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IgE-based analysis of sensitization and cross-reactivity to yellow mealworm and edible insect allergens before their widespread dietary introduction

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The European Commission authorized the use of dried yellow mealworm (*Tenebrio molitor* - TM) as a food ingredient under Regulation EU 2021/882. As TM emerges as an important allergen source, sensitization and allergy to TM in various populations need investigation. The aim of this study was to assess the incidence of sensitization to TM before its introduction as a food ingredient in Poland, as well as checking the occurrence of co-sensitivity to TM and other invertebrate allergenic extracts and molecules. This analysis was performed using serum allergen-specific immunoglobulin E (sIgE) results in 6,173 individuals using the ALEX2 test to detect sensitivity to TM and other related allergens. A total of 4.3% of the study population had sIgE to TM extract, with 0.7% of those individuals being mono-sensitized to TM. Sensitization to TM was most commonly associated with a positive response to house cricket and migratory locust allergens. sIgE antibodies against TM significantly more commonly ($p < 0.001$) co-occurred with sIgE against other invertebrate allergens. Patients with sIgE against TM were most likely sensitised to tropomyosins (49.1% of patients), Niemann-Pick C2 protein (43.8%), group 5/21 allergen (38.6%), class III chitinases (37.1%), and cysteine proteases (34.1%). Based on the serum asIgE levels to TM prior to this ingredient being introduced as a food in Poland, we hypothesised that this primary sensitization may be associated with invertebrate allergies. Our analysis showed that sensitisation to TM was most commonly associated with a positive reaction to house dust mites and shrimp tropomyosins. Therefore, we speculate that individuals allergic to shrimp should exercise caution when consuming foods containing TM.

Keywords Yellow mealworm, *Tenebrio molitor*, Sensitization profile, Food allergy, Component resolved diagnostics (CRD)

The rapidly growing human population raises concerns about ensuring everyone can access to nutritious food[1]. Inevitably, new, alternative sources of nutrition are being introduced. Due to their high protein content, essential amino acids, and polyunsaturated fatty acids, edible insects - whose consumption is common in Asian, South American, and African countries - seem to be a good candidate to meet this growing need[2]. Despite the potential benefits, introducing insects to the diet remains controversial in Europe.

The yellow mealworm, scientifically known as *Tenebrio molitor* (TM), classified under the order of *Coleoptera*, is an insect from the family of *Tenebrionidae* (darkling beetles) and is one of candidates for an alternative source of proteins, fiber, and minerals for the growing world population[3]. On November 24, 2020, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) adopted a scientific opinion on the safety of dried yellow

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mealworm (TM larvae) as a novel food pursuant to Regulation (EU) 2015/2283. The Panel concluded that the novel food is safe for human consumption under the proposed uses and use levels[4]. On June 1, 2021, following a positive vote of the Standing Committee on Plants, Animals, Food and Feed (Novel Food and Toxicological Safety section) from May 3, 2021, the European Commission adopted Commission Implementing Regulation (EU) 2021/882 authorizing the placing of dried yellow mealworm on the market as a novel food according to Regulation (EU) 2015/2283[5,6]. These regulations are crucial as they provide a framework for assessing the safety and potential risks associated with new foods before they are introduced to the market. The European Food Safety Authority (EFSA) and the European Commission plays a key role in this process, ensuring that any novel food, including TM, meets stringent safety standards. This regulatory oversight helps to build consumer trust and facilitates the acceptance of new food sources, which is essential for addressing the nutritional needs of a growing population.

TM is a cereal pest in its natural habitat. TM life cycle consists of four distinct phases: egg, larva, pupa, and adult[7]. The larval stage lasting 2–3 months, is the only form approved for human consumption by an EU regulation[8]. TM larvae are yellowish-brown and grow to a size of 2.0–3.5 cm[9]. TM can be consumed whole or as powder and paste, used in dishes like nutrition bars, pasta, or biscuits. The TM family is classified under Hexapoda (Insecta) in the phylum Arthropoda. Within this phylum, allergens such as tropomyosin, arginine kinases, and glutathione S-transferase have been identified[4]. Therefore, the Regulation on the safety of dried TM larvae as a novel food indicates that their consumption may lead to primary sensitization to TM. Secondary sensitization due to cross-reactivity in patients sensitized to crustaceans or house dust mites has also been considered[4,10]. Cross-reactivity may occur between TM allergens and proteins of phylogenetically related organisms of species within the same and different arthropod subphylum, such as crustaceans and dust mites[4,11,12]. Moreover, TM allergy may be due to sensitization to allergens present in the food consumed by TM larvae[4].

There has not been any particular interest in TM allergens in Poland to date. However, the EU approval of the use of this insect as a human food ingredient raised the question of whether citizens of the countries where TM-containing foods are not yet on the market have antibodies against TM. Therefore, we analyzed the rates of sensitization to a TM extract based on serum allergen-specific immunoglobulins E (sIgE) levels determined with a multiplex ALEX2 test in individuals with a suspected allergy who had undergone the test at a Polish laboratory due to suspected allergy in the period 2019 – 2022 before TM was introduced as a food ingredient in Poland. Our study seems to be the first to evaluate sensitization to TM and other invertebrates in a relatively large study population ($n = 6{,}137$). This study may serve as a benchmark for assessing the rates of sensitization to TM later on, when Polish citizens will have had exposure to this insect, and for evaluating the risk of possible allergic reactions to TM. Therefore, this study needs to be continued in the future.

Methods

Subjects and study design

The prevalence of sIgE to TM in the Polish population was assessed based on laboratory test results obtained from facilities that performed the ALEX2 test (Macro Array Diagnostics GmbH, Vienna, Austria) between 2019 and 2022, inclusively. The relevant data were obtained from the Immunology Laboratory at the Children's Memorial Health Institute in Warsaw (Poland) and from chain laboratories Diagnostyka S.A. (Poland). All test results from individuals who underwent the ALEX2 test at these facilities were included in the analysis. These individuals were undergoing allergy testing to investigate their symptoms for possible allergies, thus they are referred to as 'suspected allergy patients'. Patient identification was not possible based on the obtained data. The only accessible data were the patient's age and sex and the levels of sIgE against the evaluated allergen extracts and molecules.

All procedures and methods employed in this study were conducted in strict adherence to the relevant guidelines and regulations, ensuring full compliance with ethical standards and research protocols. This study was approved by the Ethics Committee at the Medical University in Lublin (approval No. KE-0254/86./03/2023).

Multi-parameter ALEX2 test

The ALEX2 test is a multiplex third-generation tool for measuring sIgE levels against allergen extracts and molecules. The ALEX2 test can be used for simultaneously detecting sIgE against 295 allergens, including 117 extracts and 178 molecules derived from various allergen types, such as foods, animals, plants, and molds. Test results are quantitative and are expressed in kilo-units of sIgE antibodies per liter (kU/L).

Following the recommended normal ranges of sIgE specified by the ALEX2 test manufacturer, sIgE levels of ≥ 0.3 kU/L were considered positive. This threshold represents the cutoff for sIgE positivity, with results above this value being classified as positive for the evaluated allergens. The test results were exported from MADx Raptor Software into Excel spreadsheets.

For the purpose of our analysis, the analyzed allergens were stratified into invertebrate allergens (which included herring worm (*Anisakis simplex*) [rAni s 1, rAni s 3], crab [Chi spp.], brown shrimp [rCra c 6], lobster [Hom g], shrimp [Lit s], shrimp [Pan b], black tiger shrimp [nPen m 1, rPen m 2, rPen m 3, rPen m 4], house cricket [Ach d], migratory locust [Loc m], American cockroach [rBla g 1, rBla g 2, rBla g 4, rBla g 5, rBla g 9, Per a, rPer a 7], mealworm [Ten m], bee venom [Api m, nApi m 1, rApi m 10], hornet venom [Dol spp], common wasp venom [Ves v, rVes v 1, rVes v 5], *Polistes dominula* venom [Pol d, rPol d 5], fire ant [Sol spp.], *Argas reflexus* [rArg r 1], *Blomia tropicalis* [rBlo t 5, rBlo t 10, rBlo t 21], *Dermatophagoides farinae* [rDer f 1, rDer f 2], *Dermatophagoides pteronyssinus* [rDer p 1, rDer p 2, rDer p 5, rDer p 7, rDer p 10, rDer p 11, rDer p 20, rDer p 21, rDer p 23], *Glycyphagus domesticus* [rGly d 2], *Lepidoglyphus destructor* [rLep d 2], *Tyrophagus putrescentiae* [Tyr p, rTyr p 2], common mussel [Myt e], oyster [Ost e], scallop [Pec spp.], clam [Rud spp.], squid [Lol spp.]) and other allergens (i.e., all allergens evaluated as part of an ALEX2 test except for invertebrate allergens).

	Number of subjects		
6,173	Females	3143	50.9%
	Males	3030	49.1%
	Children	3143	50.9%
	Adults	3030	49.1%
Age [years]	Median	Min	Max
	17.0	1.0	92.0

Table 1. Study group characteristics, including the number of participants, gender and age distribution. *%* percent, *Min* minimum, *Max* maximum.

Tenebrio molitor:	Age				Test U	
	Me	Q_1	Q_3	*IQR*	*Z*	*p-value*
Negative	16.0	6.0	36.0	30	-2.83	0.005
Positive	24.0	8.0	37.0	29		

Table 2. The median age of individuals with positive and negative test results for *Tenebrio molitor* allergens, $n = 6173$. *Me* median, *Q1* first quartile, *Q3* third quartile, *IQR* interquartile range, *Z* Mann-Whitney U test value.

Statistical analysis

To illustrate a sensitization to yellow mealworm, the following descriptive statistics were used in this analysis: frequency, percent, and mean. For data with a non-normal distribution, the median (Me) was chosen as the preferred measure of central tendency. Accompanying statistics for data dispersion included the first quartile (Q1), third quartile (Q3), interquartile range (IQR), minimum value (Min), and maximum value (Max). Non-parametric statistical tests were applied to these variables. Where necessary, exact numbers (n) with percentages (%) of all data or a specific fraction, as stated accordingly, were provided. The 95% confidence intervals (95% CI) were used. The chi-square test of independence was used to determine the statistical significance of differences in distribution between nominal variables. The Spearman rank correlation coefficient was used to test for differences between quantitative variables. The Mann–Whitney U test was used to test for the significance of the differences between the two groups in the results measured on a quantitative scale. The adopted level of significance was $p = 0.05$. Statistical analyses were conducted with IBM SPSS Statistics software, version 24.0.0.0.

Results

Subjects' characteristics

We analyzed the test results of 6,173 subjects, out of which 3,143 (51%) were female. The median subject age was 17 years. The youngest respondent was 1 year old, and the oldest was 92.0 years old (Table 1).

Elevated levels of sIgE against at least one allergen extract/component were detected in the sera of 4,220 subjects (68.36%). There were 267 positive results (4.3%) to a TM extract (+ sIgE TM), with the median sIgE level of 1.14 kU/L (Q1: 0.46 kU/L, Q3: 3.77 kU/L), (95% CI [3.7, 6.3])(Table S1). There was a significant difference in subject age between the groups stratified by the levels of sIgE against TM ($p = 0.005$). The median age of those with a negative test result was 16 years (IQR = 30). However, the median age of subjects with a positive result was higher at 24 years (IQR = 29) (Table 2).

A positive test result to TM was most commonly associated with a positive test result to house cricket (216/267; 80.9%) and migrant locust (169, 63.3%). Nearly 6% of all evaluated subjects (358/6,173) tested positive for sIgE (≥ 0.3) against *Acheta domesticus* and/or *Locusta migratoria* and/or TM allergens. Other common associations were between positive sIgE to TM and positive sIgE to grass and tree pollens (rPhl p 1 β-expansin), 58.1%; nLol p 1 (β-expansin) 53.6%; rBet v 1 (PR-10 subfamily), 49.1%; rFag s 1 (PR-10 subfamily), 45.7% and rCyn d 1 (β-expansin), 44.9%; and other.

Analysis of the prevalence and levels of sIgE against a TM extract revealed significant ($p < 0.001$) co-occurrence of TM sIgE with sIgE antigens against allergens of other invertebrates, such as migratory locust (*Locusta migratoria*), crab (*Chionoecetes spp.*), house cricket (*Acheta domesticus*), fire ant (*Solenopsis spp.*), american cockroach (*Periplaneta americana*), common mussel (*Mytilus edulis*), northern shrimp (*Pandalus borealis*), oyster (*Ostrea edulis*), squid (*Loligo spp.*), and lobster (*Homarus gammarus*) (Table 3).

Analysis of sIgE double-positivity to TM extract and allergen molecules of other invertebrates showed TM sIgE to most commonly co-exist with sIgE antibodies against 4 out of 5 evaluated tropomyosins: rPer a 7 (rho = 0.92; $p < 0.001$), nPen m 1 (rho = 0.91; $p < 0.001$), rAni s 3 (rho = 0.88, $p < 0.001$), and rDer p 10 (rho = 0.84, $p < 0.001$). Other allergen molecules that showed a sIgE response in correlation with that to TM, in decreasing order, were rBlo t 5 (mites, group 5/21 allergen) (rho = 0.72; $p < 0.001$), rPol d 5 (antigen 5) (rho = 0.66; $p = 0.002$), rBlo t 10 (tropomyosin) (rho = 0.5; $p = 0.002$), rTyr p 2 (NPC2 family) (rho = 0.5; $p = 0.009$), rPen m 2 (arginine kinase) (rho = 0.36; $p = 0.026$), rDer p 20 (arginine kinase) (rho = 0.34; $p = 0.015$), and rDer f 1 (cysteine protease) (rho = 0.24; $p = 0.032$) (Table 4).

Phylum	Class	Order	Family	Species	Allergen name	% of positive test	Correlation coefficient* *p-value* (two-tailed test)
Arthropoda	Malacostraca	Decapoda	Majidae	*Chionoecetes spp.*	Chi spp.	32.60%	0.79
							< 0.001
			Nephropidae	*Homarus gammarus*	Hom g	34.50%	0.52
							< 0.001
			Penaeidae	*Litopaenaeus s.*	Lit s	27.00%	0.85
							< 0.001
				Pandalus borealis	Pan b	28.50%	0.64
							< 0.001
	Insecta	Orthoptera	Gryllidae	*Acheta domesticus*	Ach d	80.90%	0.77
							< 0.001
			Acrididae	*Locusta migratoria*	Loc m	63.30%	0.84
							< 0.001
		Blattodea	Blattidae	*Periplaneta americana*	Per a	21.30%	0.67
							< 0.001
		Coleoptera	Tenebrionidae	*Tenebrio molitor*	Ten m		1
							.
		Hymenoptera	Apidae	*Apis mellifera*	Api m	8.20%	0.04
							0.848
			Vespidae	*Dolichovespula spp.*	Dol spp	3.00%	− 0.29
							0.493
				Vespula vulgaris	Ves v	12.40%	− 0.01
							0.972
				Polistes dominulus	Pol d	5.60%	0.38
							0.16
			Formicidae	*Solenopsis richteri & Solenopsis invicta*	Sol spp.	10.50%	0.69
							< 0.001
	Arachnida	Sarcoptiformes	Acaridae	*Tyrophagus putrescentiae*	Tyr p	29.60%	0.15
							0.182
Mollusca	Bivalvia	Mytilida	Mytilidae	*Mytilus edulis*	Myt e	17.60%	0.65
							< 0.001
		Ostreida	Ostreidae	*Ostrea edulis*	Ost e	27.00%	0.62
							< 0.001
		Pectinida	Pectinidae	*Pecten spp.*	Pec spp.	8.60%	0.51
							0.013
		Veneroidea	Veneridae	*Ruditapes spp.*	Rud spp.	22.10%	0.52
							< 0.001
	Cephalopoda	Teuthida	Loliginidae	*Loligo spp.*	Lol spp.	25.50%	0.62
							< 0.001

Table 3. The prevalence of co-sensitization between *Tenebrio molitor* and other invertebrate allergens, and the relationship between quantitative variables. *Spearman rank correlation coefficient.

An analysis of patients with sIgE against TM showed two individuals (0.7%) monosensitized to Ten m (with no positive result against other ALEX2 tested allergens). These individuals were children aged 4 and 5 years, with anti-TM sIgE levels of 0.30 and 0.31 kU/L, respectively. In the subgroup of sIgE against TM there were also eight individuals (3.0%) with no positive result to any invertebrate allergens but with positive results to other allergens. Therefore, there were a total of 10 individuals (3.7%) with no positive test result to invertebrate allergens but with a positive test result to TM. In most cases, there were statistically significant differences between the individuals with a negative and those with a positive test result to TM allergens in terms of the rates of positive test results to other invertebrate allergens ($p < 0.001$). Higher rates of positive test results to each allergen were observed in the group of individuals with a positive test result to TM allergens. Out of the 267 individuals with a positive test result to Ten m, there were 202 individuals (75.65%) with a positive test result to any dust mite allergen (all dust mite allergens in this case were molecules, and the test included 18 of them). On average, an individual with a positive test result to Ten m had six positive test results to the evaluated dust mite allergens. The greatest number of individuals were Ten m-positive, with sIgE against two mite molecules (20.0%). More than half (53.0%) of Ten m-positive individuals showed from 1 to 5 positive test results to any of the evaluated dust mite molecules. Detailed characteristics of the co-occurrence of sIgE against TM and dust mite molecules have been included in Supplementary Table S2.

Phylum	Class	Order	Family	Species	Allergen name	Protein Family	% of positive test	Correlation coefficient*
								p-value (two-tailed test)
Nematoda	Secernantea	Ascaridida	Anisakidae	*Anisakis simplex*	rAni s 1	Serine protease inhibitors	3.00%	0.17
								0.693
					rAni s 3	Tropomyosin	38.60%	0.88
								< 0.001
Arthropoda	Malacostraca	Decapoda	Crangonidae	*Crangon*	rCra c 6	Troponin C	8.20%	− 0.06
								0.805
			Penaeidae	*Penaeus monodon*	nPen m 1	Tropomyosin	36.00%	0.91
								< 0.001
					rPen m 2	Arginine kinase	14.20%	0.36
								0.026
					rPen m 3	Myosin light chain 2	4.90%	0.2
								0.523
					rPen m 4	Sarcoplasmic calcium binding protein	5.60%	0.06
								0.84
	Insecta	Blattodea	Blattidae	*Blatella germanica*	rBla g 1	Nitrile specifier, microvilli-like protein with unknown function	0.40%	
					rBla g 2	Inactive aspartic protease	2.20%	− 0.03
								0.957
					rBla g 4	Lipocalin	8.60%	0.05
								0.816
					rBla g 5	Glutathione S-transferase	0.40%	
					rBla g 9	Arginine kinase	21.70%	0.33
								0.013
				Periplaneta americana	rPer a 7	Tropomyosin	39.00%	0.92
								< 0.001
		Coleoptera	Tenebrionidae	*Tenebrio molitor*	Ten m			1
								.
		Hymenoptera	Apidae	*Apis mellifera*	nApi m 1	Phospholipase A2	5.20%	0
								0.994
					rApi m 10	Icarapin var.2	5.20%	− 0.35
								0.221
			Vespidae	*Vespula vulgaris*	rVes v 1	Phospholipase A1	4.90%	0.16
								0.609
					rVes v 5	Antigen 5	19.10%	− 0.08
								0.597
				Polistes dominulus	rPol d 5	Antigen 5	7.10%	0.66
								0.002
	Arachnida	Ixodida	Argasidae	*Argas reflexus*	rArg r 1	Lipocalin	7.10%	0.17
								0.499
		Sarcoptiformes	Echimyopodidae	*Blomia tropicalis*	rBlo t 5	Group 5/21 allergen	38.60%	0.72
								< 0.001
					rBlo t 10	Tropomyosin	13.10%	0.5
								0.002
					rBlo t 21	Group 5/21 allergen	10.50%	0.35
								0.07
			Pyroglyphidae	*Dermatophagoides farinae*	rDer f 1	Cysteine protease	29.60%	0.24
								0.032
					rDer f 2	NPC2 family	40.10%	0.15
								0.117
			Pyroglyphidae	*Dermatophagoides pteronyssinus*	rDer p 1	Cysteine protease	33.30%	0.02
								0.869
					rDer p 2	NPC2 family	39.70%	0.17

Continued

Phylum	Class	Order	Family	Species	Allergen name	Protein Family	% of positive test	Correlation coefficient*
								p-value (two-tailed test)
								0.088
					rDer p 5	Group 5/21 allergen	21.00%	0.00
								0.993
					rDer p 7	Bacterial permeability increasing like protein	15.00%	− 0.17
								0.307
					rDer p 10	Tropomyosin	39.30%	0.84
								< 0.001
					rDer p 11	Paramyosin	4.90%	0.48
								0.096
					rDer p 20	Arginine kinase	19.10%	0.34
								0.015
					rDer p 21	Group 5/21 allergen	15.70%	0.18
								0.263
					rDer p 23	Peritrophin-like protein domain	37.10%	0.08
								0.416
			Glycyphagidae	*Glycyphagus domesticus*	rGly d 2	NPC2 family	28.10%	0.04
								0.756
				Lepidoglyphus destructor	rLep d 2	NPC2 family	32.60%	0.09
								0.421
			Acaridae	*Tyrophagus putrescentiae*	rTyr p 2	NPC2 family	9.70%	0.5
								0.009

Table 4. The prevalence of co-sensitization between *Tenebrio molitor* and other invertebrate allergens, categorized into protein families. * Spearman rank correlation coefficient.

Analysis of the rates of sIgE against TM and the rates of sIgE against individual protein molecules revealed that a positive test result to TM was most commonly associated with a positive test result to the following families: tropomyosin (49.1% of patients), NPC2 (43.8%), group 5/21 allergen (38.6%), class III chitinases (37.1%), and cysteine proteases (34.1%). Analysis of the cases with positive sIgE against the tropomyosins assessed with the ALEX2 test (rAni s 3, nPen m 1, rPer a 7, rBlo t 10, rDer p 10) showed that 131 out of 267 individuals with a positive test result to TM (49.1%) had a positive test result to at least one tropomyosin. In contrast, the proportion of individuals with sIgE to tropomyosins and a negative test result to TM was merely 1.3%. This difference is statistically significant ($p < 0.001$) (Table 5).

Higher levels of sIgE against tropomyosins than those against TM were found in the case of allergens rPer a 7, rAni s 3, and rDer p 10 (59.6%, 51.5%, and 50.5% of comparisons, respectively). Conversely, higher levels of sIgE against TM than against the evaluated tropomyosin family allergens were more common in the case of tropomyosins of the mites Blomia tropicalis (57.1% of comparisons) and of black tiger shrimp nPen m 1 (49.0% of comparisons). The median sIgE levels for these allergens were as follows: nPen m 1 (3.35 kU/L), rPer a 7 (3.80 kU/L), rAni s 3 (3.69 kU/L), and rDer p 10 (3.40 kU/L), compared to the median sIgE level for TM (1.14 kU/L) (Table 6).

A total of 136 patients (50.94%) with a positive test result to TM had no positive test result against any of the five evaluated tropomyosins. The greatest proportion of patients (27.72%) with positive sIgE against TM and at least one positive test result to tropomyosins had positive test results to four of the evaluated tropomyosins (Fig. 1). Analysis of concurrent sIgE against tropomyosins and TM showed that sensitization to TM was most commonly associated with rAni s 3, nPen m 1, rPer a 7, and rDer p 10 allergens (26.97%). In 5.99% of individuals with sIgE against TM there were also sIgE against all other evaluated tropomyosin molecules (rAni s 3, nPen m 1, rPer a 7, rBlo t 10, and rDer p 10), and in 3.75% of individuals with sIgE against TM there were concurrent sIgE against rBlo t 10 (Table 7).

Concentrations of Individual Molecules in the Case of a Positive Result for Tenebrio molitor [sIgE levels (kU/L)].

Discussion

Our study results lead us to a conclusion that the prevalence of TM sensitization among Polish patients is not high. This study may serve as a benchmark for assessing the rates of sensitization to TM later on, when Polish citizens will have had exposure to this insect, and for evaluating the risk of possible allergic reactions to TM. Therefore, this study needs to be continued in the future. Our study results lead us to a conclusion that the prevalence of TM sensitization among Polish patients is not high. In our study conducted in Poland from 2019

			sIgE against *Tenebrio molitor*		
			Negative	**Positive**	
Tropomyosin	Negative	Number	5,828	136	2.28%
		%	98.7%	50.9%	
	Positive	Number	78	131	60.68%
		%	1.3%	49.1%	
Chi-square test of independence			$\chi^2 = 1{,}780.07$; $p < 0.001$		

Table 5. Distribution of positive and negative test results to tropomyosin in individuals stratified by their test result to *Tenebrio molitor*.

Molecule:	Me	Q_1	Q_3	Min	Max
nPen m 1	3,35	1,42	11,06	0,31	68,83
rPer a 7	3,80	1,35	9,82	0,30	69,95
rAni s 3	3,69	1,37	11,69	0,30	66,18
rDer p 10	3,40	1,21	9,42	0,30	69,87
rBlo t 10	1,93	0,59	7,76	0,31	41,41

Table 6. Measures of Central Tendency and Dispersion. *Me* median, *Q1* first quartile, *Q3* third quartile, *Min*. minimum value, *Max*. maximum value.

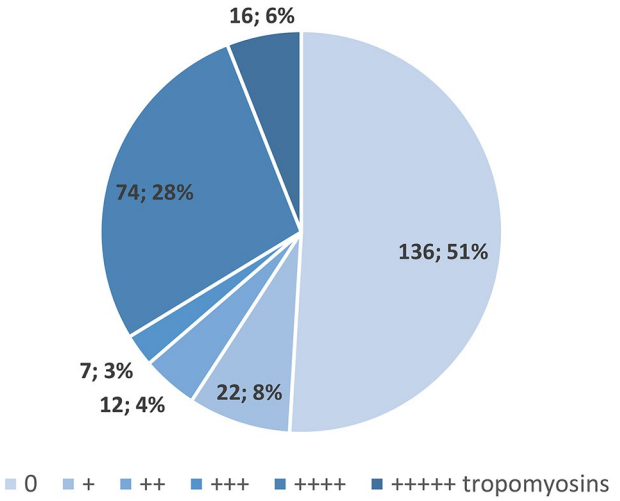

Fig. 1. The number of tropomyosin molecules (out of those evaluated with the ALEX2 test, e.g., rAni s 3, nPen m 1, rPer a 7, rBlo t 10, and rDer p 10) present concurrently with a positive test result against *Tenebrio molitor*.

to 2022, the prevalence of sensitization was slightly over 4% of patients. In contrast, in Italy, in the central and southern regions, this rate was significantly higher, reaching 7.65%. These differences may be due to varying dietary habits and the specific geographical and climatic conditions characteristic of the studied populations[13]. The prevalence of sensitization in Poland was similar to TM extract sensitization of 5% (3/58) reported by Swedish authors, who analyzed serum levels of sIgE against TM extracts in pet shop workers[14]. Somewhat lower rates of sensitization to TM were observed in a group of 64 individuals exposed to live fish bait, two of whom (3%) showed positive serum sIgE against a TM extract[15].

Reports of allergic reactions following insect consumption are scarce and come mostly from Asian countries[10,16]. A review of 141 articles published in China in 1980–2007 yielded 358 cases of anaphylactic shock in response to consumed food, with 17.6% ($n = 63$) of those cases caused by insect consumption. However, none of the cases listed in that review were related to TM consumption[17]. A survey study conducted on 1,059 inhabitants of Laos showed allergic symptoms following insect consumption in 7.6% of the respondents. Just as in the review of cases from China, none of the evaluated individuals reported TM as a cause of their allergic reaction following insect consumption[18]. Several research teams attempted to assess the prevalence of sensitization to allergens of insects from the *Tenebrioninae* family in groups of patients who had been exposed (via inhalation or direct contact) to allergens of these insects as part of their occupation or pastime[2]. Those studies showed the prevalence of sensitization (assessed with skin prick testing or serum sIgE levels) to range from 3 to 50%[19,20].

We assessed the rates of co-sensitization to TM and other invertebrate allergens detected with the ALEX2 test. Poles with sIgE against TM extract were most likely to be co-sensitized to house crickets (80.9%) and/or migratory locusts (63.3%). Only two of the 267 individuals with a positive test result to TM showed sensitization to TM only, whereas eight individuals had serum sIgE against a TM extract with a lack of sIgE against allergens of other invertebrates. These individuals most likely had primary sensitization to TM. There have been two original articles reporting a total of six cases of primary sensitization to TM[16]. Nebbia et al. presented two cases of individuals with occupational exposure to TM flour, who reported symptoms of allergic rhinitis, pruritus, and erythema at their workplace. Both individuals developed symptoms of oral allergy syndrome following a meal containing TM proteins, despite having consumed other insects (e.g. crickets) in the past, with good tolerance. These patients had serum sIgE against TM but did not exhibit sensitization to shrimp or house dust mites. Due to the lack of sensitization to inhalant or food allergens in the study group, the authors of the article suggested that the symptoms may have been due to primary sensitization to TM. Moreover, they identified a cockroach-allergen-like (CAL) protein, which likely contributed to this primary sensitization[21]. Broekman et al. identified other proteins that may be responsible for the development of primary TM sensitization in four mealworm

	rAni s 3	nPen m 1	rPer a 7	rBlo t 10	rDer p 10						
sIgE against *Tenebrio molitor*										136	50.94%
	■					2	0.75%			131	49.06%
		■				2	0.75%				
			■			3	1.12%				
				■		10	3.75%				
					■	5	1.87%	22	16.79%		
	■	■				1	0.37%				
	■		■			3	1.12%				
	■			■		1	0.37%				
	■				■	1	0.37%				
		■	■			1	0.37%				
		■		■		1	0.37%				
			■		■	2	0.75%				
				■	■	2	0.75%	12	9.16%		
	■		■	■		1	0.37%				
	■		■		■	3	1.12%				
	■			■	■	1	0.37%				
		■	■		■	1	0.37%				
			■	■	■	1	0.37%	7	5.34%		
	■	■	■	■		1	0.37%				
	■	■		■	■	1	0.37%				
	■	■	■		■	72	26.97%	74	56.49%		
	■	■	■	■	■	16	5.99%	16	12.21%		

Table 7. The ALEX2 test results positive for sIgE against tropomyosins in the 267 individuals with a positive result to *Tenebrio molitor*.

farmers. Two of those people reported symptoms of inhalant allergy at their workplace, where mealworms were farmed. The remaining two individuals who farmed mealworms as a hobby reported consuming larger quantities of mealworms and for a longer time than patients with occupational allergy, which resulted in the development of food allergy, which was confirmed with a double-blind placebo-controlled oral food challenge. The authors of those case reports ruled out a cross-reaction with shrimp by obtaining a negative oral food challenge with shrimp in all four individuals. Although one of the patients reported mild symptoms of allergic rhinitis and conjunctivitis following exposure to house dust mites, his allergy profile was not suggestive of a possible cross-reaction between mites and TM. That patient was sensitized to Der p 1 and Der p 2 proteins (found in mite excretions); however, he had no serum sIgE against Der p 10, a tropomyosin protein found in invertebrate muscles. Moreover, Broekman et al. detected serum sIgE against previously unidentified proteins (representatives of larval cuticle proteins A1A, A2B, and A3A) in all four patients. Consequently, the authors proposed that these proteins may contribute to the development of primary sensitization to TM and evoke symptoms of both inhalant and food allergies[22]. It is worth noting that primary sensitization to TM is not indicative of sensitization to other insect species, which was demonstrated by Broekman et al. in a group of four patients with primary TM sensitization[23]. Despite positive basophil activation test (BAT) results to all evaluated insects (mealworm, house cricket, giant mealworm, lesser mealworm, African grasshopper, large wax moth, and black soldier fly), there were no serum sIgE antibodies against allergens of any of these evaluated insects, which may suggest that larval cuticle proteins play a larger role in primary sensitization to TM than tropomyosins or arginine kinases. Larval cuticle proteins were found not only in TM, but also in crickets and giant mealworms; however, sequence homology between proteins of various species is low[23].

In our study sensitization to TM in the study group was most commonly accompanied by sensitization to the house cricket (in over 80% of individuals with positive sIgE against TM) and the migratory locust (in over 60% of those sensitized to TM). Sensitization to various insects in one individual may be due to high structural similarity between insect proteins. In the case of people sensitized to several insect species and other invertebrates, it is important to consider cross-reactivity between panallergens—such proteins as tropomyosins

and arginine kinases—which are major allergens in insects, crustaceans, mollusks, and nematodes of the genera *Anisakis* and *Ascaris*[16,24]. The ALEX2 test helps determine sIgE against five tropomyosin molecules: nPen m 1, rPer a 7, rDer p 10, rBlo t 10, and rAni s 3. Our study showed that nearly a half of individuals sensitized to TM showed co-sensitization to at least one of the evaluated tropomyosins. The second most common concurrent sensitization was to NPC2 family proteins (over 40% of patients), followed by group 5/21 allergen (nearly 40% of patients), class III chitinases (over one-third of patients), and cysteine protease (over 30% of patients). It is worth noting that our study showed the strongest correlation between sIgE to a TM extract and tropomyosin molecules. Moreover, most patients co-sensitized to TM and any of the tropomyosins had serum sIgE antibodies against 4 out of the 5 tropomyosins assessed with the ALEX2 test. The results of our study may have been affected by the high structural similarity between tropomyosins of various species and the resulting cross-reactivity[16]. Importantly, studies conducted by other authors focus mainly on TM sensitization in patients with symptomatic allergy to crustaceans or house dust mites. Broekman et al. evaluated patients with an allergy to shrimp who underwent a double-blind placebo-controlled oral food challenge with TM. After ingesting TM proteins, nearly 90% of patients (13 out of 15) developed allergy symptoms, and sensitization to TM was demonstrated with a positive skin prick test in all 15 patients[25]. A study conducted on patients with a house dust mite allergy showed serum sIgE antibodies against TM extracts subjected to various thermal processing protocols in 10 out of 11 patients[11]. Broekman et al. emphasized that patients with an allergy to shrimp are four times more likely to be sensitized to TM than patients with an allergy to house dust mites (88% and 22%, respectively)[26]. Similar findings were presented by Barre et al. Those authors evaluated 13 patients with an allergy to house dust mites and demonstrated sensitization to TM in only two of those patients; whereas 20 out of 21 patients with a shrimp allergy showed sensitization to TM[27]. The differences in the rates of concurrent TM allergy between patients with an allergy to house dust mites and to shrimp demonstrated by other authors may be due to a closer taxonomic relationship between TM and shrimp than between TM and mites[2]. Moreover, exposure to shrimp and TM allergens occurs by ingestion, whereas exposure to house dust mite allergens occurs by inhalation, which may also play a role in the development of co-sensitization[2]. Our study showed no significant discrepancies in the rates of sensitization to TM and the rates of sensitization to shrimp or house dust mite tropomyosins. Based on the evaluated relevant sIgE levels, the rates of co-sensitization to nPen m 1 or rDer p 10 on one hand and TM extract on the other were 36.0% ($n = 96$) and 39.3% ($n = 105$), respectively.

Cross-reactivity between invertebrate proteins is not confined to tropomyosins and arginine kinases. The complexity of cross-reactions between invertebrate proteins has been well illustrated by the case of a male with a severe anaphylactic reaction following the first-in-a-lifetime meal containing insect proteins (TM and house cricket)[28]. Earlier, due to his allergy symptoms, the patient had excluded mussels, crabs, and snails from his diet. His skin prick test result to house dust mite extract was negative, and that to shrimp extract was positive. Further diagnostic tests included BATs with snail, shrimp, mealworm, and cricket extracts. Since the shrimp extract was the only one that produced a negative result, an oral shrimp challenge was performed, and it yielded a negative result. The authors of that study concluded that proteins of other families may be involved in allergic reactions, even producing severe symptoms. Slightly over a half of our study group (136/267) sensitized to a TM extract showed no serum sIgE against any of the five tropomyosins assessed with the ALEX2 test. This may be explained by a paper by Broekman et al., who suggested that it is larval cuticle proteins and not tropomyosins or arginine kinases that play the key role in primary sensitization to TM[23]. Barre et al. observed that allergenic insect proteins are still poorly understood, and the identification of more molecules is needed, which should improve our understanding of the pathogenesis of allergic reactions to insects. In 2019, Barre et al. described a group of proteins, classified as minor allergens, which seem to be characteristic of insects exclusively. These proteins are chemosensory proteins, odorant-binding proteins, and hexamerins[27,29]. Although chemosensory proteins of various insect species differ in their amino acid sequences, their tertiary structure is similar. This is also true of odorant-binding proteins, which are circular molecules of 13–14 kDa in weight. Finally, hexamerins of various insects have similar amino acid sequences. Apart from the proteins described above, Barre et al. also listed apolipophorin III, larval cuticle proteins, and receptors for activated protein kinase as proteins characteristic of insects[29].

Knowing protein structure is crucial for predicting cross-reactions between different allergens, as in the case of chemosensory proteins[27]. On the other hand, even a high structural similarity of allergenic proteins does not necessarily result in cross-reactivity. This is consistent with study results by Francis et al., who observed no cross-reactions between the arginine kinases of *Acheta domesticus* and of TM, despite their high structural similarity[30]. A similar phenomenon is observed in tropomyosins; despite their high structural similarity, slight discrepancies in the structure of sIgE-binding sites may result in a lack of reaction between allergens and antibodies[1,31]. Therefore, the likelihood of cross-reactions between proteins of different species from the same group should be assessed circumspectly[31].

The primary or cross-sensitization may differ in different populations depending on the dietary habits or geographical zone. A recent study which evaluated allergic population of Central and Southern Italy demonstrated the presence of sensitization to TM in 7.65% and to any insect food allergens in 9.4% subjects[13].

Strengths

A strong aspect of this study is its focus on sensitization to yellow mealworm, an edible insect for which there is very little research available regarding allergies. The study concentrates on a new food ingredient that has recently been approved for consumption, allowing for the assessment of sensitization status before its widespread introduction into the diet. Another significant strength is the number of participants, which includes sIgE results from 6,173 patients. Additionally, the use of the modern, third-generation ALEX2 test for detecting allergen-specific IgE is noteworthy. The study also considers cross-reactivity analysis with other allergen extracts from crustaceans and includes an examination of co-sensitization within specific allergen protein families, such as

tropomyosins and arginine kinases, providing detailed data on the patterns of hypersensitivity co-occurrence and offering detailed insights into co-sensitization patterns.

Limitations

This study has several limitations. Firstly, the sample is geographically limited to Poland, which may restrict the generalizability of the findings to other populations with different dietary habits and environmental exposures. Additionally, the reliance on serological tests for detecting specific IgE antibodies introduces the potential for measurement errors, such as false positives or false negatives. Furthermore, the study lacks clinical data, as it is based solely on serological test results rather than actual clinical cases of allergies. Moreover, the data only included age, sex, and sIgE levels for the allergens evaluated, without information on the clinical symptoms of the patients. Another significant limitation is that the presence of sIgE does not always correlate with clinical allergy, meaning that sensitization identified in the study does not confirm allergic disease. Additionally, the retrospective nature of the study limits the ability to establish causality or temporal relationships. These factors collectively suggest that while the study identifies potential sensitizations, it does not confirm allergic disease. These limitations may affect the interpretation of the prevalence and significance of sIgE to TM in the studied population.

Conclusions

Our study evaluated the prevalence of TM sensitization prior to TM being introduced as a food ingredient in Poland. This prevalence, assessed based on serum sIgE against TM in Polish patients, was over 4%. Mono-sensitization to TM was very rare. Study participants asIgE against TM extract were most likely co-sensitized to house crickets and/or migratory locusts. Co-sensitization rates between TM and protein families emerge in the following declining order: tropomyosins, NPC2 family proteins, group 5/21 allergens, class III chitinases, and cysteine proteases. Higher levels of sIgE against tropomyosins than those against TM were observed for the following allergens: rPer a 7, rAni s 3, and rDer p 10. Conversely, higher levels of sIgE against TM than against the tropomyosin family allergens were observed in the case of rBlo t 10 and nPen m 1. Due to the fact that TM has not been part of the diet in Poland, we suspect that primary sensitization to TM may be associated with sensitization to invertebrates. As analysis of co-sensitization to TM and individual protein families revealed, the presence of asIgE to house dust mite and shrimp tropomyosins was most commonly observed among study participants sensitized to TM. Taking into account these findings, we would like to hypothesize that individuals with an allergy to shrimp should be careful when consuming foods containing TM. However, we must remember that the presence of asIgE is not equal with allergy, and clinical symptoms after exposure to allergen source is crucial in establishing therapeutic recommendations. That is why more research on the correlation between sensitization and allergy to various sources of tropomyosins, including both clinical trials and in vivo testing, should be performed.

Our study paves the way for determining the allergenic significance of TM and may show the potential, future development of TM sensitization or allergy in Poland after TM-containing foods are introduced into the market. There is a need for further studies on the prevalence of sIgE against TM and its individual allergen molecules, their correlation with clinical symptoms, and cross-reactions with house dust mites and shrimp. However, it is currently impossible to routinely assess sIgE against individual TM proteins.

Data availability

The data that support the findings of this study are available from the corresponding author, [E.M.], upon reasonable request.

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Author contributions

M.E.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review and editing; Ch.M.: Writing – original draft, Writing – review and editing; G.W.: Writing – original draft, Writing – review and editing; W.J.: Investigation, Writing – original draft, Writing – review and editing; K.D.: Data curation, Writing – original draft, Writing – review and editing S.J.: Data curation, Writing – original draft, Writing – review and editing;, C.B.: Writing – original draft, Writing – review and editing; K.K.: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review and editing. All authors reviewed the manuscript.

Declarations

Competing interests

Emilia Majsiak reports a relationship with EMMA MDT sp. z o.o. that includes: board membership, employment, and equity or stocks. 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.

Additional information

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