

ACRYLAMIDE FORMATION DURING HEAT TREATMENT OF PLANT-BASED MEAT AND CHEESE ALTERNATIVES

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ABSTRACT

Plant analogues of animal foods are currently being increasingly produced worldwide to supply the market with alternative products made from plant-based ingredients. In this study, the acrylamide (AA) content was determined by coupled liquid chromatography with mass spectrometry before and after heat treatment of plant-based meat product alternatives (PBMA; burger patties, steaks, nuggets, schnitzels), authentic meat products (nuggets, strips, schnitzels), plant-based cheese alternatives (PBCAs) and authentic cheese. In PBMA without breadcrumb coating (burgers, steaks), the AA concentrations before as well as after heat treatment remained below the method limit of quantification (20 µg/kg), while in PBMA with breadcrumb coating such as nuggets or schnitzels, frying statistically significantly ($p < 0.05$) increased the AA content from 28.5 (min-max <20-98) µg/kg to 44.5 (28-170) µg/kg. A similar trend was observed for authentic meat products with breadcrumb coating – increase of AA after heat treatment from 15.5 (<20-33) µg/kg to 36.5 (<20-64) µg/kg. In both PBCAs and authentic cheese, frying in breadcrumb coating also increased AA concentrations (to 20.0 (<20-42) µg/kg and 31.5 (<20-39) µg/kg, respectively). It was found that the source of the AA increase during heat treatment was therefore the breadcrumb coating, not the plant analogues themselves or authentic meat products or cheeses. Only in PBCAs without breadcrumb coating produced from minced almonds, heat treatment increased AA contents to as much as 1,217 µg/kg, which is already a high-risk concentration. Our results imply the need for deeper investigation into AA content in heat-treated PBCAs made from minced almonds.

Keywords: analogues, burger, steak, nugget, schnitzel, frying, baking

INTRODUCTION

Acrylamide (AA) is a highly permeable toxic compound with potential carcinogenic, neurotoxic and genotoxic effects as well as reproductive and developmental toxicity. It can enter the body directly through the respiratory tract, digestive tract, skin and other tissues, and the health risks potentially associated with this compound should not be underestimated (Fan *et al.*, 2023; Michalak *et al.*, 2019; Swiacka *et al.*, 2024). Acrylamide is formed in a wide variety of foods, especially during the heating of carbohydrate and protein foods to temperatures exceeding 120 °C, which occurs during frying, baking and roasting. It is formed during the Maillard reaction of free amino acids (especially asparagine) with reducing sugars (mainly glucose and fructose) (Demir and Agaoglu, 2021; Hossain *et al.*, 2024; Pandiselvam *et al.*, 2023). Additionally, in the absence of asparagine, acrolein and ammonia can lead to AA formation in lipid-rich foods. It is known that acrolein and acrylic acid are produced by the degradation of lipids (triglycerides) subjected to high temperatures. Degradation of amino acids with ammonia can then give rise to acrylamide formation via thermal decomposition. Amino acids such as glutamine, cysteine, and aspartic acid have also been found to produce low amounts of acrylamide (Crawford *et al.*, 2019; Gräfenhahn and Beyrer, 2024; Kumari *et al.*, 2022). According to Regulation 2017/2158, significant sources of AA include potato chips or French fries and similar products made from potato dough, bread, breakfast cereals, roasted coffee and coffee substitutes, or fine bakery wares such as various crackers, biscuits, or wafers.

Plant analogues of animal foods are currently being increasingly produced worldwide to supply the market with alternative products made from plant-based ingredients that resemble animal-based foods in their character, imitating the appearance, colour, texture and taste of classic meat or dairy products, but which were obtained in a way other than animal husbandry (Osiecka *et al.*, 2024; Windhorst, 2021). Plant-based meat analogues (PBMA) may present an alternative to meat that may help to satisfy the future demand of the increasing population for proteins, acting as a source of proteins for vegetarians and potentially easing the burden on the environment associated with the excessive consumption of meat and the animal welfare problems associated with their slaughter (Fu *et al.*, 2023; Pospiech *et al.*, 2023). Due to their high protein

contents, soybean protein concentrate, soybean protein isolate, and pea protein are commonly used for the production of PBMA. Carbohydrates, such as starch, are usually added to enhance the texture and consistency of PBMA. High temperatures are applied during production to denature proteins and form a meat-like texture. Under high temperatures, lysine and arginine of soybean protein can be grafted with reducing sugars degraded from carbohydrates, increasing the risk of the formation of acrylamide and other detrimental compounds (Fu *et al.*, 2023; Osiecka *et al.*, 2024). Meat-like flavour in plant-based substitutes is then often achieved by high-temperature cooking processes (such as frying, baking or roasting) through the formation of flavour-active compounds in thermally induced reactions, such as the Maillard reaction. This process is the second step bearing acrylamide formation risk (McClements and Grossmann, 2021; Pospiech *et al.*, 2023).

Plant-based cheese alternatives (PBCA) are also available on the market, typically based on nuts, oils, grains, soy and other plant products. They are produced using techniques similar to those used in the production of processed dairy cheese. An emulsion made of oils and proteins from plant sources, water, emulsifiers, stabilizers, and natural flavourings forms the basis of the production of PBCAs. Some products may also contain preservatives, such as olive extract or sorbic acid, and colouring agents, such as beta-carotene or annatto. Potato starch provides anti-clumping properties, while tapioca starch adds elasticity to the product. Subsequent heat treatment, used to solidify the product, can pose a risk of acrylamide formation (Craig *et al.*, 2022; Grossmann and McClements, 2021).

This work aimed to determine the AA content in plant-based meat and cheese analogues before and after heat treatment and to compare it with AA content in authentic meat and cheese products of corresponding types before and after the same heat treatment to which the PBMA/PBCAs were subjected.

MATERIAL AND METHODS

Sample preparation

A total of 39 different samples were purchased in the Czech retail network. Of those, 11 samples represented plant-based alternatives to cooked meat products

(vegan burgers, vegetarian steaks or plant-based schnitzels). Following the detection of elevated acrylamide content in products containing breadcrumb coating, six samples of battered meat products intended for pan frying were subsequently procured to allow the comparison of the results. Moreover, sixteen samples of PBCAs (mimicking cheese types of different hardness) suitable for heat treatment by grilling, frying in breadcrumbs coating or (in a grated form) for baking on top of a dish were acquired from the retail. Six samples of semi-hard cheeses that can be fried in breadcrumbs were purchased for comparison with PBCAs. The specific types of products in each group and their compositions are shown in Tables 1-4.

Half of each product was heat treated according to the manufacturer-recommended instructions on the packaging. Meat products, their vegetable analogues, and cheese samples were pan-fried in vegetable oil (200 °C, average 5 minutes per side), vegetable analogues of cheese in a frying pan (200 °C, average 5 minutes per side) or in an oven (200 °C, 7-22 minutes) as prescribed by the manufacturer (see Table 3 for details). Subsequently, the acrylamide content of the untreated and heat-treated samples was determined.

Acrylamide analysis

The determination of acrylamide was carried out based on the method described in the EN 16618:2015 standard (**European Standard, 2015**). The analysed sample was thoroughly homogenised. Approximately 1 g (with an accuracy of 0.01 g) of each sample was weighed into an extraction tube. 100 µL of internal standard solution (isotopically labelled acrylamide ¹³C₃, Sigma-Aldrich, Saint Louis, MO, USA) and 200 µL of ultrapure water (MILLI-Q PLUS, Merck KGaA, Darmstadt, Germany) was added, the tube was shaken on an IKA minishaker (IKA®-Werke GmbH & Co. KG, Staufen, Germany) and left to stand for 10 minutes.

Next, 9 mL of ultrapure water was added to the sample and the extraction tube was placed on a head-over-head shaker (Heidolph Scientific Products GmbH, Schwabach, Germany) and shaken for about 5 min at a speed of approximately 100 rpm. Subsequently, 5 mL of hexane p.a. (Lach-Ner, s.r.o., Neratovice, Czech Republic) and 10 mL of acetonitrile (LC-MS quality; Honeywell, Charlotte, North Carolina, USA) were added. The extraction tube was then shaken vigorously by hand, after which 1.5 g of sodium acetate (p.a.; Lach-Ner, s.r.o., Neratovice, Czech Republic) and 6 g of anhydrous magnesium sulfate (p.a.; Lach-Ner, s.r.o., Neratovice, Czech Republic) were added and the extraction tube was placed into the head-over-head shaker again and shaken for about 5 min at a speed of 100 rpm. Then, the tube was centrifuged for 5 min at 3,500 rpm (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). After centrifugation, the upper hexane layer was removed and 2 mL of the acetonitrile layer was purified on the SPE Oasis® MCX cartridge 3 cc/60 mg, 60 µm (Waters Corporation, Milford Massachusetts, USA). The purified extract was blown dry at approximately 40 °C with a nitrogen stream (nitrogen 4.8, AIR PRODUCTS spol. s r.o., Děčín, Czech Republic). The residue was dissolved in 1 mL of 0.1% formic acid for LC-MS (Merck KGaA,

Darmstadt, Germany) and used for analysis by liquid chromatography with mass detection.

The chromatographic analysis was performed on a Waters Acquity UPLC I-Class liquid chromatograph equipped with the ACQUITY UPLC HSS C18 SB column (100 mm x 2.1 mm; 1.8 µm; Waters Corporation, Milford, Massachusetts, USA) using gradient elution with mobile phases 0.1% formic acid/methanol for LC-MS (Honeywell, Charlotte, North Carolina, USA) (98/2 → 10/90), flow rate 0.2 mL/min, injection 2 µL, column temperature 30 °C. Detection was performed using a Xevo TQ-S triple quadrupole detector (Waters Corporation, Milford, Massachusetts, USA) based on the triple quadrupole principle coupled with the chromatograph in MRM mode, ESI+, m/z 72 → 55 and 27 for acrylamide and 75 → 58 for the internal standard (¹³C₃ labelled acrylamide), respectively. The certified reference standard of acrylamide Cat. No. 23701 (Supelco, Saint Louis, Missouri, USA) was used for calibration. The limit of quantification is 20 µg/kg, and the method repeatability is 8%.

Statistical analysis

Statistical evaluation was performed using the F-test and paired or non-paired t-test (as appropriate) in UNISTAT 6.5 software (Unistat, London, United Kingdom). Values below the limit of quantification were considered to be 50 % of the LOQ (10 µg/kg) for statistical analyses.

RESULTS AND DISCUSSION

Acrylamide content in plant-based meat analogues

Table 1 shows the acrylamide (AA) content in each plant-based alternative before heat treatment (i.e. as purchased) and after heat treatment according to the manufacturer's recommendations. The table shows a fairly wide range of values, ranging from values below the limit of quantification of the method used (20 µg/kg) up to the highest value of 98 µg/kg for the plant alternative before heat treatment and 170 µg/kg after heat treatment. The amount of AA in breadcrumb-coated products (sample numbers SN 6-11) was higher than in steak or burger products (SN 1-5). In the breadcrumb-coated products (SN 6-11), AA concentrations were mostly above the limit of quantification even before heat treatment (25-98 µg/kg) and after it, it increased to 28-170 µg/kg, while in breadcrumb-free products (SN 1-5), AA concentrations were below the limit of quantification even after heat treatment. This might be caused by the fact that breadcrumbs further increase the content of saccharides in the product, which could contribute to AA formation. Moreover, most breadcrumb-coated products are already pre-heat treated by the manufacturer, which represents an additional step during which high temperature can increase the AA formation.

Table 1 Composition of analysed plant-based meat alternatives and their acrylamide content

Sample number	Product type	Breadcrumbs / batter base	Protein	Oil	Acrylamide content (µg/kg)	
					before heat treatment	after heat treatment
1	steak	no	wheat, soy	sunflower	< 20	< 20
2	steak	no	soy, wheat	rapeseed	< 20	< 20
3	burger	no	pea, rice	rapeseed	< 20	< 20
4	burger	no	pea	sunflower	< 20	< 20
5	burger	no	soy	rapeseed, coconut	< 20	< 20
6	nuggets	corn	pea, wheat	sunflower	63 ± 9.5	86 ± 12.9
7	nuggets	corn	soy, wheat	sunflower	26 ± 3.9	42 ± 6.3
8	nuggets	corn	soy, pea	olive	< 20	28 ± 4.2
9	nuggets	buckwheat, corn	soy, cashew nuts	olive	31 ± 4.7	47 ± 7.1
10	schnitzel	corn	soy, wheat	sunflower	25 ± 3.8	42 ± 6.3
11	schnitzel	corn	pea, wheat	sunflower	98 ± 14.7	170 ± 25.5

Osiecka et al. (2024) analysed 7 pea-based meat alternatives in the forms of burgers, meatballs, gyros or chicken-like pieces, so this PBMA were without breadcrumb coating. The AA content in all of these products was below the limit of quantification of the method used (< 20 µg/kg), similar to our study. **Fu et al. (2023)** determined the content of AA in 15 PBMA samples from China's retail in the forms of meatballs, ground meat, steaks, burger patties, chicken breasts and sauced beef in the as-purchased condition to range from 31.81 to 186.70 µg/kg, with the average content at 68.55 µg/kg. Compared with the results of our study, their values appear to be consistent rather with PBMA's coated in breadcrumbs than burger patties or steaks. **Abdullaieva et al. (2024)** reported the highest AA content in any of the 16 PBMA's from Denmark's retail analysed in their study to be 65.7 ± 6.6 µg/kg, increasing to 119 ± 12 µg/kg after heat treatment. **Pospiech et al. (2024)** investigated AA in 4 plant burger analogues, which are commercially available on the European Union market. Before heat treatment, AA concentrations ranged approx. 8-24 µg/kg, with the lowest concentrations found in a soy protein-based burger and the highest in a pea protein-based burger. Pan-frying (200 °C, 5 minutes on each side) revealed the highest AA increase in soy flour- and sunflower protein-based patties, both of which contained approx. 14.5 µg/kg before, which

increased to 72.3 (soy flour-based) and 69.2 (sunflower protein-based) µg/kg after frying. In our study, AA concentrations in burger patties were below the detection limit of 20 µg/kg both before and after frying; we, therefore, cannot discuss the influence of product composition on AA formation during frying. We can, however, draw some conclusions regarding nuggets and schnitzels, where wheat protein appears to be associated with higher AA content. **Ma et al. (2024)**, however, claim that the AA content is also influenced by the technological process to which the plant protein is subjected prior to the PBMA production itself. They evaluated the effect of different parameters of protein extrusion and found that higher extrusion temperature and lower feeding speed of the raw material were beneficial to reducing the acrylamide content. The humidity of the material being extruded also influenced the AA formation (interestingly, a U-shaped curve was observed as 20 and 60 % humidity both led to a lower AA production than 40 %). **Squeo et al. (2023)** also compared four categories of used proteins; namely, they compared native legume flours, dry-fractionated proteins, wet-extracted proteins, and texturized vegetable proteins. In their study, the lowest AA was detected in legume flours category (280 ± 98 µg/kg), while the highest in the wet-extracted proteins category (451 ± 186 µg/kg). The highest concentration detected in any of

their samples was 748 µg/kg, the minimum one (in a sample of the legume flour) was 185 µg/kg. Assuming that the final content of the proteins they analyzed in the final PBMA products is approx. 20-30 % (Gräfenhahn and Beyrer, 2024), the AA content originating from the wet-extracted proteins (max. AA concentration of 748 µg/kg) could be up to approx. 150 µg/kg, while when using protein components from the legume flour category (max. AA 185 µg/kg), the AA content in the final product could be approx. 40 µg/kg. The type of protein used for the production of plant-based alternatives can, therefore, influence the AA content in the final product.

Acrylamide content in meat products

Finding out that the acrylamide content of the vegetable alternatives is significantly affected by the use of breadcrumb coating, meat products of similar type (nuggets, strips, schnitzel) were also analysed to compare acrylamide formation (see Table 2). The AA content before cooking was very low, below the limit of quantification in some products and ranging between 21 and 33 µg/kg in others. Pan-frying led to a statistically significant increase in AA concentrations (p < 0.05) to values of 30-64 µg/kg. This is clearly visible in Figure 1, which compares the AA concentrations of breadcrumb-coated plant analogues and meat products (nuggets, strips and cutlets). This figure also demonstrates that the AA content in authentic meat products prior to the heat treatment is generally lower than in plant-based alternatives and that the latter shows a wider range of AA concentrations. Frying statistically significantly (p < 0.05) increases AA concentrations in both types of products, again with higher variability in PBMA, probably depending on the used

raw materials and their processing, as discussed above. The differences between authentic and plant-based meat products at any time point (before or after heat treatment) were, however, not significant, which was most likely caused by the low number of samples and the aforementioned high variability of AA content in PBMA.

For breadcrumb-coated meat products (chicken schnitzel) from the Turkish market, Ölmez et al. (2008) reported values similar to our findings (34 µg/kg). Demir and Agaoglu (2021), however, reported much higher values for these products, namely 172.83 ± 13.32 µg/kg for 6 samples of nuggets and 149.60 ± 6.07 µg/kg for 5 samples of schnitzels from retail in Turkey. The formation of AA in breadcrumb coatings prepared from wheat, corn or rice is also supported by the results of Crawford et al. (2019), who determined the AA content in flatbreads baked for 2 minutes at 195.5 °C. Flatbreads from whole wheat flour contained 21.3 ± 0.6 µg/kg AA, flatbreads from yellow corn meal 8.8 ± 1.1 µg/kg and flatbreads from brown rice flour 8.1 ± 0.35 µg/kg AA. According to Li et al. (2021), AA in corn flatbreads of different colors (blue, red, white, yellow) baked at 215 °C can reach values of 26.5 ± 2.7 µg/kg to 56.5 ± 9.8 µg/kg. Michalak et al. (2019) reported the AA content in 10 samples of soft wheat bread purchased from supermarkets in Poland 55.0 ± 24 µg/kg and in 10 samples of crisp wheat bread 312 ± 56 µg/kg. Takatsuki et al. (2003) determined 35 µg/kg AA in one sample of wheat bread-crumbs purchased from retail markets in Tokyo (Japan), in other samples the amount of AA was between 9-30 µg/kg. In samples of wheat breadcrumb coating originating from prepared foods, these authors determined 32-53 µg/kg AA.

Table 2 Composition of analysed authentic meat products and their acrylamide content

Sample number	Product type	Breadcrumbs / batter base	Meat	Oil	Acrylamide content (µg/kg)			
					before treatment	heat	after treatment	heat
12	nuggets	wheat, corn	chicken	sunflower	21 ± 3.2		34 ± 5.1	
13	nuggets	wheat	chicken	sunflower, rapeseed	22 ± 3.3		39 ± 5.9	
14	nuggets	wheat, corn	chicken	rapeseed	< 20		30 ± 4.5	
15	strips	rice, corn	chicken	sunflower	< 20		47 ± 7.1	
16	strips	wheat	chicken	sunflower	< 20		< 20	
17	schnitzel	wheat, corn	chicken	rapeseed	33 ± 5.0		64 ± 9.6	

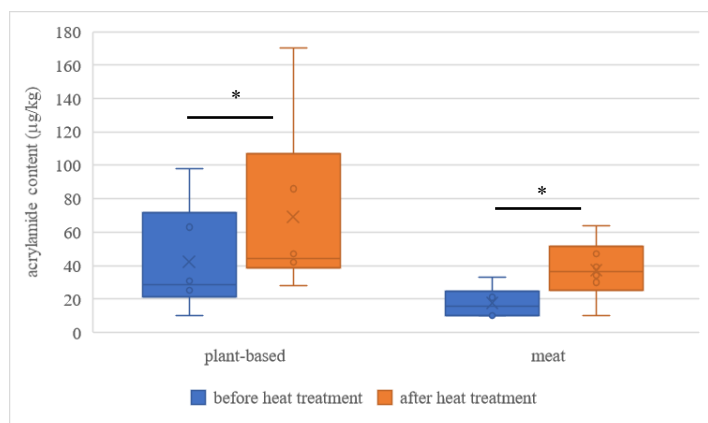


Figure 1 Acrylamide content in breadcrumb-coated meat products (nuggets, strips and schnitzels) and their plant-based alternatives.

Legend: * above the boxes indicate statistically significant differences (p < 0.05)

Acrylamide content in plant-based cheese analogues

Unlike in PBMA, the AA content of almost all plant-based cheese analogues (PBCAs) was below the limit of quantification before heat treatment (Table 3). The semi-hard cheese analogue coated in breadcrumbs and precooked by the manufacturer was the only exception, likely due to the pre-heat treatment by the manufacturer; still, the AA content was only slightly above the limit of quantification (21 µg/kg). Even after heat treatment (carried out by pan-frying or oven-baking as per manufacturer’s instructions), AA climbed above the limit of quantification. These samples were either breadcrumb-coated and pan-fried (AA 20-42 µg/kg) or contained ground almonds; in such samples, the AA content increased proportionally to the almond content. Samples containing approx. 10-11 % of ground almonds showed an increase in AA to levels of 21-29 µg/kg, while in the sample containing 91 % of almonds, concentration increased to a record high of 1,217 µg/kg. This is a very high concentration comparable with the foods considered to be high-risk from the perspective of increased AA content, such as

French fries, in which AA concentrations were reported to reach as much as 1,068 µg/kg (samples from ten restaurants in Madrid, Spain, Mesias et al., 2019), or as much as 1,411 µg/kg (30 samples from street vendors in Addis Ababa, Ethiopia, Deribew and Woldegiorgis, 2021), eight samples of potato crisps containing on average 834 µg/kg, with maximum concentrations of up to 2,336 µg/kg (Ölmez et al., 2008), seven samples of potato chips purchased from local supermarkets of India containing AA in the range of 1,064-6,391 µg/kg (Pal Murugan et al., 2019) or vegetable crisps (11 samples from the European Union), with average concentrations reaching up to 1,846 µg/kg (Mustatea and Popa, 2015). The increased AA formation in the PBCA with high almond content was even visually detectable – rapid browning was observed in this sample, indicating the formation of Maillard reaction products, while other PBCA analogues not containing almonds preserved their yellow colour. Mesias et al. (2024) also determined differences in AA content in roasted almonds depending on the roasting conditions: the darker the almonds, the more AA (mean of 480 µg/kg); ground roasted almonds contained more AA (414 µg/kg) than whole roasted almonds (280 µg/kg). Similarly, Apaydin et al. (2024) found that the darker colour of roasted nuts and almonds corresponds with higher AA content – the darkest roasted cashew contained the highest AA concentration (148 µg/kg), the same was true for almonds (120 µg/kg). The connection between higher AA formation in products with a darker surface is also confirmed by EFSA CONTAM Panel (2015). Our results show that even though the AA content in PBCAs has not been much discussed in the scientific literature so far, a deeper investigation of these products, especially the almond-based ones, is highly desirable.

Acrylamide content in authentic cheese samples

Based on the detection of AA formation in breadcrumb-coated pan-fried PBCAs, a comparison with authentic cheeses was performed (similar to meat samples). Results are presented in Table 4 and statistical comparison with PBCAs is shown in Figure 2. It is obvious that no statistically significant difference in AA concentrations between breadcrumb-coated pan-fried PBCAs and cheese samples was observed (range of <20-42 µg/kg in PBCAs and <20-39 µg/kg in cheeses), but frying of battered cheeses led to a significant (P < 0.01) increase in AA concentrations. From the perspective of AA formation, therefore, PBCAs do not pose a greater risk than authentic cheeses.

Table 3 Composition of analysed plant-based cheese alternatives and their acrylamide content

Sample number	Product type	Heat treatment	Main components	Acrylamide content (µg/kg)	
				before heat treatment	after heat treatment
18	Halloumi-style, for grilling	pan	modified starch, coconut fat, plant protein	< 20	< 20
19	Halloumi-style, for grilling	oven	rapeseed oil, starch, modified starch	< 20	< 20
20	Camembert-style, soft-ripened	oven	shea butter, almonds (10 %), starch	< 20	21 ± 3.2
21	Camembert-style, for grilling	pan	shea butter, almonds (10 %), starch	< 20	29 ± 4.4
22	semi-soft, for grilling	oven	cashew nuts (87 %)	< 20	< 20
23	Mozzarella-style, soft	oven	coconut oil, cashew nuts, rice	< 20	< 20
24	Mozzarella-style, soft	oven	almonds (11 %), shea butter, almond protein	< 20	21 ± 3.2
25	Mozzarella-style, grated	oven	modified starch, coconut oil (13 %), sunflower oil (5 %), pea protein	< 20	< 20
26	Parmesan-style, hard	oven	cornstarch, chickpea flour, coconut oil	< 20	< 20
27	Parmesan-style, grated	oven	modified starch, coconut oil (10 %)	< 20	< 20
28	Parmesan-style, grated	oven	almonds (91 %)	< 20	1,217 ± 182.6
29	Gouda-style, semi-hard	pan (in breadcrumbs)	coconut oil (21 %), modified starch	< 20	21 ± 3.2
30	Gouda-style, semi-hard	pan (in breadcrumbs)	coconut oil (21 %), starch, modified starch	< 20	< 20
31	semi-hard	pan (in breadcrumbs)	coconut oil (25 %), modified starch	< 20	< 20
32	semi-hard, breadcrumb-coated, pre-fried	pan	coconut oil (25 %), modified potato starch, potato starch and protein, breadcrumbs (wheat flour)	21 ± 3.2	42 ± 6.3
33	semi-hard, breadcrumb-coated, pre-fried	pan	coconut oil (25 %), modified potato starch, potato starch and protein, breadcrumbs (wheat flour)	< 20	20 ± 3.0

Table 4 Composition of analysed authentic cheese products and their acrylamide content

Sample number	Product type	Fat in dry matter of cheese (%)	Breadcrumbs / batter base	Oil	Acrylamide content (µg/kg)	
					before heat treatment	after heat treatment
34	semi-hard	30	wheat flour and breadcrumbs, eggs	rapeseed	< 20	32 ± 4.8
35	semi-hard	30	wheat flour and breadcrumbs, eggs	rapeseed	< 20	28 ± 4.2
36	semi-hard	30	wheat flour and breadcrumbs, eggs	rapeseed	< 20	34 ± 5.1
37	semi-hard, breadcrumb-coated, pre-fried	30	wheat flour and breadcrumbs, eggs	rapeseed	22 ± 3.3	39 ± 5.9
38	semi-hard, breadcrumb-coated, pre-fried	30	wheat flour and breadcrumbs, corn starch	rapeseed, sunflower	< 20	< 20
39	semi-hard, breadcrumb-coated, pre-fried	45	wheat flour and breadcrumbs, eggs	rapeseed	< 20	31 ± 4.7

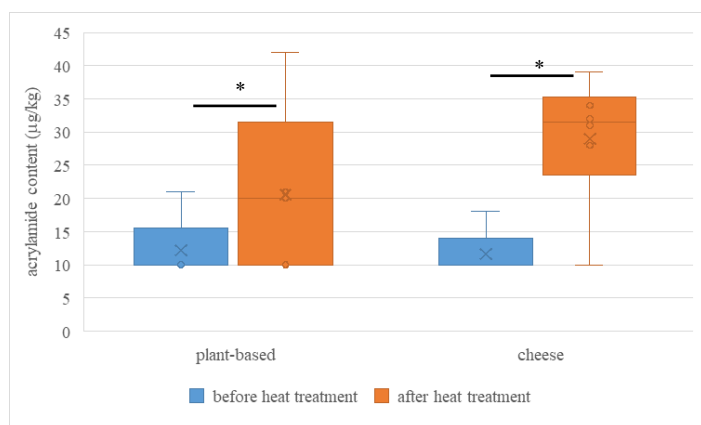


Figure 2 Acrylamide content in breadcrumb-coated pan-fried cheeses and their plant-based alternatives.

Legend: * above the boxes indicate statistically significant differences ($p < 0.01$).

CONCLUSION

When analysing the acrylamide content in plant alternatives of meat products before and after heat treatment, the AA content in burger- or steak-type products did not increase above the method's limit of quantification (20 µg/kg), while the PBMA in the form of nuggets or schnitzels that are coated in breadcrumbs showed a statistically significant increase in AA concentrations after pan-frying ($p < 0.05$). A similar trend was observed also in authentic meat products coated in

breadcrumbs (nuggets or schnitzels) after pan-frying. Breadcrumb-coated plant cheese analogues and authentic cheeses also showed an increase in AA concentration when pan-fried. While the increase was not statistically significant for samples of plant-based alternatives, a significant AA increase after frying was detected in authentic cheese samples ($p < 0.01$). It follows from the above that the source of the AA increase during pan-frying was therefore the breadcrumb coating, not the plant analogues themselves or authentic meat products or cheeses. Overall, the AA content of the plant alternatives of meat products and cheeses as well as authentic meat products and cheeses fried in breadcrumbs does not reach values comparable to foods at risk of high AA content, such as French fries or potato crisps. Importantly, however, AA concentrations in baked plant cheese analogues made from ground almonds increased in line with the proportion of almonds in the product, with a maximum AA content of 1,217 µg/kg, which is already comparable to the AA content in the aforementioned high-risk foods. This suggests the need for further research on the AA content in heat-treated PBMA-containing ground almonds.

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