molecular formulae, fragment ions, and retention times and further complemented by structure confirmation using advanced NMR methods.

4. Results and discussion

4.1. Acrylamide mitigation effect of phenylacrylic acids

Acrylamide concentrations in the extracts are shown in Fig. 1. Four subsequent test series were carried out in duplicate with batches of potatoes and sweet potato, respectively. The corresponding glucose and fructose values were 0.22, 0.28; 0.38, 0.44; 0.36, 0.58; and 1.16, 1.67 g/kg for Potato 1, Potato 2, Potato 3, and Sweet potato, respectively. A clear acrylamide mitigation was not detected. According to common statistical benchmarks ($\alpha = 0.05$) the Welch-test indicated no significant differences between the treatments in each of the series. The analysis of the three pooled potato series indicated normal distribution. ANOVA determined no significant difference between the treatments (p-value > 0.05). This was in contradiction to the results published previously (Xu and An, 2016; Yuan et al., 2019).

In the next step, extracts were analysed for adducts. Small signals with the respective m/z value (nominal mass) of protonated molecular ions were detected for adducts of phenolic hydroxyl groups with acrylamide for *p*-coumaric and ferulic acid, but not for cinnamic and caffeic acid (Fig. 2 and Table 1). There was also no signal for a possible double addition product of caffeic acid. Furthermore, not even trace signals were found for postulated adducts with 3-oxopropanamide. Therefore, the examination of a pathway with a putative participation of 3-oxopropanamide was no longer pursued.

4.2. Reaction of acrylamide with phenylacrylic acids in a model system

To confirm the formation of acrylamide adducts with phenylacrylic acids in fried potatoes and sweet potatoes the reaction was simulated using pure phenylacrylic acid and acrylamide in a model system on 10% hydrated silica gel (Schieberle et al., 2005). The surface of French fries is the area of major acrylamide formation (Gökmen et al., 2006). According to literature, frying decreased the water content in French fries from 68.09% to 18.41% (Romani et al., 2009), while the surface concentration decreased to 2% or less (MacMillan et al., 2008). Therefore, the water content on the surface of French fries during the frying process should pass through the water concentration chosen for the model. LC-MS analysis of the respective reaction mixture showed intense [M + H$^+$] signals for Michael-addition derived adducts at 236 m/z for *p*-coumaric acid, 266 m/z for ferulic acid, 252 m/z for caffeic acid, and, surprisingly, also at 220 m/z for cinnamic acid. Supposed nucleophilic centres of phenylacrylic acids are the phenolic hydroxy groups, and therefore no reaction was expected for cinnamic acid due to the missing hydroxyl group. Furthermore, because of the +M-effect of hydroxy groups, electron-rich carbon atoms in *ortho* position may react as nucleophiles

Table 1
Postulated adducts of phenylacrylic acids with acrylamide in fried potato and sweet potato (measured according to 2.7).

	Cinnamic acid	*p*-Coumaric acid		Caffeic acid		Ferulic acid	
	–	a) –O–(CH$_2$)$_2$–CONH$_2$		a) –O–(CH$_2$)$_2$–CONH$_2$		a) –O–(CH$_2$)$_2$–CONH$_2$	
R$_1$	–H	b) –OH		b) –OH		b) –OH	
R$_2$	–H	–H		–OH		–OCH$_3$	
R$_3$	CH=CH–CONH$_2$	b) –CH=CH–CONH$_2$		b) –CH=CH–CONH$_2$		b) –CH=CH–CONH$_2$	
m/z [MH$^+$]	218	a) 236	b) 234	a) 252	b) 250	a) 266	b) 264
Potato	–	a) +	b) –	a) –	b) –	a) +	b) –
Sweet Potato	–	a) +	b) –	a) –	b) –	a) +	b) –

Putative structure based on a) Yuan et al. (2019), b) Xu and An (2016); (+) ion detected, (−) not detected.

Table 2

Measured accurate mass of the protonated molecule ions, elemental composition and most abundant fragment ions of reaction products of phenylacrylic acids and acrylamide in a model system. Accurate mass is described as mass-to-charge-ratio [m/z]. The errors in atomic mass units (amu) between measured and calculated mass are shown in parenthesis.

Adduct of (Retention time)	[M + H$^+$]	[BFI][a]	[M + H$^+$] - [BFI]
p-Coumaric acid (15.2 min)	236.0912 (−0.0010) $C_{12}H_{13}NO_4$+H$^+$	147.0438 (−0.0008) $C_9H_7O_2$	89.0484 (+0.0007) $C_3H_7NO_2$
Ferulic acid (15.7 min)	266.1026 (−0.0002) $C_{13}H_{15}NO_5$+H$^+$	177.0545 (−0.0006) $C_{10}H_9O_3$	89.0481 (+0.0004) $C_3H_7NO_2$
Caffeic acid (12.5 min)	252.0858 (−0.0014) $C_{12}H_{13}NO_5$+H$^+$	163.0388 (−0.0007) $C_9H_7O_3$	89.0470 (−0.0007) $C_3H_7NO_2$
[Z,E] Cinnamic acid (17.1 min) (18.6 min)	220.0974 (0.0000) $C_{12}H_{13}NO_3$+H$^+$	131.0495 (−0.0002) C_9H_7O	89.0479 (+0.0002) $C_3H_7NO_2$

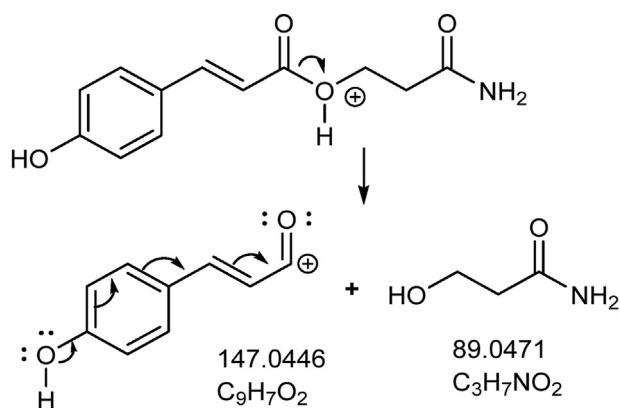[a] BFI = base peak fragment ion.

Fig. 3. p-Coumaric acid acrylamide ester adduct: Ionisation, base ion formation and resonance stabilisation. Calculated exact mass and elemental composition are shown under each structure.

with acrylamide. At elevated temperatures and a low water activity the carboxylate group of phenylacrylic acids may react as a nucleophile in an oxa-Michael addition (Xingyu et al., 2019; Jha et al., 2017).

If phenolic hydroxyl groups were favoured nucleophiles in this reaction, the addition of two acrylamide molecules would be expected for caffeic acid, as it was reported for all four hydroxy-groups of rosmarinic acid (Yuan et al., 2019). However, the extracted ion chromatogram did not show any hint for a participation of both phenolic hydroxy groups in acrylamide interception.

Each reaction mixture was then analysed by UPLC-QToF-MS/MS (Table 2). The accurate mass determination of the reaction products confirmed the elemental composition (molecular formula) of each postulated Michael product. Analytical standards for structure confirmation were not available. Hence, the position of the acrylamide addition remained unknown and required additional MS/MS data. Parameters (collision energy: 10 eV) for collision induced dissociation (CID) were tuned to cleave mainly the weakest bonds within the product molecules. Beside less abundant nonspecific neutral losses of water and ammonia, MS/MS conditions resulted in base ions, of which the accurate masses and elemental compositions are shown in Table 2. More indicative was the difference of protonated molecular ions and the respective base ion, which was 89 amu for each of the acrylamide adducts of the four phenylacrylic acids used in the model system. In each case the accurate mass of this neutral loss corresponded to $C_3H_7NO_2$, which must be attributed to 3-hydroxypropanamide. Such a cleavage can be explained only for oxa-Michael-addition products resulting from the reaction of the carboxylate group of the respective phenylacrylic acid with acrylamide. The fragmentation of the ester and the resonance stabilisation of the resulting base ion are shown exemplarily for p-coumaric acid in Fig. 3. The delocalisation of the positive charge over the entire molecule strongly favours this fragmentation reaction in CID. For respective Michael-adducts of acrylamide with phenolic hydroxy groups or with nucleophilic carbon atoms of the aromatic ring a fission of 89 amu is unlikely. The latter, upon cleavage of a supposed ether bridge, would not contain a 3-hydroxypropanamide moiety, as for phenol ethers a fission of a phenol moiety (cleavage of the bond between oxygen and the carbon side chain) is preferred. Furthermore, the formation of a propanamide ester explains, why the reaction of cinnamic acid generated a Michael-product.

4.3. Synthesis of phenylacrylic acid esters with 3-chloropropanamide

To reinforce the hypothesis of an ester rather than phenol ether formation, the postulated adducts were synthesized along a nucleophilic substitution reaction instead of the presumed oxa-Michael-type addition in the model system. Both reaction mechanisms are compared in Fig. 4. 3-Chloropropanamide was reacted with the respective phenylacrylic acid
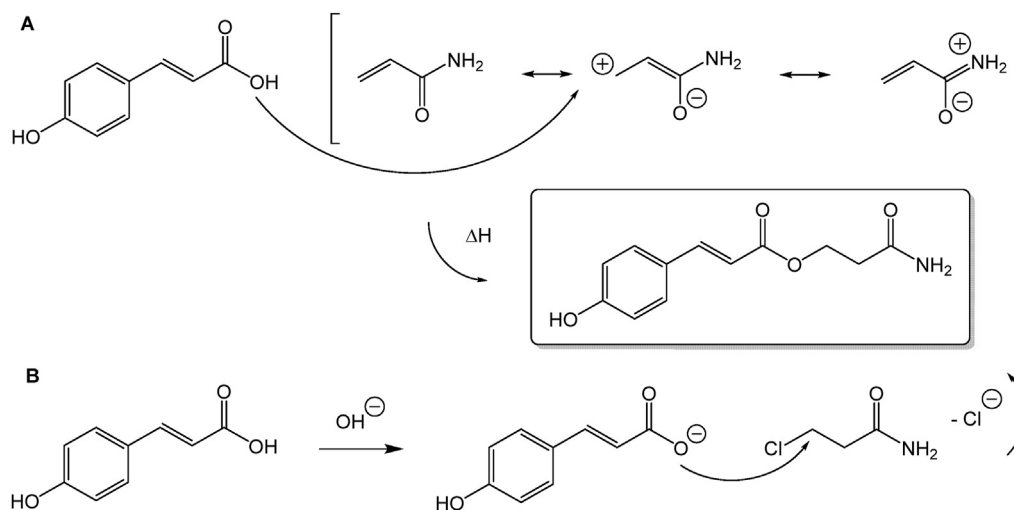

Fig. 4. Reaction mechanism of p-coumaric acid with acrylamide (A) and 3-chloropropanamide (B). Reaction A follows an oxa-Michael-mechanism, reaction B follows SN2.

Table 3

Evaluation of phenylacrylic acid adduct formation in potato and sweet potato.

Retention time [min]	Ø Accurate mass [m/z]	Calculated molecular formula	Found in	Identified substance
12.5	181.05	$C_9H_9O_4^+$	C1, C2, C3	caffeic acid
13.5	252.09	$C_{12}H_{14}NO_5^+$	C2, C3	3-amino-3-oxopropyl-3-(4-dihydroxyphenyl)acrylate
14.3	258.07	$C_{12}H_{13}NO_4Na^+$ $C_{10}H_8N_7O_2^+$	UP, USP, A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3	not identified
14.8	165.05	$C_9H_9O_3^+$	A1, A2, A3	*p*-coumaric acid
15.2	236.09 258.07	$C_{12}H_{14}NO_4$ $C_{12}H_{13}NO_4Na^+$	A2, A3	3-amino-3-oxopropyl-3-(4-hydroxyphenyl)acrylate
15.6	195.07	$C_{10}H_{11}O_4^+$	B1, B2, B3	ferulic acid
15.7	288.09	$C_{13}H_{15}NO_5Na^+$	B2, B3	3-amino-3-oxopropyl-3-(4-hydroxy-3methoxyphenyl)acrylate
18.3	242.08	$C_{12}H_{14}NO_3^+$ $C_{10}H_8N_7O^+$	UP, USP, A1, A2, A3, B1, B2, B3, C1, C2, C3, D1, D2, D3	not identified
18.6[a]	242.08	$C_{12}H_{14}NO_3^+$	D2, D3	3-amino-3-oxopropyl-3-acrylate
19.6	149.06	$C_9H_9O_2^+$	D1, D2, D3	cinnamic acid

UP = Untreated fried potato.
USP = Untreated fried sweet potato.
A1 = Fried (sweet) potato + *p*-coumaric acid.
A2 = Fried (sweet) potato + *p*-coumaric acid + acrylamide.
A3 = Fried (sweet) potato + *p*-coumaric acid + 3-chloropropanamide.
B1 = Fried (sweet) potato + ferulic acidB2 = Fried (sweet) potato + ferulic acid + acrylamide.
B3 = Fried (sweet) potato + ferulic acid + 3-chloropropanamide.
C1 = Fried (sweet) potato + caffeic acid.
C2 = Fried (sweet) potato + caffeic acid + acrylamide.
C3 = Fried (sweet) potato + caffeic acid + 3-chloropropanamide.
D1 = Fried (sweet) potato + cinnamic acid.
D2 = Fried (sweet) potato + cinnamic acid + acrylamide.
D3 = Fried (sweet) potato + cinnamic acid + 3-chloropropanamide.
 [a] Traces of the respective *Z*-isomer were detected as well.

according to an S_N2 mechanism to give the presumed esters (Fig. 4B). Indeed, this reaction resulted in the same phenylacrylic acid adducts (retention time, accurate mass, and fragment ions) as the one with acrylamide in the model system (Fig. 4A). Standing for all propanamide esters, the *p*-coumaric acid derivative was purified and submitted to NMR analysis.

4.4. Structure elucidation via NMR

Besides [1]H- und [13]C-1d spectra two-dimensional spectra as DQF-COSY and heteronuclear ^{13}C-edited HSQC and HMBC were measured. All signals of the [1]H- and [13]C-1d spectra were assigned as follows: **^{1}H-NMR** (600 MHz, 298 K, MeOD, MeOH = 3.32 ppm): δ 7.64 (d, 1H, $J =$ 15.9 Hz, 5-H), 7.46 (m, 2H, 3-H), 6.82 (m, 2H, 2-H), 6.33 (d, 2H, $J =$ 15.9 Hz, 6-H), 4.44 (t, 2H, $J =$ 6.3, 8-H), 2.63 (t, 2H, $J =$ 6.3 Hz, 9-H) ppm.
^{13}C-NMR (151 MHz, 298 K, MeOD, MeOD = 48.0 ppm): δ 174.4 (s, C-

10), 167.7 (s, C-7), 160.0 (s, C-1), 145.4 (s, C-5), 129.8 (s, C-3), 125.8 (s, C-4), 115.4 (s, C-2), 113.5 (s, C-6), 60.3 (s, C-8), 34.5 (s, C-9) ppm. All spectra (5 figures) and the atom assignment (1 figure) are shown in the supplementary material. Long range correlations in the HMBC spectrum showed a [3]J_{CH}-coupling of the saturated protons (4.44 ppm, 8-H) with the carboxylic carbon (167.7 ppm, C-7). Therefore, the esterification took place between the carboxylate function of *p*-coumaric acid and the chloride of the 3-chloropropanamide. The structure of the *p*-coumaric acid adduct was hereby confirmed to be 3-amino-3-oxopropyl-3-(4-hydroxyphenyl)acrylate. As the other phenylacrylic acid adducts were synthesized along the same reaction pathway and products showed identical MS/MS-fragmentation patterns, they were most likely the corresponding propanamide esters.

4.5. Phenylacrylic acid ester formation in fried potato and sweet potato

When treated with 0.1 mol/L of *p*-coumaric- or ferulic acid, fried potatoes and sweet potatoes showed signals at the nominal masses indicative for Michael-addition products with acrylamide (Table 1). The $[M + H^+]$-signals were too small to obtain good MS/MS–spectra. The signal-to-noise ratio was too low to identify any fragments. Therefore, the synthesized standards were added to prove the identity of the presumed phenylacrylic acid propanamides. However, UPLC-QToF-MS/MS chromatograms showed clearly that the retention times of the assumed acrylamide adducts in the fried samples did not match with the retention times of the respective standards, and the accurate mass of the presumed ferulic acid adduct did not fit to the postulated adduct.

In a follow-up experiment, potato and sweet potato cubes treated with phenylacrylic acids, acrylamide, and 3-chloropropanamide were analysed *via* UPLC-QToF-MS/MS. Retention time, accurate mass, and elemental composition are shown in Table 3.

Raw samples of both, potato and sweet potato cubes, contained no free phenylacrylic acids and no acrylamide adducts. However, fried samples without addition of phenylacrylic acids showed peaks at m/z of assumed adducts with acrylamide for *p*-coumaric and cinnamic acid, which were not identical with the ester adducts synthesized. These compounds were detected in any fried sample irrespectively of any supplementation and the peak areas did not increase or decrease. However, propanamide esters of phenylacrylic acids were not detected (Table 3). The accurate mass of the unknown compounds matched well with the respective elemental composition of the acrylamide adducts of *p*-coumaric and cinnamic acid, but other meaningful suggestions for the elemental compositions are possible. For instance, m/z 258.0733 matches with $C_{12}H_{13}NO_4Na^+$, the sodium adduct of 3-amino-3-oxopropyl-3-(4-hydroxyphenyl)acrylate, as well as with $C_{10}H_8N_7O_2^+$. Because not even traces of the protonated adduct were measured, it was considered to be more likely $C_{10}H_8N_7O_2^+$, possibly an advanced glycation end product (AGE) formed along the Maillard-reaction. High amino group and reducing sugar concentrations combined with the frying process provide suitable conditions for the formation of AGE (Van Nguyen, 2006). The same suggestion was made for 242.0783 m/z, which matched with the sodium adduct of 3-amino-3-oxopropyl cinnamate. No protonated adduct was measured, and $C_{10}H_8N_7O^+$ would also fit.

In all samples, the respective adducts were detected beside non-reacted phenylacrylic acids (Table 3). Identical sample masses were weighted in to enable a comparison of the peak areas. The fivefold concentration (Area) of adducts was found with 3-chloropropanamide compared to the obviously less reactive acrylamide (Fig. 5). These results evidenced that a formation of ester adducts of phenylacrylic acids with acrylamide was basically possible, but irrelevant under real-life conditions.

5. Conclusion

Natural phenolic antioxidants were recurrently supposed to mitigate acrylamide formation in food. Contradictory to some data in literature,
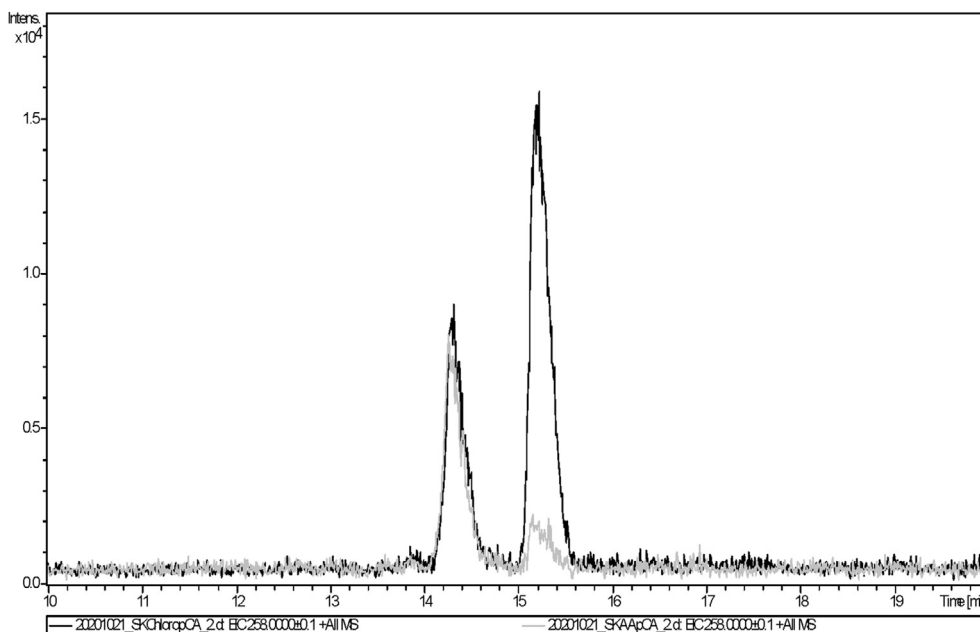
Fig. 5. UPLC-HR-MS-qToF extracted ion chromatogram (EIC) at 258 ± 0.1 m/z of two samples (overlaid). Grey: Sweet potato cubes dipped in 0.1 mol/L *p*-coumaric acid and acrylamide, which were fried for 6 min at 180 °C. Black: Sweet potato cubes dipped in 0.1 mol/L *p*-coumaric acid and acrylamide, which were fried for 6 min at 180 °C. The peak at 14.3 has not been identified. It was presumed to be an AGE. The peak at 15.2 min is the sodium adduct of 3-amino-3-oxopropyl cinnamate.

the present study showed that hydroxylphenyl acrylic acids, namely *p*-coumaric, ferulic, and caffeic acid, and cinnamic acid were not suitable additives for the lowering of acrylamide concentration in fried potato and sweet potato. Previously postulated Michael-adducts of phenols and acrylamide were neither found in fried products nor in a model system. The reaction products of phenylacrylic acids with acrylamide in a model system were unambiguously identified as 3-amino-3-oxopropyl-phenylacrylates by *de novo* synthesis, MS/MS and NMR. Their possible formation in fried potato and sweet potato was confirmed, provided that both, acrylamide and the respective phenylacrylic acid were supplemented in high concentration (0.1 mol/L, over solubility limit) before frying (Xu and An, 2016). In non-supplemented samples no adduct formation was detected, which was in line with the absence of detectable amounts of free phenylacrylic acids in raw potato and sweet potato and relatively low acrylamide concentrations in fried products (17–264 µg/kg) compared to the high supplementation of 0.1 mol/L acrylamide, which led to barely detectable amounts of 3-amino-3-oxopropyl-phenylacrylates. This study concludes that the recurrently reported acrylamide mitigating effect of herbal and fruit extracts containing phenols cannot be attributed to the abundant phenylacrylic acids, but must result from other, perhaps more suitable constituents. Generally, effective mitigation appears more effective with the prevention of acrylamide formation early in the processes (production parameters, asparaginases) or during the frying (addition of competing nucleophiles, for example amines or thiols) and less practical by intercepting acrylamide formed in the course of the production process. However, this generalization needs to be verified for other foods.

CRediT authorship contribution statement

Eric Rottmann: Project administration, Methodology, Experimental, Investigation, Formal analysis, Visualization, Writing – original draft. **Kristina Volkmann:** Formal analysis, Data curation, Validation. **Jörg Fohrer:** Formal analysis, Data curation, Validation, Writing – review & editing. **Ulrich Krings:** Writing – review & editing, Writing – original draft, Data curation. **Ralf G. Berger:** Developed the, Conceptualization, Supervision, Project administration, Writing – review & editing, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://do i.org/10.1016/j.crfs.2021.04.005.

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