Fagopyrum 42 (2): 41-47 (2025)

Research Paper

Antigenotoxic Effects of Tartary and Common Buckwheat Extracts, Rutin, and Quercetin on DNA Damage Induced by the Dietary Mutagen Acrylamide

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DOI https://doi.org/10.3986/fag0048

Received: June 11, 2025; accepted June 27, 2025
Key words: common buckwheat, Tartary buckwheat, DNA damage, acrylamide, antigenotoxic

ABSTRACT

The antigenotoxic effects of methanolic extracts of Tartary (*Fagopyrum tataricum* Gaertn.) and common buckwheat (*Fagopyrum esculentum* Moench) flour were evaluated against acrylamide-induced DNA damage. Acrylamide, a toxic food contaminant, was first identified in 2002 following its detection in Swedish food products. Our findings demonstrate that extracts from both buckwheat species significantly reduced DNA strand breaks. Tartary buckwheat contains higher levels of rutin, quercetin, and polyphenols, and exhibits greater antioxidant activity compared to common buckwheat. Due to endogenous rutin-degrading glucosidase activity, part of the rutin was enzymatically converted into quercetin. Processing generally decreased antioxidant activity, with the exception of wheat bread, where a slight increase was observed, likely attributed to Maillard reaction products.

We confirmed that acrylamide induces genotoxic effects in HepG2 cells at all tested concentrations (0.3125, 0.625, 1.25, and 2.5 mM) after 24 hours of exposure, and that methanolic buckwheat extracts effectively reduced the formation of acrylamide-induced DNA damage. The extract from Tartary buckwheat demonstrated the highest antigenotoxic activity, surpassing even pure rutin or quercetin at higher concentrations. These results suggest that although thermal processing can generate potentially harmful compounds, such as acrylamide, food matrices may simultaneously contain bioactive components capable of counteracting or mitigating such adverse effects.

INTRODUCTION

Acrylamide is a chemical compound typically formed in starchy foods subjected to hightemperature processing such as baking, frying, and roasting. It is generally absent in foods that are boiled or prepared using microwave heating (Capuano & Fogliano, 2011). It forms as a byproduct of the Maillard reaction, occurring between naturally present carbohydrates and amino acids. While some Maillard reaction products exhibit beneficial antioxidant, antimicrobial, or anti-allergenic properties (van Boekel et al., 2010), others, especially those formed during thermal processing, can have mutagenic, carcinogenic, or cytotoxic effects (Knize et al., 1999). These include heterocyclic amines, nitrosamines, polycyclic aromatic hydrocarbons, 5hydroxymethylfurfural, and acrylamide.

Acrylamide is considered one of the most important heat-induced food contaminants. It has been detected in a wide range of food products, including potato chips, French fries, cornflakes, bread, cookies, and coffee, as well as in roasted nuts, olives, and—unexpectedly—dried fruits (Capuano & Fogliano, 2011; Lupăescu & Oroian, 2025). Numerous studies have confirmed its neurotoxic, mutagenic, and carcinogenic properties (Shipp et al., 2006¸ Govindaraju et al., 2024; Đekić et al., 2025).

Growing consumer interest in healthy eating, has revived attention toward traditional crops. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) and common buckwheat (*Fagopyrum esculentum* Moench) are two such underutilized crops, recognized for their high nutritional value and potential healthpromoting properties. In Europe, buckwheat is cultivated in several countries, including Russia, Belarus, Ukraine, Poland, Croatia, Slovenia, Austria, Denmark, and France, among other countries (Kreft, 1995; Jha et al., 2024).

Buckwheat is known for its excellent nutritional profile, particularly its richness in polyphenols, notably the flavonoids rutin and quercetin (Fabjan et al., 2003; Kreft et al., 2020). The aim of this study was to evaluate the potential antigenotoxic effects of methanolic extracts from buckwheat-based food products (bread, cookies) containing rutin and quercetin, against acrylamideinduced DNA damage in HepG2 cells *in vitro*.

MATERIALS AND METHODS

Initially, we assessed DNA damage in HepG2 cells induced by acrylamide at concentrations ranging from 0.3125 mM to 2.5 mM. Subsequently, we evaluated whether cotreatment with methanolic extracts of common and Tartary buckwheat in the presence of 2.5 mM acrylamide, after 24-hour incubation could reduce DNA damage. A methanolic extract from wheat, which lacks rutin and quercetin, was included as a control.

Tartary buckwheat, common buckwheat and wheat flour extracts

Tartary buckwheat (TB) and common buckwheat (CB) were cultivated in the experimental field of the Biotechnical Faculty, University of Ljubljana, Slovenia, sown in June and harvested in August. Seeds were air-dried at 30°C until constant weight and milled using a Hawos Billy Mill 200 (Getreidemühlen Reisinger, Ybbsitz, Austria) to obtain TB and CB flour with a particle size of <0.236 mm. Commercial wheat (W) flour (type 850) was purchased from Mlinotest (Ajdovščina, Slovenia) and had flour particle size <0.200 mm. All flour samples are stored at University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia. Wheat was selected as a control material due to its compositional similarity to buckwheat but with negligible or non-detectable flavonoid content and markedly lower antioxidant capacity compared to buckwheat. This allowed exclusion of possible matrix effects when comparing the biological activities of flavonoid-rich material.

Methanolic extracts were prepared by adding 25 mL of 80% methanol (HPLC grade) in water to 1 g of each flour sample. The mixtures were shaken at room temperature for 8 hours at 250 rpm. Extracts were then filtered through filter paper (70 g/m^2, Macherey-Nagel, Germany), aliquoted, and stored at –20°C until further use.

Genotoxicity testing

The potential genotoxicity of wheat (W), common buckwheat (CB) and Tartary buckwheat (TB) extracts, as well as pure rutin (R) and quercetin (Q), was evaluated using the comet assay. HepG2 cells were seeded into 12-well microtiter plates (Corning Costar Corporation, Corning, NY, USA) at a density of 40,000 cells/well. After 24 h of incubation at 37°C in 5% CO_2 and humidified atmosphere, the growth medium (William's E) was replaced with a fresh medium, containing W, CB and TB extracts (0.04 %, 0.2 % and 1 % (v/v)), rutin (4, 20 and 100 μM) or quercetin (2, 10 and 50 μM). Cells were then incubated for an additional 4 and 24 hours under the same conditions. A negative control (non-treated cells),

a solvent control (1 % methanol in growth medium) and a positive control (benzo[a]pyrene [BaP], 30 μM) were included in each experiment. At the end of the exposure (4 and 24 h), cells were harvested, and DNA damage was determined using the comet assay as described by Moller et al (2020). with minor modifications.

Comet assay

The comet assay was performed as described by Møller et al. (2020). Briefly, 30 μL of cell suspension was mixed with 70 μL of 1% low melting point agarose and immediately added to fully frosted microscope slides precoated with a layer of 1% normal melting point agarose. Cells were then lysed (2.5 M NaOH, 0.1 EDTA, 0.01 M Tris and 1% Triton X-100, adjusted to pH 10) for 1 h at 4°C. Afterwards, the slides were rinsed with distilled water, placed in electrophoresis buffer (1 mM EDTA, 300 mM NaOH, pH 13) for 20 min to allow DNA unwinding, and then electrophoresed for 20 min at 25 V and 300 mA. Subsequently, the slides were neutralized with 0.4 M Tris buffer (pH 7.5), stained with ethidium bromide (5 μg/mL), and images captured using a fluorescence microscope (Nikon Eclipse 800). Images of 50 randomly selected nuclei per slide were analyzed using Comet Assay IV software (Perceptive Instruments, UK). The percentage of tail DNA was used as the measure of DNA damage. Three independent experiments were performed for each treatment condition.

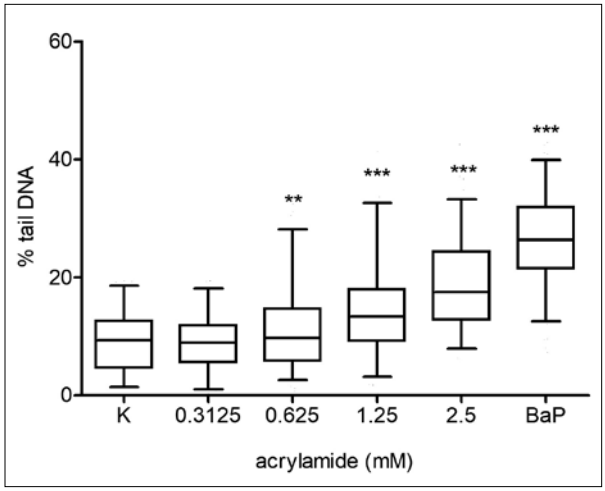

Figure 1. *Effect of acrylamide at graded concentrations (0.3125, 0.625, 1.25, and 2.5 mM) on the formation of DNA strand breaks in HepG2 cells following 24 h exposure. Benzo[a]pyrene (BaP, 30 μM) was used as a positive control for genotoxicity. DNA damage was assessed with the comet assay and is expressed as percent of tail DNA. Fifty cells were analysed per experimental point in each of the three independent experiments. Data are presented as quantile box plots. The edges of the box represent the 25th and 75th percentiles, the median is a solid line through the box, and the error bars represent 95% confidence intervals. Significant difference (1-way ANOVA; Dunnet's Multiple Comparison test) between treated cells and vehicle control (K) is indicated by **P < 0.01 and ***P < 0.001.*

Statistical evaluation

The comet assay results, differences between treatment groups within each experiment were analyzed using non-parametric analysis of variance (Kruskal-Wallis test). Dunnett's multiple comparison test was used for the evaluation of differences between solvent control (1% methanol) and sample groups; *P < 0.05; **P < 0.01; ***P < 0.001 was considered statistically significant.

RESULTS AND DISCUSSION

Our results confirmed that acrylamide, at concentrations between 0.625 and 2.5 mM, induced DNA strand breaks in HepG2 cells after 24 hours of exposure. For antigenotoxicity assessment, we selected 2.5 mM acrylamide, the concentration that exhibited the most pronounced genotoxic effect (Figure 1). These findings align with previous studies documenting acrylamide's genotoxicity in hepatic cell lines, confirming its potential risk as a food contaminant (Shipp et al,. 2006).

As shown in Figure 2, the methanolic extract from Tartary buckwheat provided the most effective protection against acrylamide-induced DNA damage, significantly reducing DNA strand breaks even at the lowest tested concentration (0.008 % (v/v)). This was followed by the extracts from common buckwheat, while the wheat extract demonstrated the weakest protective capacity. The superior antigenotoxic activity of Tartary buckwheat may be attributed to its higher polyphenolic content, particularly rutin and quercetin, known for their antioxidant and DNA-protective properties. The weaker effect of wheat extract supports the idea that flavonoid content plays a critical role in modulating genotoxic effects.

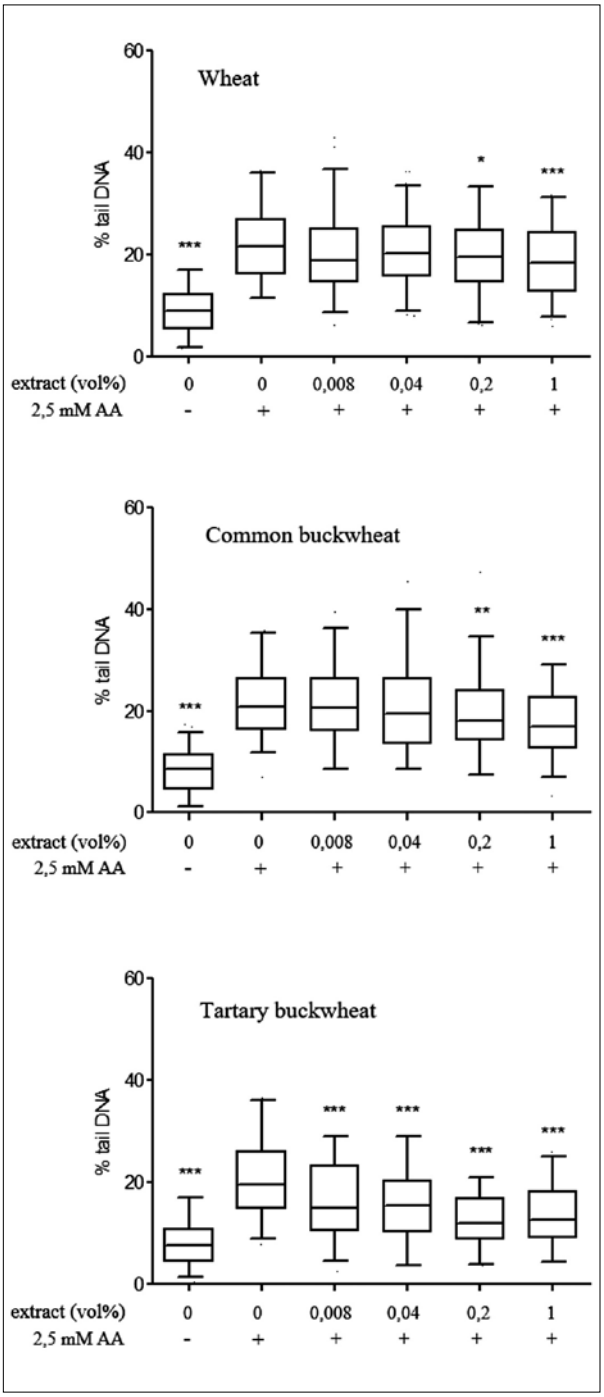

Figure 2. *Antigenotoxic effects of methanolic extracts from wheat, common buckwheat, and Tartary buckwheat in HepG2 cells. DNA damage was induced by acrylamide (AA, 2.5 mM). DNA damage was assessed with the comet assay and is expressed as percent of tail DNA. Fifty cells were analysed per experimental point in each of the three independent experiments. Data are presented as quantile box plots. The edges of the box represent the 25th and 75th percentiles, the median is a solid line through the box, and the error bars represent 95% confidence intervals. Significant difference (1-way ANOVA; Dunnet's Multiple Comparison test) between treated cells and vehicle control (0 extract, - 2.5 mM AA) is indicated by **P < 0.01 and ***P < 0.001. (see legend below Figure 1).*

When comparing the antigenotoxic potential of pure rutin (100 µM) and quercetin (50 µM), both flavonoids significantly reduced acrylamide-induced DNA damage in HepG2 cells (Figure 3). Notably, the protective effect of the buckwheat extracts was comparable to or even greater than that of the pure flavonoids, despite the lower concentrations of rutin and quercetin in the extracts. This suggests that additional compounds within the extracts may contribute synergistically to the observed DNA protection. Such synergy has been reported in complex plant matrices, where minor phenolics, vitamins, and other bioactive molecules enhance the overall antioxidant capacity (Alexander et al., 2023). It was suggested that a

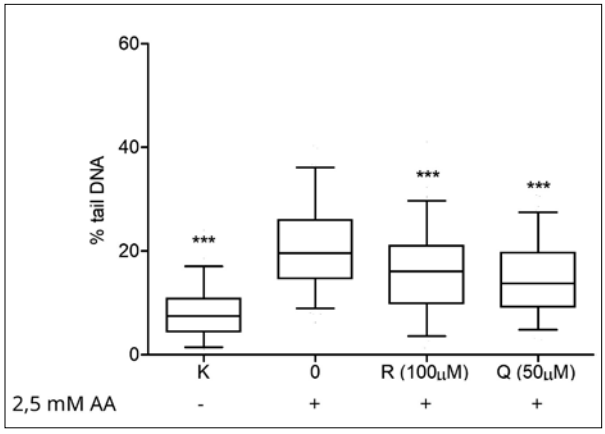

Figure 3. *Antigenotoxic effects of rutin (R) and quercetin (Q) in HepG2 cells. DNA damage was induced by acrylamide (AA; 2.5 mM). DNA damage was assessed with the comet assay and is expressed as percent of tail DNA. Fifty cells were analysed per experimental point in each of the three independent experiments. Data are presented as quantile box plots. The edges of the box represent the 25th and 75th percentiles, the median is a solid line through the box, and the error bars represent 95% confidence intervals. Significant difference (1-way ANOVA; Dunnet's Multiple Comparison test) between treated cells and vehicle control (K) is indicated by **P < 0.01 and ***P < 0.001.*

polyherbal kaempferol and quercetin-rich cocktail could treat Alzheimer's Disease related brain damage. In this way a mixture of natural flavonoids with synergistic effects could be supporting an alternative treatment to currently available medicines (Alexander et al., 2023). It was pointed out by Rahmatkar et al. (2024) that oxidative stress and neuroinflammation play a crucial role in neurodegenerative conditions. Based on the ethnomedical claims and available literature Rahmatkar et al. (2024) suggested that neuroprotective efficacy of a Tartary buckwheat seed extract could have effects against acrylamide induced neurotoxicity. Further investigations are needed to identify the specific compound(s) responsible for the observed antigenotoxic activity and to elucidate their mechanisms of action.

These findings are encouraging, as they demonstrate that bioactive compounds naturally present in foods — such as those in buckwheat — can counteract the harmful effects of substances formed during food processing, thereby mitigating or neutralizing their impact.

CONCLUSION

Our study confirmed the genotoxic potential of acrylamide in HepG2 cells at all tested concentrations after 24-hour exposure, reinforcing concerns about the health risks posed by this common food contaminant. Importantly, methanolic extracts from buckwheat significantly reduced acrylamide-induced DNA damage, with Tartary buckwheat extract exhibiting the strongest antigenotoxic activity — even greater than that of pure rutin or quercetin. Such pronounced activity points to the presence of multiple bioactive components in Tartary buckwheat that synergistically enhance protection at the cellular level.

These findings highlight the promising role of naturally occurring compounds in foods as effective dietary agents to mitigate the genotoxic effects of contaminants formed during thermal processing, such as acrylamide. Incorporating such antioxidant-rich plant materials into the diet could represent a valuable strategy for reducing genotoxic risk associated with processed foods. Moreover, this study provides a basis for further research aimed at identifying and characterizing the specific bioactive constituents responsible for this protective effect, as well as understanding their mechanisms of action in relevant biological systems.

Future investigations should also consider more complex *in vitro* test systems such as 3D cell models and the impact of food matrix and processing on the bioavailability and efficacy of these compounds, ultimately contributing to the development of safer and healthier food products.

ACKNOWLEDGEMENT

Funding: This work was the result of a study financed by the Slovenian Research and Innovation Agency, through the program ARIS P1-0245 (Ecotoxicology, Toxicogenomics and Carcinogenesis).

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IZVLEČEK

Antigenotoksični učinki izvlečkov tatarske in navadne ajde, rutina in kvercetina na poškodbe DNA, povzročene s prehranskim mutagenom akrilamidom

Raziskali smo antigenotoksične učinke metanolnih izvlečkov moke tatarske in navadne ajde proti poškodbam DNK, ki jih povzroča akrilamid. Akrilamid je toksičen kontaminant v hrani, prvič identificiran leta 2002 po njegovem odkritju v živilih na Švedskem. Naši rezultati so pokazali, da tako tatarska kot navadna ajda pomembno zmanjšata obseg poškodb DNK. Znano je, da tatarska ajda vsebuje višje koncentracije rutina, kvercetina in polifenolov ter izkazuje večjo antioksidativno aktivnost v primerjavi z navadno ajdo. Zaradi prisotnosti encima rutin glikozidaze, se je del rutina pretvoril v kvercetin. Antioksidativna aktivnost se je po obdelavi večinoma zmanjšala, izjema je bil pšenični kruh, kjer je prišlo do rahlega povečanja, verjetno zaradi Maillardove reakcije. Med termično obdelavo živil z visokim deležem ogljikovih hidratov se med termično obdelavo pri visoki temperaturi (pečenje, cvrtje, praženje) kot stranski produkt Maillardove reakcije, ki poteka med sladkorji in aminokislinami, tvori akrilamid. Le-ta ima v večjih količinah škodljiv vpliv na zdravje, saj deluje mutageno, kancerogeno in nevrotoksično.

Potrdili smo, da akrilamid povzroča genotoksične učinke v celicah HepG2 pri vseh testiranih koncentracijah (0,3125; 0,625; 1,25 in 2,5 mM) po 24-urni izpostavitvi ter da metanolni ekstrakti ajde učinkovito zmanjšajo obseg z akrilamidom povzročene poškodbe DNK. Pri tem je bil najbolj učinkovit metanolni ekstrakt tatarske ajde, ki je bil pri tem celo učinkovitejši kot sam rutin ali kvercetin v višjih koncentracijah. Rezultati kažejo, da kljub nastajanju potencialno škodljivih spojin, kot je akrilamid, med termično obdelavo hrane, lahko živilske matrice hkrati vsebujejo bioaktivne spojine, ki te negativne učinke omilijo ali celo izničijo.