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A preliminary study of the effects of transformations induced by gamma-ray treatment on the detection of *Acheta domesticus* and *Tenebrio molitor* allergenic proteins by enzyme-linked immunosorbent assay (ELISA) techniques

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Abstract

Introduction. With the global population projected to reach approximately 9 billion by 2050, there's a growing need to explore alternative food sources. Insects have emerged as a potential solution to meet food demand, offering a substitute for conventional livestock. However, a primary safety concern surrounding these novel foods is their allergenic potential, especially given the absence of standardized testing methodologies. To mitigate this risk, food irradiation has been explored as a method to reduce allergenicity in insects intended for human and animal consumption.

Material and methods. This study utilized an enzyme-linked immunosorbent assay to determine the allergenic proteins in specific insect types after irradiation treatment.

Results and discussion. Significant differences in detectable protein levels were observed between *Tenebrio molitor* and *Acheta domesticus* samples, but no significant differences in protein content were found between food and feed samples of both species under identical irradiation conditions. Further research is required to ensure the protocol's suitability for more complex food matrices.

Key words

- edible insects
- food contamination
- food hypersensitivity
- enzyme-linked immunosorbent assay (ELISA)
- food irradiation

INTRODUCTION

The global food system faces increasing challenges as it strives to meet the nutritional needs of an ever-growing population within the constraints of environmental sustainability. It has been estimated that global population will reach 9.8 billion in 2050, so requiring an increase of livestock production of about 40% with respect to 2019 [1]. However, traditional livestock production, the main source of protein at the time, is associated with significant environmental impacts, namely high greenhouse gas emissions, extensive land and water use, and significant contributions to deforestation and biodiversity loss [2]. Insect consumption has therefore been proposed as a viable means to address these major challenges [3]. In fact, insect farming may represent a significant opportunity to reduce the impact of agriculture on climate change, mainly because insects emit fewer greenhouse gases than traditional livestock [2, 4]. Moreover, insects can efficiently convert feed into protein while requiring minimal land and water for breeding. From a nutritional perspective, insects are a rich source of micro- and macromolecules, making them a valuable addition to the human diet. Particularly, they are rich in high-quality protein whose average con-

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tent is of about 40%, ranging from 20% up to over 70%, depending on the species and the development stage at the time of harvesting [5]. Currently, edible insects are a dietary component for about 2 billion consumers, although consumption in Europe and the Americas is low [3]. Nonetheless, the global edible insects' market is expected to reach USD 16.39 billion by 2032 [6].

In Europe, edible insects are considered novel food, namely food product that does not have a history of human consumption within Europe and that was not significantly consumed before May 1997, when the first Novel Food regulation came into place. Therefore, their introduction as a food in the market must follow the Novel Food Regulation 2015/2283 [7] and its implementing Regulations 2468/2017 and 2469/2017 [8, 9]. Nowadays, four insect species, namely Tenebrio molitor (Coleoptera: *Tenebrionidae*), *Alphitobius diaperinus* (Coleoptera: *Tenebrionidae*), *Locusta migratoria* (Orthoptera: Acrididae), and *Acheta domesticus* (Orthoptera: Gryllidae), have been authorized as novel foods [10-15]. As well as being a viable alternative to many foods, edible insect proteins have also been studied as an alternative protein source in animal feed [6, 16]. It must be pointed out that insects intended for animal or human consumption are farmed animals; therefore, they cannot be collected from the wild and the general legislation for feeding other livestock should be applied [17]. Noteworthy, the parts of insects that can be used varies depending on the animal to be fed and whether it enters the food chain [6].

Despite the recognized benefits, there are several barriers to the widespread adoption of insect-based proteins [6]. Particularly, disgust, food neophobia, lack of interest, lack of information, and no prior experience have been reported as the most frequent factors affecting this repulsion [2, 6]. Also, the potential safety concerns represent a limitation to their use. Besides contamination with pathogens and heavy metals [1, 18], edible insects may be also a source of allergens. Indeed, the consumption of novel food insects may induce primary sensitization and allergic reactions to insect protein [19]. The primary allergenic proteins present in insects are tropomyosin and chitin. Current research points to tropomyosin as the leading allergen causing cross-reactivity among crustaceans, mollusks, mites, and cockroaches. Chitin, a polysaccharide forming the exoskeleton of insects, is another molecule of concern. Studies have revealed diverse effects of chitin on the immune system [20].

Tropomyosin was identified as a major allergen with cross-reactivity for subjects already allergic to crustacean and cross-reactivity with pan-allergens of the Arthropoda genus, namely crustaceans and house dust mites, has been reported too [21]. Therefore, the labeling of insect-based food products should include a statement near the ingredients list indicating that they may cause allergic reactions in individuals with known allergies to crustaceans and dust mites.

To overcome the safety issue due to the allergenic risk, food processing methods have been investigated to modulate the risk of cross-reactivity and allergenicity of edible insects. In fact, it has been demonstrated that food processing could have the potential to reduce the IgE-binding ability of allergens, thereby attenuating IgE-mediated allergic responses, by altering both the linear and conformational structure of epitopes [21]. In this context, previous studies have demonstrated that enzymatic hydrolysis and thermal treatments [22, 23] can effectively reduce cross-reactivity and allergenicity in edible insects [21]. Moreover, gamma radiation (1-15 kGy) has also been shown to effectively reduce the IgE-binding capacity of tropomyosin in shrimp [24].

Gamma radiation treatment is already used against microbiological contamination, to preserve the hygienic quality of food during storage and transport, ensure shelf life, and reduce the risk of foodborne illness [25]. It is toxicologically and microbiologically safe and nutritionally adequate for any food irradiated up to a maximum dose of 10 kGy [26]. Irradiation's primary advantage overheat processing or chemical treatments is its capacity to decrease microbial load without compromising product quality. Over the last two decades, the treatment with ionizing radiation has been used all over the EU [27] on a large variety of foods [28] including frog legs, poultry, fish, and vegetables. In the European Union the treatment is regulated by the Directive 1999/2/EC [29] that covers general and technical aspects of irradiation, the labelling of irradiated food, and the conditions for authorizing food irradiation.

However, up to date, no studies have investigated the effect of gamma radiation on the allergenicity of the edible insects authorized by the European Commission [30]; therefore, further experimental work is needed to determine optimal treatment conditions, ensuring safety without compromising the nutritional and organoleptic value of these foods. In this context, the present work was aimed at studying the effect of gamma radiation on the allergenicity of both food and feed samples of the house cricket (*Acheta domesticus*, Linnaeus, 1758, AD) and the mealworm (*Tenebrio molitor*, Linnaeus, 1758, TM). These two insect species are known for their high protein content and wide commercial distribution across various regions of the world. Furthermore, the European Food Safety Authority (EFSA) has recently completed safety assessments for their consumption. Particularly, *Acheta domesticus* is an orthopteran insect commonly classified under the family Gryllidae (Orthoptera-Insecta-Arthropoda-Invertebrates), likely originating from southwestern Asia. Adults measure between 16 and 26 mm in length. Their bodies are brownish to yellowish and pale, with three distinct dark crossbands on the head [31]. Although this species originates from Northern Africa and the Middle East, it is now distributed in eastern North America, Europe, and India, and has been introduced into Latin America. Ten allergenic proteins have been identified in cricket, namely hexamerin 1B precursor, hypothetical accessory gland protein (partial), myosin heavy chain isoform G, myosin heavy chain (MHC), putative arginine kinase, tropomyosin, and tropomyosin isoforms [32]. Among these cricket allergens, tropomyosin has high cross-reactivity with the serum of shrimp allergic patients [32]. *Tenebrio molitor* is an insect species that belongs to the family of Tenebrionidae (darkling beetles). Adults mea-

sure approximately 14 mm in length, with shiny dark brown to black bodies. Common names for these insects often reference the coloration of their immature stages. TM is a cosmopolitan species, naturally found in the temperate regions of Europe, but it is now distributed worldwide. The larvae feed primarily on stored grain products, making them a common pest. They also consume animal-based materials such as meat scraps, dead insects, and feathers. A variety of allergenic proteins have been identified in TM, including alpha-amylase, putative trypsin-like proteinase, putative serine proteinase truncated, cockroach allergen-like protein, larval cuticle protein A1A, larval cuticle protein A2B, and larval cuticle protein A3A [33, 34]. In addition, TM and AK in yellow mealworm strongly cross-react with IgE in patients with house dust mite (HDM) and crustacean allergy, which indicate their allergenicity [34, 35].

To achieve our objective, a crustacean allergen detection test (enzyme-linked immunosorbent assay – ELISA) was exploited to establish if the clinical cross-sensitization can also translate into analytical cross-reactivity.

MATERIAL AND METHODS

Insect sampling

Insect samples for both food and feed applications were provided by the food and feed supply chain operator Italian Cricket Farm (Via Vigone 20, 10060 Scalenghe, Turin, Italy). Each species was processed through dedicated production lines for either food or feed purposes.

The process for food production line (FO) includes the following stages:

1. insects were carefully selected at an age of 50 to 60 days, ensuring that females had not yet laid eggs;
2. the selected insects underwent a fasting period of 24 to 36 hours;
3. they were then humanely stunned by exposure to hypothermia in a cold room (2-8 °C);
4. after stunning, the insects were boiled for approximately 10 minutes.

Conversely, for the feed production line (FE), no restriction about age or sex was applied for insects. However, as for FO, they underwent a fasting period of 24 to 36 hours and the stunning process via hypothermia (2-8 °C).

In both lines, the final step involved drying the insects in a desiccator to achieve a relative humidity level of 5% before removal for further processing.

Sample irradiation

Whole dried insects were irradiated using a Nordion Gammacell 40 Cs-137 irradiator (Ottawa, 447 March Rd., Ottawa, ON, Canada, K2K 1X8), located at the Italian National Institute of Health (Istituto Superiore di Sanità, ISS). The irradiator operated at a dose rate of approximately 0.7 Gray per minute, with a variation of ±15%.

Irradiation treatments were conducted at room temperature, applying doses of about 1 (identifiable lower-level treatment) and 3 kGy (used in some countries for shrimp treatment). For each dose, approximately 100 grams of each sample type (AD and TM, both food and feed) was packaged in sterile polyethylene bags (Fisherbrand™ Twirl'EM™) and placed within the irradiation chamber. Following irradiation, samples were stored in a silica dryer at room temperature. To prepare samples for analysis, they were ground using a mortar and pestle, avoiding the use of a blender to minimize potential thermal effects and preserve sample integrity.

Sample preparation

Approximately 1 g aliquots of sample grinded in mortar were prepared for each group for subsequent analysis. For research purposes, the samples were labelled with a 6-character alphanumeric code according to the scheme *(Table 1)*: Species (AD/TM), Food (FO)/Feed (Fe), exposure (Not irradiated - 0 kGy, 1 kGy, 3 kGy).

A total of 69 samples were prepared: 33 for method validation and 36 for testing. Particularly, 20 AD and 13 TM samples were exploited to verify the manufacturer's validation report, while 18 AD and 18 TM samples for testing. Samples, no longer in their original packaging condition, were stored at room temperature.

Enzyme-linked immunosorbent assay (ELISA) determination

Given the lack of specific methods and certified reference materials for detecting insect presence using the ELISA technique, as well as the reported cross-reactivity of insect proteins with crustacean ones, the Ridascreen® FAST Crustacean Enzyme Immunoassay Kit (Art. R7312) supplied by R-Biopharm Italia Srl (Via Morandi, 10, 20077 Melegnano MI, Italy) was utilized to determine insect allergenic proteins. This sandwich immunoassay kit is designed for the quantitative detection of crustaceans that may be present either as ingredients or contaminants in both raw and cooked food products. It identifies crustacean proteins, mainly

Table 1
Sample code assignment

Species	Intended use	Sample labelling		
		Not irradiated	1 kGy	3 kGy
Acheta domesticus (AD)	Food (FO)	AD FO 0k	AD FO 1k	AD FO 3k
	Feed (FE)	AD FE 0k	AD FE 1k	AD FE 3k
Tenebrio molitor (TM)	Food (FO)	TM FO 0k	TM FO 1k	TM FO 3k
	Feed (FE)	TM FE 0k	TM FE 1k	TM FE 3k

tropomyosin from the troponin-tropomyosin-complex, a protein recognized as the primary allergen in this group of organisms, characterized by heat resistance, and therefore suitable for the detection of crustaceans in food samples.

The assay operates within a range of 2-160 mg of crustaceans per kg of food (ppm). The reliability of the kit was evaluated by comparing key validation parameters – such as selectivity, limits of detection (LOD), limits of quantification (LOQ), repeatability, and recovery – provided by the manufacturer with our experimental findings. The kit instructions also report minor cross-reactivity with arthropods, due to similarities in protein composition. For locusts, the manufacturer reports a cross-reactivity value of 23,600 mg protein/kg for pure samples diluted at a ratio of 1:160.

Experiments were conducted following the manufacturer's guidelines. Particularly, 1 g of a representative sample was mixed with 20 ml of preheated Allergen Extraction Buffer (GFl 1002, Gesellschaft für Labortechnik GmbH, Burgwedel, Germany) at 60 °C. The mixture was vigorously agitated and incubated for 10 minutes at the same temperature in a water bath. After incubation, the samples were cooled in an ice bath and centrifuged for 10 minutes at 2500 rpm at 4 °C using a refrigerated centrifuge (ALC 4237R, ALC Apparecchi per Laboratori Chimici, Milan, Italy). Approximately 2 ml of the extract was then subjected to high-speed centrifugation at 12000 rpm for 10 minutes in reaction caps using a microcentrifuge. One hundred microliters of the extract were added to each well for the assay.

The testing methodology utilizes microtiter strips coated with specific antibodies for crustacean proteins. When standards and samples are added to these wells, any crustacean proteins present will bind to the antibodies. During a washing step, unbound components are removed. An antibody conjugated to peroxidase is then introduced, binding to the antibody-antigen complex, forming an antibody-antigen-antibody (sandwich) complex. Any unbound conjugate is eliminated in another washing step.

Crustacean proteins are detected by adding a Substrate/Chromogen solution, in which the peroxidase conjugate catalyzes the conversion of the chromogen into a blue product. The addition of a stop solution induces a color shift from blue to yellow, and photometric measurement is conducted at 450 nm. The absorbance measured correlates directly with the crustacean protein concentration in the sample, expressed in mg/kg. The instrumental results are then converted to grams of protein per kilogram (g/kg), accounting for the sample dilutions (1:25 for AD and 1:25-1:150 for TM).

RESULTS AND DISCUSSION

Validation

The reliability of the kit was evaluated by comparing its performance with experimental results relating to key validation parameters (accurate identification of presence, limits of detection, LOD, and limits of quantification, LOQ, repeatability, and recovery) as specified by the manufacturer (verified in different zero matrices). According to the validation report provided by the manufacturer, the LOD is indicated to be 2 ppm, while the LOQ aligns with the method's application threshold of 20 ppm. In our protocol, the optical density (OD) measured for each blank sample was found to be either near or below that of the 0 ppm calibrators, and lower than the optical density associated with the manufacturer's LOQ (20 ppm). Under our experimental conditions, the calculated LOD and LOQ for the method (verified in a rice flour matrix free from shrimp), derived as mean ± 3 standard deviations (SD) and 10 SD, respectively, were determined to be 2 ppm and 19 ppm. In every instance examined, the negative control exhibited an optical density signal lower than that of the initial point on the curve (20 ppm). Each analytical session included a positive sample consisting of shrimp meat, which the selected kit accurately identified on all occasions. The manufacturer's validation report indicates a repeatability estimate of 6.6%, derived from determinations conducted on spiked samples prepared by the manufacturer. Within this research the repeatability of the measurement was assessed by analyzing the deviations observed between two determinations of the same AD sample within the same analytical session.

The repeatability data ranged from 1.4% (for food at 0 kGy) to 9.4% (for feed at 3 kGy). The average percentage repeatability value calculated from two measurements across all samples is 3.3%. These values are fully compliant with the specifications outlined in Standard EN 15633-1 [36], which stipulates that the repeatability value at the recovery level must be less than 20%. Additionally, the validation report from the manufacturer states that the reproducibility estimate is 10.3%. All reproducibility values observed for both food and feed categories across three irradiation levels (0, 1, and 3 KGy) were found to be below this threshold. An evaluation of recovery values was not conducted in this preliminary study exclusively assessed insects as a raw material while certified reference standards for AD and TM were unavailable. Based on the tests conducted, the method was deemed suitable for detecting the presence of insects by identifying crustacean proteins.

Experimental testing

Analytical measurements were conducted at different time intervals, with the day following the completion of 3 kGy irradiation serving as the starting point (T0). AD samples were analyzed at T0 (immediately after irradiation), as well as 7- and 28-days post-irradiation. TM samples were analyzed 18, 22, and 28 days after the irradiation endpoint. For each product type (AD and TM), both food and feed samples were analyzed to determine the concentration of crustacean proteins (g/kg) in untreated (0 kGy) and irradiated samples (1 kGy and 3 kGy). *Table 2* provides obtained data for AD and TM samples, respectively, across the different irradiation treatments (0, 1, and 3 kGy).

The analysis revealed varying levels of crustacean proteins in AD samples, ranging from 1.2 g/kg in irradiated feed samples (AD FE 1K) to 3.7 g/kg in not irradiated food samples (AD FO 0K). The data demonstrated a

Table 2
Crustacean content (grams per kilogram) found in non-irradiated and irradiated (1 kGy and 3 kGy) *Acheta Domesticus* (AD) and *Tenebrio Molitor* (TM) food and feed samples

	g/kg (mean ± partial dispersion)				
Sample code	**day 0**	**day 7**	**day 18**	**day 22**	**day 28**
AD FO 0K	3.7±0.02	2.9±0.07			1.3±0.02
AD FO 1K	2.9±0.06	1.9±0.12			2.1±0.09
AD FO 3K	2.8±0.12	2.5±0.02			1.3±0.03
AD FE 0K	2.1±0.01	1.5±0.04			1.5±0.02
AD FE 1K	1.5±0.01	1.2±0.04			1.9±0.04
AD F3 3K	1.9±0.12	2.8±0.35			1.2±0.11
TM FO 0K			4.7±0.17	5.4±0.15	5.6±0.17
TM FO 1K			5.4±0.25	7.4±0.05	1.2±0.03
TM FO 3K			5.7±0.01	4.5±0.05	1.0±0.01
TM FE 0K			6.3±0.17	6.6±0.07	6.5±0.12
TM FE 1K			6.4±0.13	8.1± 0.36	7.5±0.25
TM FE 3K			5.6±0.08	4.0±0.25	5.5±0.12

significant variability, particularly with respect to reproducibility. While repeatability within the same sample and analytical run was relatively low (1.4-9.4%), reproducibility across different analytical runs of the same sample was higher, ranging from 8% to 30% for both food and feed AD samples.

The data demonstrated that there were no statistically significant differences in protein concentrations between food and feed. Furthermore, no significant differences in protein content were observed between food and feed samples of both AD and TM under identical irradiation conditions (0, 1, and 3 kGy). Conversely, a significant difference in detectable crustaceans' protein levels was observed between TM and AD samples, with TM samples showing significantly higher levels (Kruskal-Wallis test, p=5.676E-05), as illustrated in *Figure 1* [37]. To the best of our knowledge, there are not many studies that have evaluated the effect of irradiation on the allergenic effect of insect proteins. In a 2023 study, Yang *et al*. concluded that high doses of radiation induce structural changes in tropomyosin (unfolding or aggregation), which result in the reduction of the IgE-binding capacity of tropomyosin.

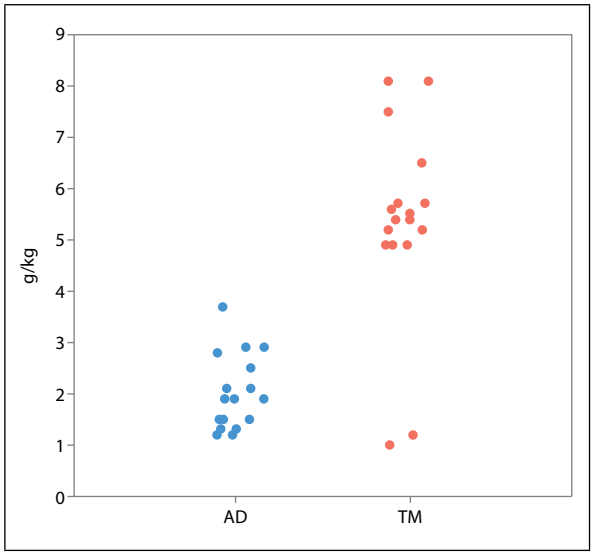

Figure 1
Density distributions (Jitter plot) of crustacean protein content (g/kg) in irradiated and non-irradiated *Acheta Domesticus* (AD) and *Tenebrio Molitor* (TM) food and feed samples.

The interpretation of this scientific evidence is complex and multifaceted. And some aspects warrant further investigation.

Firstly, the protein content and composition can vary significantly among different crustacean and insect's species. Moreover, the antibody used in the assay may exhibit varying affinities for proteins from different species. Consequently, different crustacean species may yield different results, as the calibration of the assay was performed using a limited set of representative species (Ridascreen RIDASCREEN® FAST Crustacean -2nd generation- Art. No. R7312 product information's). In addition, the inherent heterogeneity of protein content across different insect species significantly complicates the correlation between the ELISA-derived total allergenic protein levels and the precise protein composition of each specific insect matrix. Consequently, the experimental data do not elucidate whether the observed discrepancy is attributable to a genuine difference in total protein.

Numerous analogous studies have yielded inconsistent results. Rothman [38] reported protein concentrations of 42.2% ± 20.2% for adult Coleoptera, 37.3% ± 13.3% for immature Coleoptera, and 49.0% ± 23.0% for adult Orthoptera. These findings align with the study

by Rumpold [39], which reported mean protein values of 40.69% for Coleoptera and 61.32% for Orthoptera, and with van Huis [40], who reported a protein content on a dry matter basis for insects ranging from 7% to 91%. These findings were further corroborated by Churchward-Venne [41], who highlighted a protein concentration of 65.0% for AD and 58.1% for TM. Da Silva [21] reported a protein content range of 64.4%-70.7% for AD and 65.6% for TM. In two 2021 opinions [10, 11], EFSA reported a protein content for dried AD (4-5 weeks old) and TM (~11 weeks old) ranging from 59.5% to 60.8% and 55.5% to 58.8%, respectively. Yang [25] found a protein content of 46.44% for TM samples and 71.7% for AD. Subsequently, García-Vaquero [42] reported a protein content on a dry matter basis of 44.8% to 66% for TM and 62.57% to 70.7% for AD. These data are partially confirmed by Liang *et al*. [43], who highlighted a protein concentration of 40.69% for Coleoptera and 61.32% for Orthoptera.

However, a significant challenge in comparing results across these studies arises from the lack of standardized methodologies for protein quantification. Notably, a consensus on the optimal nitrogen-to-protein conversion factor for protein determination remains elusive and there is still no consensus about what the right nitrogen to protein ratio is to be used, when protein is determined by means of Dumas or Kjeldahl methods.

Furthermore, the protein and amino acid content of edible insects is quite variable, likely due to factors such as diet, developmental stage, geographic location, seasonal variations, and processing procedures [41]. Additionally, a direct comparison between literature-reported protein content values and allergenic protein data obtained from ELISA assays is complicated by the lack of comprehensive information regarding the composition of the standards and antibodies used in these assays. While ELISA kits can detect a broad spectrum of crustacean proteins, including tropomyosin and chitin, the specific quantitative contribution of each protein remains uncertain [21].

CONCLUSION

Globalization has accelerated the spread of dietary habits. In many cases, the nutritional and safety implications of consuming these novel foods have not yet been fully assessed. Insects, for example, have recently been approved as novel foods within the EU, but their consumption is already widespread globally. As a potentially more sustainable alternative to traditional protein sources, insects have garnered significant attention. However, there are still knowledge gaps in the scientific understanding of insect consumption. Existing studies often present conflicting results, highlighting the need for further research. Primarily, allergenicity is a significant food safety issue that requires further scientific investigation. The allergenic potential of edible insects is a significant concern as crustacean food allergy affects up to 4% of the population in different regions of the world. Given the high prevalence of crustacean food allergy, this poses a significant threat to individuals already allergic to crustaceans who may also react upon consuming novel insect protein-based foods. On the other hand, numerous studies have produced often contradictory results regarding the effectiveness of food technology techniques (such as irradiation) in reducing the allergenicity of a food. This pilot research delved into the food safety aspects of insect consumption and aimed to verify the possibility of using a simple analytical technique for the determination of insects in foods and to evaluate the effect of gamma irradiation on the allergenicity of *Acheta domesticus* (cricket) and *Tenebrio molitor* (mealworm) samples. At the current state of knowledge, no harmonized official analytical methods are available to determine allergenic insect proteins in food. Nor are certified reference materials and inter-laboratory proficiency schemes available. In this context, our study showed that an ELISA kit for the determination of crustaceans was able to detect certain insects (AD and TM) due to cross-reactivity and that irradiation did not significantly affect insect detection. Furthermore, no significant differences were found between samples intended for animal consumption and those intended for human consumption that underwent additional heat treatment (boiling). However, a key finding was significantly higher levels of detectable crustacean proteins in TM samples compared to AD ones, although it should be considered that the inherent complexity and heterogeneity of insect samples (both AD and TM) presented challenges in sample handling and analysis.

Research has confirmed the difficulties generally encountered in homogenizing complex matrices such as insects and dried products in general. Homogenization processes were performed manually to avoid overheating the insect aliquots, which could potentially degrade proteins. The limitation of this treatment is the possibility of obtaining finely homogenized samples. In this sense, a possible methodological evolution of this study is represented by the possibility of increasing the sample size and evaluating alternative homogenization methods. Advances in homogenization techniques for such samples can enhance the quality of analytical data, improving precision and enabling the detection of significant differences in allergenic protein levels. Further improvements in analytical performance could be achieved using more advanced analytical methods, certified reference materials, and proficiency testing. Also, future research in this area may explore the potential of using sieved insect flours. This aspect may influence the dispersion of the analytical data obtained. At last, the possibility of extending the methodology to complex foods that may contain insects as ingredients and as contaminants should be also explored. This latter assessment could represent a further step for the enhancement of scientific knowledge in this area of research and an increase in the actions carried out to protect allergic consumers.

Conflict of interest statement

The Authors declare that there are no conflicts of interest.

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