Food Research International 242 (2026) 119880

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Influence of apple pomace and asparaginase on acrylamide levels in cookies and behavior during simulated gastric digestion: risk assessment perspectives☆

Sumeyra Sevim [a,b], Arife Macit [c], Miguel A. Faria [a,*], Olga Viegas [d], Sara C. Cunha [a], Mevlude Kizil [e], Isabel M.P.L.V.O. Ferreira [a,*]

[a] LAQV/REQUIMTE, Laboratory of Bromatology and Hydrology, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Portugal
[b] Department of Nutrition and Dietetics, Faculty of Health Sciences, Ankara Medipol University, Ankara, Turkey
[c] Department of Nutrition and Dietetics, Faculty of Health Sciences, Munzur University, Tunceli, Turkey
[d] Faculty of Nutrition and Food Sciences, University of Porto, Portugal
[e] Department of Nutrition and Dietetics, Faculty of Health Sciences, Hacettepe University, Ankara, Turkey

ARTICLE INFO

Keywords:
Acrylamide intermediates
GC–MS/MS
In vitro semi-dynamic digestion
Monte Carlo
Risk assessment
Stomach

ABSTRACT

Cookies are widely consumed baked products; however, their contribution to dietary acrylamide (ACR) intake raises increasing health concerns. This study investigated the effects of apple pomace addition and asparaginase treatment on ACR levels in cookies, the fate of ACR during simulated gastric digestion using the INFOGEST semi-dynamic model, and the associated health risks of ACR exposure from cookie consumption. Risk assessment was conducted for adults (18–65 years) and children (3–10 years) using Monte Carlo simulation. Three formulations were evaluated: a control cookie, a cookie in which wheat flour was partially replaced with apple pomace, and a cookie containing asparaginase. Cookies formulated with apple pomace contained significantly lower ACR levels than the control (132 ± 8 μg/kg *vs.* 215 ± 11 μg/kg; $p < 0.05$), although the reduction was less pronounced than in cookies containing asparaginase (65 ± 14 μg/kg). During gastric digestion, ACR exhibited formulation-dependent release kinetics. Nevertheless, the total ACR released at the end of digestion was similar for control and pomace cookies, likely due to additional ACR formation in the stomach, despite differences in release patterns throughout digestion. Margin of Exposure (MOE) values for children remained well below 10,000, indicating a potential carcinogenic risk, although reductions of up to 59% were observed for control and pomace cookies after digestion. Target Hazard Quotient (THQ) values showed a slight increase after digestion in both age groups but remained below 1, suggesting no concern for chronic toxicity. Control cookies indicated a potential carcinogenic risk for children (ILCR = 1.56×10^{-4}), whereas pomace and asparaginase formulations reduced this risk (ILCR = 9.59×10^{-5} and 4.72×10^{-5}, respectively). However, simulated gastric digestion increased ILCR values for both control and pomace cookies (2.24×10^{-4} and 2.33×10^{-4}, respectively). These findings indicate that dietary exposure to ACR cannot be assessed solely based on its concentration in food, and that digestive kinetics should be incorporated into health risk assessment.

1. Introduction

Cookies have become one of the most popular snacks in the world and play a crucial role in the casual food sector because of their wide range of flavors and long shelf life (Yang et al., 2022). However, cookies present certain nutritional drawbacks due to their high fat and sugar content. Additionally, the potential formation of acrylamide (ACR), a hazardous dietary compound generated during thermal processing under low-humidity conditions is of toxicologic concern. Acrylamide forms when free asparagine and reducing sugars react at temperatures exceeding 120 °C. (González-Mulero et al., 2023). ACR has been confirmed as a probable human carcinogen and classified in Group 2A by the International Agency for Research on Cancer due to its toxicity (IARC, 1994). According to the EC (2017), cookies are among the foods

☆ This article is part of a Special issue entitled: 'ICFC2025' published in Food Research International.

* Corresponding authors.
E-mail addresses: mfaria@ff.up.pt (M.A. Faria), isabel.ferreira@ff.up.pt (I.M.P.L.V.O. Ferreira).

https://doi.org/10.1016/j.foodres.2026.119880
Received 30 December 2025; Received in revised form 23 June 2026; Accepted 27 June 2026
Available online 30 June 2026

requiring monitoring to assess ACR risk and implement mitigation measures as their consumption can contribute up to 56% to ACR intake in children (over 2 years of age) (Abt et al., 2019) and increase the health risks (Hoseini, Shahidi, Shariatifar, Ahmadi & Sharifi Soltani, 2025.

The food sector has recently suggested mitigation techniques to lower the amounts of ACR in its products while keeping the quality standards unaltered. These include modifying the formulations or the processing parameters (reducing pH, baking temperature, and duration), as well as including food ingredients that have been shown to mitigate the ACR formation (Sarion, Codină, & Dabija, 2021). Because formulation modifications can regulate the presence of ACR-forming components before baking, optimizing formulations such as partially or fully replacing key ingredients is crucial in managing ACR levels (Mesias, Delgado-Andrade, & Morales, 2022).

In parallel, efforts to improve the nutritional profile of cookies and crackers by enriching them with protein and fiber have increased, with a growing trend in the food industry towards products with high fiber content (Luo et al., 2017; Suo et al., 2025; Yang et al., 2022). In this context, the addition of natural compounds as possible inhibitors of the Maillard reaction is being studied because they are readily accepted as food ingredients by consumers (Champrasert, Orfila, & Suwannaporn, 2022; López-Ruiz et al., 2023; Ma et al., 2022). In this regard, apple pomace, rich in pectin and antioxidants, may increase the nutritional value of cookies while also contributing to the reduction of ACR formation, thereby improving food safety and reuse of industrial bioresidues (Llavata, Picinelli, Simal, & Cárcel, 2022; Pascoalino et al., 2025). Previous investigations proved that soluble dietary fibers reduce ACR content in bakery products (Torres, Dueik, Carré, & Bouchon, 2019) and the addition of lyophilized and washed apple pomace provided fiber enrichment and a reduction of ACR up to 60% (López-Ruiz et al., 2023). The use of antioxidants to mitigate acrylamide formation in cookies has also been reported previously, particularly through the addition of spices such as saffron, cinnamon, and turmeric (Arabameri et al., 2026). Moreover, the addition of the enzyme asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) is another well-known strategy that efficiently reduces ACR formation without influencing on the sensory quality (Anese, Quarta, & Frias, 2011; Gazi, Göncüoğlu Taş, Görgülü, & Gökmen, 2023). This enzyme hydrolyzes asparagine into aspartic acid and ammonia before heating or processing and reduces ACR levels by 40–90% in bakery products (Anese, Quarta, & Frias, 2011; Gazi, Göncüoğlu Taş, et al., 2023; Mohan Kumar, Shimray, Indrani, & Manonmani, 2014), being more efficient than the apple pomace. However, this strategy is expensive, hindering the production of low-price foodstuffs and is perceived by consumers as a synthetic compound addition (Claus, Carle, & Schieber, 2008), diminishing the product acceptability.Given the widespread occurrence of ACR in processed foods and its potential adverse health effects, risk assessment has become an essential component of ACR safety studies. Consequently, estimating dietary exposure and characterizing the associated health risks are important for evaluating the safety of food products and the effectiveness of mitigation strategies. Risk assessment is commonly performed by calculating dietary intake based on ACR concentrations and food consumption data, followed by the determination of indicators such as the Margin of Exposure (MOE), Incremental lifetime Cancer Risk (ILCR) and Hazard Quotients (HQ) (EPA, 2021). These approaches provide valuable information on the potential health implications of ACR exposure and support the development of strategies aimed at reducing consumer risk. However, limited information is available regarding the potential formation, degradation, or transformation of ACR during gastrointestinal digestion, which may influence its actual bioaccessible levels and consequently affect exposure estimates.

The aim of this study was to conduct a comprehensive health risk assessment of ACR exposure though three different cookie formulations: a control formulation, a formulation in which apple pomace partially replaces wheat flour, a formulation similar to the control formulation, but incorporating asparaginase. The innovative and comprehensive approach of the present study focused on assessing: *(i)* the effectiveness of natural *versus* synthetic-perceived compounds in reducing ACR levels; *(ii)* the fate of ACR following simulated gastric digestion; and *(iii)* the health risk assessment of ACR exposure from cookies ingestion. ACR levels in the cookie samples and digests was assessed by gas chromatography coupled with MS/MS (GC–MS/MS) after solid-liquid extraction and derivatization with xanthydrol using a methodology previously validated in our lab for cookies and digests matrixes (López-Ruiz et al., 2023).

A semi-dynamic *in vitro* approach was used to evaluate the fate of ACR during gastric digestion, enabling a more physiologically relevant simulation of digestion since the gastric pH and the digestive enzymes are added gradually A probabilistic risk assessment was conducted using the Monte Carlo simulation method (10,000 iterations) to estimate dietary exposure and associated health risks for two population groups: adults aged 18–65 years (assumed body weight of 70 kg) and children aged 3–10 years (assumed body weight of 12 kg).

2. Materials and methods

2.1. Materials and reagents

Acrylamide (ACR) standard (99% of purity) and L-asparagine amidohydrolase (EC 3.5.1.1) were purchased from Sigma-Aldrich (St. Louis, MO, USA). ACR$^{13}C_3$ IS (99% of purity) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). For the ACR extraction of the analytes 1,2-dichloroetane (≥99%), diethylene glycol (99%), xanthydrol (98%), hydrochloric acid (37%) and methanol (MeOH) HPLC grade were acquired from Sigma-Aldrich. Ethyl acetate (AcOEt) pesticide residue analysis grade was acquired from Fluka, potassium hydroxide (KOH) and sodium chloride (NaCl) were purchased from VWR (Leuven, Belgium). Potassium carbonate (K_2CO_3), ammonium bicarbonate ($NH4HCO_3$), and sodium bicarbonate ($NaHCO_3$) were acquired from Panreac (Barcelona, Spain). Ultrapure water (0.054 μS/cm) was supplied by a "Seral" system (SeralPur Pro 90 CN, Germany). For digestion porcine α-amylase, pepsin, bile, and pancreatin extracts were provided by Sigma-Aldrich. Pomace from apples was prepared in the laboratory by washing and lyophilizing apples.

2.2. Cookies formulation and preparation

Ingredients for cookies were purchased in the local markets of Porto (Portugal). Cookies were prepared according to López-Ruiz et al. (2023). Three different formulations of cookies were prepared, control cookies as positive control, cookies with asparaginase as negative control, and cookies with apple pomace, in those cookies prepared: 60 g of flour was replaced with 60 g of apple pomace. This ratio was set to achieve a final pectin content of 5%. Apple pomace, a by-product from the apple juice industry (kindly provided by Sumol+Compal®) lyophilised and washed to remove free sugars, was used as a source of fiber and partially replaced wheat flour. To prepare the formulation of cookies added with asparaginase (Sigma A3809–100 UN) the enzyme was added to control formulation at a concentration of 500 U/kg of flour. To ensure a homogeneous distribution in the dough, asparaginase was dispersed in the aqueous phase before being added to the dry ingredients (Anese, Quarta, & Frias, 2011). Later, cookies were processed as described in detail in López-Ruiz et al. (2023) (Suplemmentary Figs. S1–S3) (López-Ruiz et al., 2023). Finally, some cookies were crushed, homogenized, and introduced into a plastic pot until the extraction and analysis. The rest was preserved in its original state for *in vitro* digestion.

2.3. Semi-dynamic in vitro digestion

To simulate the structural changes in the food matrix and pH in stomachal content, a standardized semi-dynamic protocol designed by

Mulet-Cabero et al. (Mulet-Cabero et al., 2020) was followed. The fat, protein, and carbohydrate contents obtained from the proximate analysis of cookies were used to calculate the energy value needed for simulating gastric digestion. The total amount of cookies (60 g) was considered as real *in vivo* cookie proportion to allow the scale down to an *in vitro* simulation using 5 g, using the protocol's supplementary information of Mulet-Cabero et al. (Mulet-Cabero et al., 2020). Electrolyte simulated salivary fluid (eSSF) and electrolyte simulated gastric fluid (eSGF) were prepared following the procedure suggested by the INFOGEST 2.0 protocol (Mulet-Cabero et al., 2020). Supplementary Table S1 shows oral and gastric conditions for the INFOGEST *in vitro* semi-dynamic digestion for all cookies. The cookies were mincing and added with water before oral digestion. The oral digestion fluid was prepared by adding eSSF, 0.3 M $CaCl_2(H_2O)_2$, amylase solution (Sigma Aldrich, A3176, 10 U/mL), and ultrapure water. To simulate the oral phase, the food was mixed with the oral digestion fluids and mixed at 37 °C for 2 min, and then the mix transferred into the vessel to simulate the gastric phase. Digestion was performed in a v-form titration vessel with thermostat jacket (ref:6.1418.150; Metrohom, Herisau, Switzerland), thermostatised at 37 °C, and equipped with a stirrer paddle at 15 rpm. It contained the basal simulated gastric fluid (10%, SGF) resembling the *in vivo* fasting conditions. The remaining 90% of SGF which contains eSGF, $CaCl_2(H_2O)_2$, water and HCl were added at a constant ratio by separated devices. The total amount of 1 M HCl used for gastric digestion was estimated by testing the volume needed to reach an end pH of 2. Porcine pepsin (Sigma Aldrich, P7012, 2500 U/mL) was added by a proper secretion rate of each cookie. The gastric emptying time and aliquot were calculated for a total of four gastric emptying points, named GE1–GE4 (Fig. S4), and they were collected using a glass pipet every gastric emptying time. Right after, the pH was measured and NaOH (0.5 M) was added to increase the pH to 7 to inactivate pepsin activity. All samples were digested with triplicate as described above. Fig. 1 displays the methodology's overall layout.

2.4. Acrylamide quantification in cookies and in stomach bioaccessible fractions

Acrylamide quantification in cookies and in stomach bioaccessible fractions were performed as described in detail in López-Ruiz et al. (2023) by GC–MS after derivatization with xanthydrol (López-Ruiz et al., 2023) with small modifications. Briefly, a 3 g sample was used for digests, while a 2 g was used for cookies. After spiking with 200 µL of $ACR^{13}C_3$ at 10 mg/L, 2.5 mL of 1,2-dichloroethane were added to digested samples. The solvent was separated and evaporated in N_2 stream, the residual fraction dissolved in 200 µL of ethyl acetate, then 1 µL was injected for GC–MS analysis. The limits of detection (LOD), quantification (LOQ) were 4 µg/kg and 25 µg/kg, respectively. RSD for six independent assays was lower than 8%, and the recovery ranged between 85 and 98%.

2.5. Acrylamide exposure and health risk assessment

The exposure analysis was carried out for children and adults. The following formula was used to determine the estimation of daily intake (EDI) of ACR from cookies consumption based on the amounts of ACR in cookie samples, daily dietary status, and body weight (Basaran & Sadighara, 2024):

$$EDI = \frac{C \times IR}{BW}$$

where C is the concentration of ACR (mg/kg); IR is the daily consumption (kg/day); BW is the body weight (kg). The daily average consumption of cookies for children and adults was obtained from the Portuguese National Food, Nutrition, and Physical Activity Survey (IAN-AF 2015–2016) (17.4 g/day and 11.5 g/day, respectively) (IAN-AF, 2018). The body weight for children and adults was set at 12 and 70 kg respectively according to Committee (2012). The potential health risk associated with cookies consumption was assessed using a margin of exposure (MOE) as a probabilistic method. The following equation was used to determine the neurotoxic and carcinogenic risk values of ACR exposure using the MOE method:

$$MOE = \frac{BMDL10}{EDI}$$

$BMDL_{10}$ is the value benchmark dose limit for a 10% increase in cancer risk (µg/kg bw/day). The $BMDL_{10}$ for ACR is 0.17 mg/kg bw/day for neoplastic effects and 0.43 mg/kg bw/day for non-neoplastic

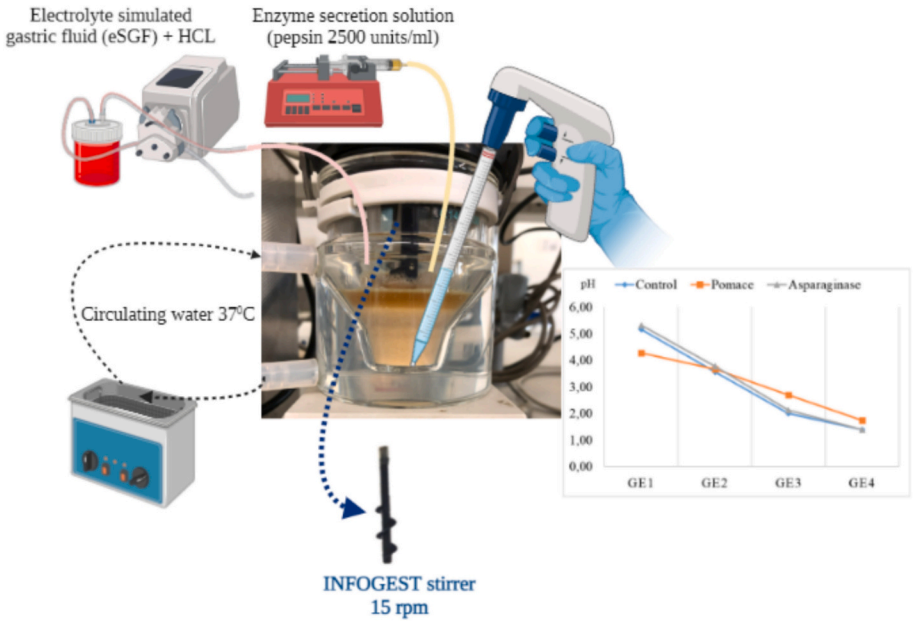

Fig. 1. The titration vessel, the two pumps for adding fluids and enzymes, and the printed stirrer set at 15 rpm are highlighted in this schematic representation of the gastric phase of the INFOGEST semi-dynamic method. The pH of each of the four gastric emptying points was measured. This figure was prepared with the online BioRender program.

(neurotoxic) effects. MOE values above 10,000 and 125 indicate low carcinogenic and neurotoxic risk, respectively.

Carcinogenic health risk was also evaluated with the lifetime carcinogenic risk method. For this purpose, the Incremental Lifetime Cancer Risk (ILCR) probability was assessed to demonstrate the cancer risk associated with exposure to ACR using following formula (EPA, 2021):

$$ILCR = EDI \times SF$$

where EDI is the estimation of daily intake and SF is the slope factor, which is 0.5 mg/kg bw/day for ACR (Aghvami et al., 2023). It is deemed safe if ILCR is less than 1.00×10^{-6}, a possible and substantial risk if ILCR is between 1.00×10^{-4} and 1.00×10^{-6}, and a serious health risk if $1.00 \times 10^{-4} <$ ILCR (Basaran & Sadighara, 2024).

Moreover, Target Hazard Quotient (THQ), which is the ratio of exposure to the toxic substance to the reference dose—the maximum level at which no negative health impacts are anticipated—was used to evaluate non-carcinogenic health risk. While THQ < 1 shows no health risk, THQ ≥ 1 suggests a possible health issue that is not carcinogenic (Aghvami et al., 2023). THQ was determined by the following equation:

$$THQ = \frac{EDI}{RfD}$$

where, RfD is the oral reference dose for ACR elated to degenerative nerve changes is 0.002 mg/kg bw/day (Basaran & Sadighara, 2024).

2.6. Statistical analysis

For each cookie, the digestion experiments were performed in triplicate. Data are described as mean ± standard deviation. One-way analysis of variance (ANOVA) was performed using SPSS 23.0 statistical software (IBM Corp., Armonk, NY, USA), and Tukey's multiple comparison test was employed to find significant differences ($p < 0.05$) between cookies and between the gastric emptying. The Pearson correlational coefficient was used to assess the relationship between pH and ACR concentration in each gastric emptying aliquot. A $P < 0.05$ was considered statistically significant. A Monte Carlo simulation consisting of 10,000 iterations was conducted using Crystal Ball software (version 7.2, Oracle) to perform a quantitative uncertainty analysis in the public health risk assessment.

3. Results and discussion

3.1. Reducing acrylamide levels in cookies by recipe modification

Adding 500 U/kg of asparaginase significantly decreased the ACR levels in cookies to 65 ± 14 μg/kg compared to the 215 ± 11 μg/kg in the control formulation. Anese, Quarta, Peloux, and Calligaris (2011) reported that adding 500 U/kg asparaginase is enough to significantly reduce ACR level in cookies. Literature reveals that ACR reductions achieved by asparaginase were 96 and 54% in rotary cut biscuits and wire cut cookies, respectively (Gazi, Göncüoğlu Taş, et al., 2023). Moreover, asparaginase combined with phenolic compounds (ellagic and gallic acids) or calcium salts ($CaCO_3$ and $CaCl_2$) reduces effectively ACR formation in wheat biscuits, with the ellagic acid-asparaginase combination providing the highest reduction rate at 89% (Musa, Oellig, & Scherf, 2025). The reduction in ACR levels observed in the present study (approximately 70%) is consistence with results reported in the aforementioned papers. However, enzymes are more expensive than alternative mitigation strategies and are therefore not appropriate for use in producing low-cost food products (Claus et al., 2008). Despite the reduction in ACR promoted by the addition of asparaginase, consumers tend to prefer the use of natural compounds as ingredients over synthetic additives, or those perceived as such, such as asparaginase. (Ma et al., 2022). In the present work, a 39% reduction of ACR content was achieved through the addition of apple pomace (132 ± 8 μg/kg). Although relevant and statistically significant ($p < 0.05$) it was not as substantial as the 70% reduction observed in cookies treated with asparaginase (65 ± 14 μg/kg). The partial replacement of wheat flour by apple pomace in the formulation may cause a decrease in ACR precursors, but the most relevant effect should be due to fiber increase, specially pectin. López-Ruiz et al. (2023) assessed the impact of partial replacement of wheat flour by different dietary fibers, namely, k-carrageenan, arabinogalactan and pectin on ACR content of biscuits. The addition of 5 g/100 g of pectin provided higher reduction of ACR then similar addition of other sources of fiber. Moreover,pectin derived from apple pomace, particularly lyophilized forms with reduced sugar content, significantly reduced ACR levels whereas, untreated dehydrated apple pomace caused a huge increase in ACR due to its high content of reducing sugars (López-Ruiz et al., 2023). Hölzle et al. (2025) demonstrated that flaxseeds or chia seeds, which are notable for their high fiber content and functional effects, when added to cookies increased ACR levels. However, adding organic acids to cookies containing chia seeds decreased ACR production. The authors stated that ACR formation increased with seed addition due to higher free asparagine content, phenolic compounds, unsaturated fatty acids, and reduced moisture, whereas organic acids decreased ACR levels by lowering pH and reducing the initial step of the Maillard reaction (Hölzle et al., 2025).

3.2. Effect of cookies formulation on acrylamide levels during gastric digestion

The gastrointestinal tract undergoes various changes during digestion, particularly concerning to pH. The gastric phase is the most drastic. These conditions can significantly influence the behavior and fate of ACR (Aktağ, Hamzalıoğlu, Kocadağlı, & Gökmen, 2022). To investigate this effect, we employed the semi-dynamic INFOGEST method, focusing on the gastric phase to better replicate physiological conditions and monitor ACR fate under realistic pH dynamics physiological (Mulet-Cabero et al., 2020). This method mimics the physiological pH dynamics within the stomach and enables sampling points throughout the process. The total gastric digesting time and the corresponding emptying times were determined according to the nutritional value of each cookie and are presented in Supplementary Table S1. Cookies with asparaginase and control cookies had an average gastric digestion time of 16.5 min (16.03 and 16.98 min, respectively), while cookies with pomace, with a slightly lower caloric content, required a shorter although quite similar time (14.27 min).

The ACR content in each of the 4 gastric emptying (GE) aliquots during the gastric digestion was assessed for all samples and are shown in Fig. 2 and Table 1. The amount of ACR in each gastric empty for cookies with asparaginase was under the LOD. For cookies with pomace, the release of ACR from the first to the last GE point decreased, whereas the release of ACR from the first to the GE3 point for control cookies increased then decreased in the last GE points. The total ACR released after gastric digestion is similar for control cookies and cookies with pomace (308 ± 37 and 321 ± 23 μg/kg respectively). The cumulative ACR formation and percentage of ACR release from stomach to intestine (in each of the GEs) is presented in Fig. 2. It clearly shows that there is a linear increase in the cumulative release of ACR from the first GE point to the last GE point for cookies. Although the final amount released from the digestion of 5 g of cookies is around 1500 ng in both situations, the percentage of this amount released in each GE is different, which can have implications in ACR absorption dynamics. With pomace, a high percentage is released in the first GE which can promote a faster intestinal absorption in this type of matrices. Noticeably, the total amount of ACR released from both types of cookies is similar although the cookies had different initial amounts. This can be attributed to ACR formation during digestion, more than doubling in the case of samples with pomace. Some authors stated that the structure of the food matrix might influence how bioaccessible ACR occurs during digestion (González-Mulero, Mesías, Morales, & Delgado-Andrade, 2022; Sansano,

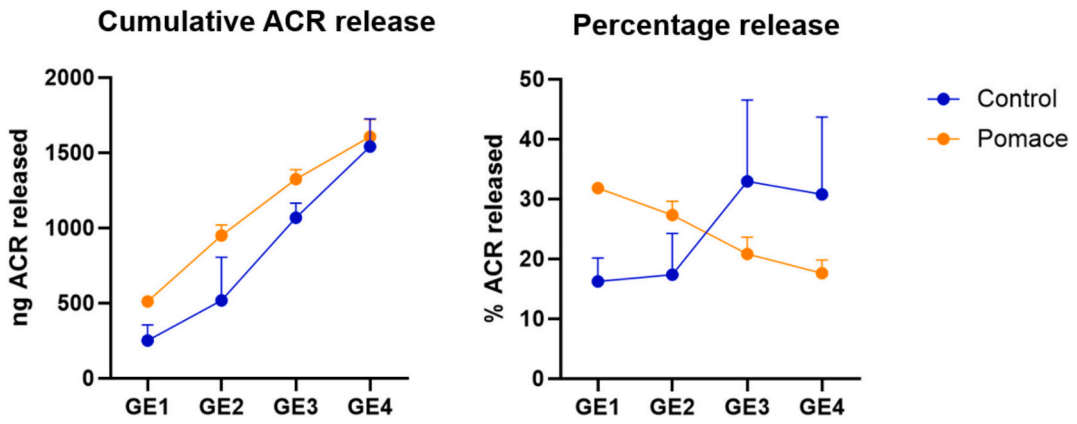

Fig. 2. The cumulative ACR release (ng) and percentage release (%) in each of the GE from the gastric digestion of 5 g of cookies. The release of ACR in cookies with asparaginase is not presented as its concentration was <LOD in all GEs.

Table 1
Concentration of ACR in cookies (before digestion), expressed as μg/kg. GE1, GE2, GE3 and GE4 amount of ACR released (ng) in each gastric emptying using 5 g of cookies. *Total ACR released to the intestine (expressed in μg/kg in cookies before digestion which would originate the values of ACR found after gastric digestion).

Type of cookies	ACR (μg/kg) (initial)	GE1 (ng ACR)	GE2 (ng ACR)	GE3 (ng ACR)	GE4 (ng ACR)	Total ACR* (μg/kg) (after digestion)
Control	**215 ± 11[a]**	250 ± 104	267 ± 184	508 ± 295	438 ± 282	**308 ± 37 (43 ± 10%)**
Asparaginase	**65 ± 14[b]**	<LOD	<LOD	<LOD	<LOD	**n.a.**
Pomace	**132 ± 8[c]**	514 ± 8	439 ± 65	334 ± 63	283 ± 50	**321 ± 23 (143 ± 2%)**
p	**0.004**					

p was calculated by ANOVA, different superscripted letters in the same column mean statistically significant difference among the values, according to the Tukey test ($p < 0.05$). Data are presented as the mean ± SD of three replicates.

Heredia, Peinado, & Andrés, 2017). Hamzalıoğlu and Gökmen (2015) also stated that depending on the dietary matrix, ACR displayed various kinetic patterns during gastric digestion. They demonstrated that Schiff base quickly vanished during the gastric phase while ACR levels rose, suggesting that gastric pH helps the conversion of intermediate products such as Schiff bases formed during thermal process into ACR (Hamzalıoğlu & Gökmen, 2015). The presence of other intermediary α-dicarbonyl compounds in samples with apple pomace (*e.g.* 3-deoxyglucosone) are also known to contribute to ACR formation at low pH and temperatures below 120 °C (Aktağ & Gökmen, 2020). Similarly, Sansano et al. (2017) found an ACR increase of 32% for fries, 83% for crackers, and 410% for sweet biscuits, after gastric digestion. Additionally, an ACR rise occurred immediately at the start of the gastric stage for some products such as sweet biscuits, while for other products it occurred after a longer gastric digestion period. This suggests that the rate of matrix degradation may be related to the kinetics of various ACR changes (Sansano et al., 2017). The dynamic changes in ACR concentration during *in vitro* gastrointestinal simulation highlight the need to revise conventional models for assessing dietary exposure. Specifically, the lack of comprehensive studies addressing the biotransformation and fate of ACR throughout the digestive process constitute a critical barrier to establishing accurate and evidence-based risk assessment (Aktağ et al., 2022). Therefore, changes in ACR during the *in vitro* digestion process should be considered in dietary exposure and risk assessment.

3.3. Dietary exposure and health risk assessment

3.3.1. Considering the ACR levels in cookies (Risk assessment 1)

The Monte Carlo method was used to determine the cumulative probability and frequency distributions of the carcinogenic and non-carcinogenic risks of being exposed to ACR in cookies. The mean of the EDI value of control cookies, cookies with pomace and with asparaginase for children were 3.12×10^{-4}, 1.92×10^{-4} and 9.43×10^{-5} mg/kg bw/day respectively, and for adults 3.54×10^{-5}, 2.17×10^{-5} and 1.07×10^{-5} mg/kg bw/day, respectively. Results are presented in Table 2. The lowest MOE value for children was observed in the control group (544.55), followed by the pomace group (886.18), while the highest value was observed in cookies treated with asparaginase (1802.18). This indicates that the control and pomace groups may pose a significant health concern in terms of neoplastic effects in children, whereas asparaginase treatment reduces the risk, despite not reaching the safety margin value of 10,000. On the other hand, no significant health risk is expected for children in terms of non-neoplastic MOE (>125). Similarly, adults are not predicted to be at substantial risk for non-neoplastic MOE. Unlike children, the MOE values of carcinogenic risk for adults in cookies treated with asparaginase is 15,888, which is above the 10,000 thresholds; this indicates a relatively low level of concern. However, MOE values for adults in the control (4806.24) and pomace (7821.49) groups are indicative of potential health concerns. Therefore, significant reduction of carcinogenic risk from the applied strategies was only noticed for adults and for the treatment with asparaginase. According to the latest EFSA (2015) report, biscuits, crackers and crispbread are among the major dietary sources of ACR exposure following fried potato products, coffee and soft bread in adults. For children (toddlers, other children, adolescents), soft bread, breakfast cereals, biscuits and other cereal or potato-based products can contribute up to 25% of the total dietary exposure. The MOE values for total dietary ACR intake range from 425 for average adult consumers to 50 for toddlers with high consumption levels, raising significant public health concerns (EFSA, 2015). Basaran and Sadighara (2024) reported equally that ACR exposure from both maize and potato crisp consumption can reach levels that are concerning in terms of carcinogenic risks whereas Basaran and Faiz (2022) stated that the ACR dietary exposure from Turkish traditional food (meat products, loaves of bread, simit, and desserts) was not of concern concerning neurotoxicity and carcinogenicity (MOE > 10,000). Arabameri et al. (2026) reported ACR

Table 2
Estimated daily intake (EDI) and health risk assessment of ACR through cookies in children and adults.

	BW[§] (kg)	IR[¥] (kg day)		Cookies type	EDI mg/kg bw/day	MOEs		THQ	ILCR
						Neoplastic	Non-neoplastic		
Children	12	0.0174	Initial[1]	Control	3.12×10^{-4}	544.55*	1377.39	0.156	1.56×10^{-4}*
				Pomace	1.92×10^{-4}	886.18*	2241.51	0.096	9.59×10^{-5}
				Asparaginase	*9.43×10^{-5}*	*1802.76**	*4559.92*	*0.047*	*4.72×10^{-5}*
			After digestion[2]	Control	4.47×10^{-4}	380.31*	961.97	0.224	2.24×10^{-4}*
				Pomace	4.66×10^{-4}	364.81*	922.75	0.233	2.33×10^{-4}*
Adults	70	0.0115	Initial[1]	Control	3.54×10^{-5}	4806.24*	12,156.95	0.018	1.77×10^{-5}
				Pomace	2.17×10^{-5}	7821.49*	19,783.76	0.011	1.09×10^{-5}
				Asparaginase	*1.07EE−05 × 10^{-5}*	*15,887.85*	*40,186.92*	*0.005*	*5.35×10^{-6}*
			After digestion[2]	Control	5.06×10^{-5}	3359.68*	8498.02	0.025	2.53×10^{-5}
				Pomace	5.28E−05 × 10^{-5}	3219.70*	8143.94	0.026	2.64×10^{-5}

BMDL10 = 0.17 mg/kg bw for neoplastic; BMDL = 0.43 mg/kg bw for non-neoplastic.
RfD = 0.002 mg/kg day; SF = 0.5 mg/kg day^{-1}.
§ Body weight according to EFSA.
¥ Daily average consumption according to Portuguese National Food, Nutrition, and Physical Activity Survey (IAN-AF 2015–2016).
1 Values used for risk assessment 1.
2 After digestion (Risk assessment 2), according to the equivalent ACR mg/kg in cookies before digestion which would originate from the values of ACR found after gastric digestion.
* Values indicating health concern.

levels formed in Iranian tahdig lead to MOE values below 10,000 for children and adults a high public health concern.

Concerning Target Hazard Quotient, the rank order for children was: control cookies (0.156) > cookies with pomace (0.096) > cookies with asparaginase (0.047), and for adults control cookies (0.018) > cookies with pomace (0.011) > cookies with asparaginase (0.005). The THQ values approach suggests that ACR exposure through cookie consumption does not pose significant non-carcinogenic health risks to consumers (THQ values <1). A similar risk profile was observed in other food products when assessed using the Target Hazard Quotient (THQ) approach, with no carcinogenic risks associated with acrylamide exposure through Iranian tahdig consumption reported (Arabameri et al., 2026).

The cumulative probability histogram and frequency distributions of ILCR indexes for the cookies are presented in Fig. 3. The rank order of ILCR associated with ACR exposure for children was control cookies (1.56×10^{-4}) > cookies with pomace (9.59×10^{-5}) > cookies with asparaginase (4.72×10^{-5}) and for adults was control cookies (1.77×10^{-5}) > cookies with pomace (1.09×10^{-5}) > cookies with asparaginase (5.35×10^{-6}). The results of the health risk assessment based on Monte Carlo simulation indicated that the ILCR index due to ingestion of ACR formation in control cookies for children were slightly higher than the commonly accepted threshold of 1.00×10^{-4}, suggesting a potential carcinogenic risk, while cookies with pomace and asparaginase did not pose a health risk to children (ILCR < 1.0×10^{-4}). Although cookies are snack products and contribute only modestly to daily dietary intake, the consumption of control cookies alone may still present a potential carcinogenic risk for children. For all other cases—including adult consumers—health risks remained within acceptable levels (THQ < 1 and ILCR < 1.0×10^{-4}). Therefore, it can be concluded that the consumption of this type of food does not pose significant health concerns for adults.

3.3.2. *Considering the average ACR in cookies after gastric digestion (Risk assessment 2)*

The results indicated that ACR levels in cookies differed from the amount reached the intestine, as additional ACR was formed in the stomach. To consider cases in which the total amount of ACR reaching the intestine for absorption is higher than the ingested amount, dietary exposure and health risk assessments were conducted using the ACR

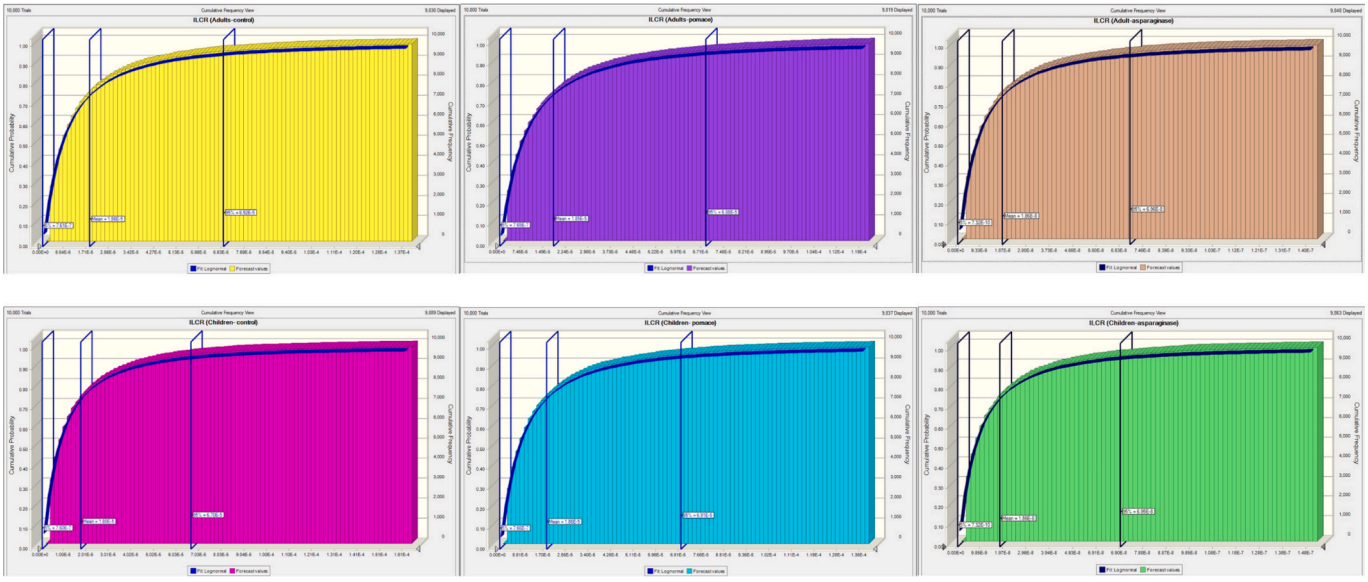

Fig. 3. Frequency of cancer risk (ILCR) and cumulative probability for ACR in cookies.

content (μg/kg) in cookies before digestion and the total ACR detected after gastric digestion (Table 1). Although certain studies found that ACR decreases after intestinal digestion, ACR is absorbed in the small intestine immediately following stomach discharge, so the formation in the stomach undoubtedly influences ACR absorption and consequently its systemic levels. Taking this into account, the outcomes of the subsequent risk assessment are particularly striking. When considering the ingested ACR and that formed during digestion, the mean of the EDI value of control cookies and cookies with pomace after digestion was similar due to the higher increase in ACR content in pomace cookies (143%). Moreover, they were higher than risk assessment 1 for children and adults (Table 2). Cookies with asparaginase were not considered for the second risk assessment since their ACR level was found below the LOD in each GE. In Children, the MOE values were well below 10,000 in all products and fell below 400 in the control and pomace samples after digestion, configuring and increased risk. In the children's group, MOE decreased by approximately 30% in the control sample, from 544.55 before digestion to 380.31 after digestion. In the pomace sample, it decreased by approximately 59%. A similar trend was observed in adults; MOE decreased by 30% in the control sample and by 59% in the pomace sample. This situation indicates that the exposure after gastric digestion poses a higher risk for both adults and children, configuring an increased potential health concern.

THQ values showed a slight increase after digestion in both age groups; however, they were below 1 in all groups before and after gastric digestion, and no significant health risk in terms of chronic toxicity is anticipated. In terms of ILCR, an increase was observed after digestion in all samples. The cumulative probability histogram and frequency distributions of ILCR indexes for the cookies after gastric digestion are presented in Fig. 4. Of particular concern is the fact that, in children, the ILCR value in control cookies showed an increase of approximately 44% from before digestion to after digestion. In the pomace sample, this value increased by approximately 143%. Whilst only control samples showed values above 1×10^{-4} based on pre-digestion ACR levels in children, in post-digestion control and pomace samples showed values similarly above 1×10^{-4}, which indicates a serious health risk. In adults, ILCR values increased by 43% in control samples and 142% in pomace samples, reaching similar levels after digestion (2.53×10^{-5} and 2.64×10^{-5}). However, this situation does not pose significant risk.

Ultimately, the digestion process resulted in a reduction of MOE in both children and adults, accompanied by a concomitant increase in THQ and ILCR values. The most critical increase in absolute risk levels was observed among children, particularly in the pomace samples. This implies that the assessment of post-digestion exposure is a critical process of comprehensive risk analysis, and the digestion dynamics should be included in risk assessment.

4. Conclusions

The present study provides comprehensive insights into the advancement of risk assessment approaches by improving exposure estimation through the consideration of changes in acrylamide (ACR) concentrations during digestion. Despite inherent limitations, such as the lack of determination of ACR precursors, which could provide further insight into the mechanisms involved in ACR formation and transformation throughout the digestive process, the findings contribute to a more realistic assessment of ACR exposure and fulfil the main objectives of this work. The study showed that apple pomace inclusion in cookie recipes resulted in a significant reduction in the amount of ACR, though not as effective as that found in asparaginase-added cookies. The current study also provides novel insights into how ACR levels change during gastric digestion, revealing that the highly acidic gastric fluids trigger ACR formation. ACR exhibits different kinetic patterns depending on the cookie formulation. However, an increase in ACR

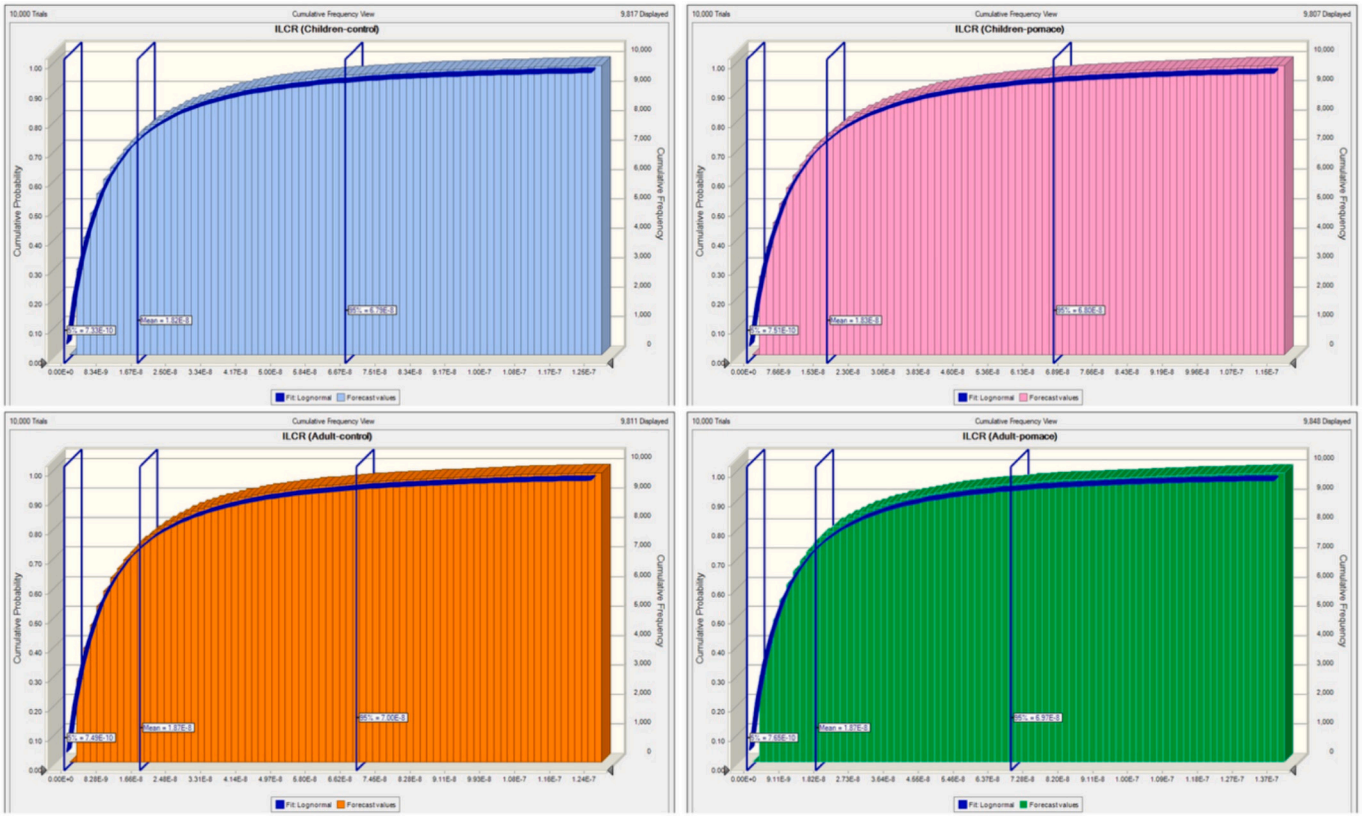

Fig. 4. Frequency of cancer risk (ILCR) and cumulative probability for ACR in cookies after digestion, according to the equivalent ACR mg/kg in cookies before digestion which would originate from the values of ACR found after gastric digestion.

concentration was consistently observed at the end of digestion across the three formulations assessed. Although the total ACR released was similar in pomace and control cookies, the proportion released at each stage varied. This stage-dependent release pattern may influence the dynamics of ACR absorption and its potential bioavailability. The semi-dynamic INFOGEST method effectively tracked the fate of ACR during the gastric phase of digestion, while accounting the specific pH changes of each cookie formulation.

Based on the initial ACR content of cookies, the consumption of control cookies may present a potential carcinogenic risk for children whereas cookies with pomace not. Notwithstanding, the amount of ACR reaching the intestine was higher than the initial ACR content of cookies, thereby, leading to an increased level of exposure. Consequently, when the risk assessment is based on ACR levels after gastric digestion, a higher health risk is observed. Specifically, the potential carcinogenic risk for children exceeded threshold values only for the control cookies before digestion; however, after digestion, an elevated carcinogenic risk was observed for both the control and pomace cookies. The carcinogenic risk is low in both situations but increased if considering the ACR formation in stomach. This means that ACR concentrations in foods may not directly correspond to dietary exposure levels due to changes occurring during digestion. Therefore, risk assessment should thus integrate digestion kinetics to provide a more accurate estimation of exposure.

CRediT authorship contribution statement

Sumeyra Sevim: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Arife Macit:** Writing – review & editing, Visualization, Resources, Investigation, Formal analysis, Data curation. **Miguel A. Farıa:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Olga Viegas:** Writing – review & editing, Methodology, Formal analysis. **Sara C. Cunha:** Writing – review & editing, Formal analysis. **Mevlude Kizil:** Writing – review & editing, Resources. **Isabel M.P.L.V.O. Ferreira:** Writing – review & editing, Supervision, Resources, Investigation, Funding acquisition.

Funding

This research was funded by Fundação para a Ciência e a Tecnologia (FCT) in the framework of the project SALIVA+ (DOI 10.54499/2022.08978.PTDC) and its FEDER component by the Innovation and Digital Transition Program (COMPETE2030), within the scope of the R&D project entitled “New Methodological Approaches to Assess the Risk of Neurodegeneration from Exposure to Mixtures of Food Contaminants”, with the acronym “neuroNAMix” and reference COMPETE2030-FEDER-00714200, operation 16003, DOI 10.54499/2023.18065.ICDT. This work received financial support from the PT National Funds (FCT/MECI, Fundação para a Ciência e Tecnologia and Ministério da Educação, Ciência e Inovação) through the project UID/50006/2025 DOI 10.54499/UID/50006/2025 - Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Miguel Faria reports financial support was provided by Foundation for Science and Technology. Sara Cunha reports financial support was provided by Foundation for Science and Technology. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Sara C. Cunha acknowledges FCT for the 2022.07841.CEECIND/CP1724/CT0014 contract. Miguel A. Faria acknowledges FCT the researcher contract through CEECINSTLA/00029/2022 DOI 10.54499/CEECINSTLA/00029/2022/CP3029/CT0002.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2026.119880.

Data availability

Data will be made available on request.

References

Abt, E., Robin, L. P., McGrath, S., Srinivasan, J., DiNovi, M., Adachi, Y., & Chirtel, S. (2019). Acrylamide levels and dietary exposure from foods in the United States, an update based on 2011-2015 data. *Food Additives & Contaminants: Part A, 36*(10), 1475–1490. https://doi.org/10.1080/19440049.2019.1637548

Aghvami, M., Mohammadi, A., Khaniki, G. J., Ahmadi, M., Moazzen, M., Arabameri, M., & Shariatifar, N. (2023). Investigation of cocoa and cinnamon effect on acrylamide formation in cakes production using GC/MS method: A risk assessment study. *Food Chemistry: X, 18*, Article 100629. https://doi.org/10.1016/j.fochx.2023.100629

Aktağ, I. G., & Gökmen, V. (2020). A survey of the occurrence of α-dicarbonyl compounds and 5-hydroxymethylfurfural in dried fruits, fruit juices, puree and concentrates. *Journal of Food Composition and Analysis, 91*, Article 103523. https://doi.org/10.1016/j.jfca.2020.103523

Aktağ, I. G., Hamzalıoğlu, A., Kocadağlı, T., & Gökmen, V. (2022). Dietary exposure to acrylamide: A critical appraisal on the conversion of disregarded intermediates into acrylamide and possible reactions during digestion. *Current Research in Food Science, 5*, 1118–1126. https://doi.org/10.1016/j.crfs.2022.07.004

Anese, M., Quarta, B., & Frias, J. (2011). Modelling the effect of asparaginase in reducing acrylamide formation in biscuits. *Food Chemistry, 126*(2), 435–440. https://doi.org/10.1016/j.foodchem.2010.11.007

Anese, M., Quarta, B., Peloux, L., & Calligaris, S. (2011). Effect of formulation on the capacity of l-asparaginase to minimize acrylamide formation in short dough biscuits. *Food Research International, 44*(9), 2837–2842. https://doi.org/10.1016/j.foodres.2011.06.025

Arabameri, M., Shariatifar, N., Asghar, H., Bahramzad, H., Beigomi, M., Moazzen, M., & Sharifiarab, G. (2026). Investigating the effect of different additives on the acrylamide formation process in bread tahdig: A health risk assessment study. *Applied Food Research, 6*(1), Article 101807. https://doi.org/10.1016/j.afres.2026.101807

Basaran, B., & Faiz, O. (2022). Determining the levels of acrylamide in some traditional foods unique to Turkey and risk assessment. *Iranian Journal of Pharmaceutical Research: IJPR, 21*(1), Article e123948. https://doi.org/10.5812/ijpr.123948

Basaran, B., & Sadighara, P. (2024). The level, human exposure, and health risk assessment of acrylamide in chips and breakfast cereals: A study from Türkiye. *Journal of Food Composition and Analysis, 134*, Article 106584. https://doi.org/10.1016/j.jfca.2024.106584

Champrasert, O., Orfila, C., & Suwannaporn, P. (2022). Acrylamide mitigation using zein–polysaccharide complex particles. *Food Hydrocolloids, 124*, Article 107317. https://doi.org/10.1016/j.foodhyd.2021.107317

Claus, A., Carle, R., & Schieber, A. (2008). Acrylamide in cereal products: A review. *Journal of Cereal Science, 47*(2), 118–133. https://doi.org/10.1016/j.jcs.2007.06.016

Committee, E. S. (2012). Guidance on selected default values to be used by the EFSA scientific Committee, scientific panels and units in the absence of actual measured data. *EFSA Journal, 10*(3), 2579. https://doi.org/10.2903/j.efsa.2012.2579

EC. (2017). European Commission, Commission recommendation (EU) 2017/2158 of 20 November 2017 on establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. *The Official Journal of the European Union, 304*(60), 24–44. http://data.europa.eu/eli/reg/2017/2158/oj.

EFSA. (2015). EFSA Panel on Contaminants in the Food Chain, Scientific opinion on acrylamide in food. *EFSA Journal, 13*(6), Article 4104. https://doi.org/10.2903/j.efsa.2015.4104

EPA. (2021). United States Environmental Protection Agency. Basic information about the integrated risk information system. . Retrieved 18 May from https://www.epa.gov/iris/basic-information-about-integrated-risk-information-system.

Gazi, S., Göncüoğlu Taş, N., Görgülü, A., & Gökmen, V. (2023). Effectiveness of asparaginase on reducing acrylamide formation in bakery products according to their dough type and properties. *Food Chemistry, 402*, Article 134224. https://doi.org/10.1016/j.foodchem.2022.134224

González-Mulero, L., Mesías, M., Morales, F., & Delgado-Andrade, C. (2022). Assessment of the acrylamide bioaccessibility in cereal and potato-based foods after in vitro digestion. *Food Research International, 161*, Article 111820. https://doi.org/10.1016/j.foodres.2022.111820

González-Mulero, L., Mesías, M., Morales, F. J., Navajas-Porras, B., Rufián-Henares, J. A., & Delgado-Andrade, C. (2023). Acrylamide bioaccessibility in potato and veggie

chips. Impact of in vitro colonic fermentation on the non-bioaccessible fraction. *Food Research International, 164*, Article 112409. https://doi.org/10.1016/j.foodres.2022.112409

Hamzalıoğlu, A., & Gökmen, V. (2015). Investigation of the reactions of acrylamide during in vitro multistep enzymatic digestion of thermally processed foods. *Food & Function, 6*(1), 108–113. https://doi.org/10.1039/C4FO00884G

Hölzle, E., Breitling-Utzmann, C., Blumberg, O., Klass, N., Remezov, A., Schödl, S., Sischka, A., Tränkle, K., Steliopoulos, P., & Oellig, C. (2025). Influence of chia and flaxseeds on acrylamide formation in sweet bakery products. *Food Chemistry, 463*, Article 141344. https://doi.org/10.1016/j.foodchem.2024.141344

Hoseini Majd, S., Shahidi, S. A., Shariatifar, N., Ahmadi, M., & Sharifi Soltani, M. (2025). Acrylamide occurrence in Iranian biscuits and its potential risk of exposure. *Food Science & Nutrition, 13*(7), Article e70480. https://doi.org/10.1002/fsn3.70480

IAN-AF. (2018). The national food, nutrition and physical activity survey, cookies intake, by age groups (IAN-AF 2015–2016). Retrieved from https://www.ian-af.up.pt/en.

IARC. (1994). Acrylamide. In *, 60. Monographs on the evaluation of carcinogenic risks to humans: some industrial chemicals* (pp. 389–433). International Agency for Research on Cancer–IARC.

Llavata, B., Picinelli, A., Simal, S., & Cárcel, J. A. (2022). Cider apple pomace as a source of nutrients: Evaluation of the polyphenolic profile, antioxidant and fiber properties after drying process at different temperatures. *Food Chemistry: X, 15*, Article 100403. https://doi.org/10.1016/j.fochx.2022.100403

López-Ruiz, R., Marin-Saez, J., Cunha, S. C., Fernandes, A., de Freitas, V., Viegas, O., & Ferreira, I. M. P. L. V. O. (2023). Fibre enrichment of cookies to mitigate acrylamide formation and gastrointestinal bioaccessibility. *LWT, 182*, Article 114835. https://doi.org/10.1016/j.lwt.2023.114835

Luo, D., Liang, X., Xu, B., Kou, X., Li, P., Han, S., Liu, J., & Zhou, L. (2017). Effect of inulin with different degree of polymerization on plain wheat dough rheology and the quality of steamed bread. *Journal of Cereal Science, 75*, 205–212. https://doi.org/10.1016/j.jcs.2017.04.009

Ma, Y., Huang, H., Zhang, Y., Li, F., Gan, B., Yu, Q., Xie, J., & Chen, Y. (2022). Soluble dietary fiber from tea residues with inhibitory effects against acrylamide and 5-hydroxymethylfurfural formation in biscuits: The role of bound polyphenols. *Food Research International, 159*, Article 111595. https://doi.org/10.1016/j.foodres.2022.111595

Mesias, M., Delgado-Andrade, C., & Morales, F. J. (2022). An updated view of acrylamide in cereal products. *Current Opinion in Food Science, 46*, Article 100847. https://doi.org/10.1016/j.cofs.2022.100847

Mohan Kumar, N., Shimray, C. A., Indrani, D., & Manonmani, H. (2014). Reduction of acrylamide formation in sweet bread with L-asparaginase treatment. *Food and Bioprocess Technology, 7*(3), 741–748. https://doi.org/10.1007/s11947-013-1108-6

Mulet-Cabero, A.-I., Egger, L., Portmann, R., Ménard, O., Marze, S., Minekus, M., … Carrière, F. (2020). A standardised semi-dynamic in vitro digestion method suitable for food–an international consensus. *Food & Function, 11*(2), 1702–1720. https://doi.org/10.1039/C9FO01293A

Musa, S., Oellig, C., & Scherf, K. A. (2025). Mitigation of acrylamide in cookies and crispbread using calcium salts and phenolic acids in combination with asparaginase as well as rosemary extract. *Food Chemistry: X, 28*, Article 102605. https://doi.org/10.1016/j.fochx.2025.102605

Pascoalino, L. A., Finimundy, T. C., Pires, T. C. S. P., Pereira, C., Barros, L., Ferreira, I. C. F. R., … Reis, F. S. (2025). Investigating bioactive compounds in apple pomace: Potential to develop high added-value products from an industrial surplus. *Food Bioscience, 63*, Article 105664. https://doi.org/10.1016/j.fbio.2024.105664

Sansano, M., Heredia, A., Peinado, I., & Andrés, A. (2017). Dietary acrylamide: What happens during digestion. *Food Chemistry, 237*, 58–64. https://doi.org/10.1016/j.foodchem.2017.05.104

Sarion, C., Codină, G. G., & Dabija, A. (2021). Acrylamide in bakery products: A review on health risks, legal regulations and strategies to reduce its formation. *International Journal of Environmental Research and Public Health, 18*(8), 4332. https://doi.org/10.3390/ijerph18084332

Suo, X., Tagliasco, M., Bonfini, M., Bonfili, L., Moreno Araiza, O., Baggio, A., … Vittadini, E. (2025). Development of sugar- and fat-reduced pulse cookies with improved predicted glycemic behavior. *Applied Food Research, 5*(1), Article 100761. https://doi.org/10.1016/j.afres.2025.100761

Torres, J. D., Dueik, V., Carré, D., & Bouchon, P. (2019). Effect of the addition of soluble dietary fiber and green tea polyphenols on acrylamide formation and in vitro starch digestibility in baked starchy matrices. *Molecules, 24*(20), 3674. https://www.mdpi.com/1420-3049/24/20/3674.

Yang, L., Wang, S., Zhang, W., Zhang, H., Guo, L., Zheng, S., & Du, C. (2022). Effect of black soybean flour particle size on the nutritional, texture and physicochemical characteristics of cookies. *LWT, 164*, Article 113649. https://doi.org/10.1016/j.lwt.2022.113649