Effect of thermal processing on mealworm allergenicity

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Scope: The growing world population requires the exploration of new sustainable protein sources to ensure food security. Insects such as mealworm are promising candidates. For safety reasons, a risk assessment, including allergy risks, is needed. Since allergenicity can be influenced by thermal processing, it is highly important to take this into account.

Methods and results: Fresh mealworm was heat processed and extracted by a sequential extraction method using in succession Tris, urea, and a combined SDS/DTT buffer. Extracts were tested using immunoblot, basophil activation test and skin prick test in 15 shrimp allergic patients, previously indicated as population at risk for mealworm allergy. Immunoblots showed a difference in IgE binding between processed and unprocessed mealworm extracts. However, this was due to change in solubility. Some allergens were soluble in urea buffer, but became more soluble in Tris buffer and vice versa. IgE binding was seen for all extracts in blot and basophil activation test. The results from 13 skin prick tests showed a skin reaction similar between processed and unprocessed mealworm.

Conclusion: Thermal processing did not lower allergenicity but clearly changed solubility of mealworm allergens. A sequential extraction method allowed for assessment of a broader protein panel.

Keywords: Food allergy / Mealworm / Sequential extraction method / Solubility thermal processing

1 Introduction

A huge shortage of protein sources for human food consumption is expected in the near future due to the growing world population [1]. Sustainable protein sources are being explored to solve the coming food insecurity problem. The larvae of the yellow mealworm beetle (Tenebrio molitor) is a good candidate and is already for sale in Great Britain, the US, and in the major supermarkets in the Netherlands and Belgium [2, 3]. However, a thorough safety assessment, and in particular an allergenicity risk assessment, is yet to be performed [4]. Allergenicity is not only a theoretical threat, since 0.1–5.7% of the pediatric and 0.1–3.2% of the adult European population has a food allergy [5]. Moreover, previously we [6] found that IgE from patients sensitized to shrimp and house dust mite (Der p 10; closely related species), binds to mealworm proteins. The relevant proteins were identified as the pan allergens tropomyosin and arginine kinase, which are major allergens in shellfish (e.g. shrimp and lobster). Allergenicity can be influenced by factors such as matrix [7] and processing—for instance, by changing protein structure and thus IgE-binding epitopes [8]. This was previously reported for other foods such as peanut, tree nuts, and apple [9–12]. Thermal processing by dry roasting enhanced allergenicity of peanut [12], while for tree nuts the allergenic properties changed in such a way that most pollen allergic patients reacting to tree nuts had no clinical reaction after eating the heat processed food [12, 13]. Thus processing may have an impact on the risk of getting an allergic reaction for mealworm. Since mealworm is closely related to shellfish one might expect that processing may alter the allergenicity of mealworm proteins in a comparable manner to shellfish.

For instance, Nakamura et al. [14] reported that thermal processing resulted in an enhanced IgE-binding capacity of scallop tropomyosin in dot blot and competitive ELISA using serum from scallop allergic patients. This enhanced capacity was suggested to be a result of glycation between free
amino acids and aldehyde or ketone groups of sugars during heating. The same group found an opposite result after Maillard reaction with squid tropomyosin [15]. Samson et al. [16] found no significant difference between boiled and raw shrimp extract using immunoblot with serum from shrimp allergic patients. However, interindividual differences in protein recognition were observed. Carnes et al. [17] reported that boiled extracts of shrimp and lobster had higher IgE-binding capacity in ELISA and recorded greater skin reactivity in skin prick test (SPT). A similar finding was observed by Liu et al. [18] when testing shrimp tropomyosin. Taken together, the results in the above-mentioned papers are to some extent contradicting. This could be due to solubility issues. Therefore, more attention should be paid to the preparation of extracts to ensure the presence of a representative set of proteins for allergenicity assessment.

Most studies reported the effect of processing using immunoblot and ELISA. Unfortunately, these methods lack information on the functionality of IgE binding, which can be measured using SPT and basophil activation test (BAT). These tests are therefore preferred over immunoblot and ELISA in allergenicity assessment. However, they cannot replace food challenges—the “gold” standard. Immunoblot, BAT, and SPT were used in this study to test the effect of processing on mealworm allergenicity. Shrimp allergic patients were tested due to the lack of a sufficient number of mealworm allergic patients. To ensure that most relevant proteins were covered, a sequential protein extraction method was used and the presence of allergens was confirmed using nanoLC–MS.

2 Materials and methods

2.1 Patient selection and screening

Three sera from patients diagnosed with shrimp allergy at the University Medical Centre Utrecht, the Netherlands, were used to test the effect of processing on protein solubility using immunoblot. For allergenicity testing, 15 adult patients diagnosed with shrimp allergy, based on suggestive history and sensitization were included. All patients reacted positive to mealworm protein in SPT and serology. All patients gave informed consent before answering the questions and for the performance of SPT and blood collection. The study was approved by the local ethics committee.

2.2 Thermal processing of mealworm

Raw and freeze dried Yellow mealworms in final larval stage were kindly provided by Dutch insect farm Kreca (Ermelo, The Netherlands). Raw mealworms (50 g) were heat processed by various methods: Blanching for 1 min at 100 °C, boiling in 300 mL water for 10 min at 100 °C, baking for 3.5 min at 1000 Watt on an induction cooker (Prima Donna tsi-199k), or frying for 30 s at 180 °C in peanut oil. All processed and unprocessed mealworms were stored at −20 °C until further use.

2.3 Mealworm extract preparation

Five grams of raw, freeze dried, and processed mealworms were extracted using a sequential protein extraction method (see Fig. 1). First, the mealworms were mixed with 25 mL ice cold Tris buffer (20 mM Tris buffer pH 7.6 containing 1 mM phenylthiocarbamide (Sigma Aldrich) and Halt Protease Inhibitor Cocktail (Thermo Scientific)). The amount of mealworm was corrected for weight gain or weight loss due to processing. Subsequently, the mealworms were disrupted, using an ultraturrax (3 × 10 s) under continuous cooling. After centrifugation (30 min, 15 000 × g at 4 °C), the supernatant was recovered.

The insoluble residue was washed once with 5 mL Tris buffer (as described above). The 30 and 5 mL supernatants were combined. Twenty-five milliliter was used for sample cleanup and concentration using TCA precipitation. Second, the remaining pellet was extracted overnight at 4 °C with 30 mL urea buffer (6 M urea in 20 mM Tris buffer pH 7.6 containing 1 mM phenylthiocarbamide and Halt Protease Inhibitor Cocktail). The sample was subsequently centrifuged and the supernatant was collected. The pellet was washed once more with 5 mL urea buffer, centrifuged, and the supernatant was combined with the 30 mL urea supernatant. Twenty-five milliliters of the extract was TCA precipitated. Tris and urea extracts were combined (1:1) for the BAT. Finally, the insoluble residue was almost completely dissolved at room temperature in 20 mL SDS/DTT buffer (20 mM Tris pH 7.6, 2% SDS, and 1% DTT) and the supernatant was collected after centrifugation. All TCA precipitated samples were redissolved in 6 M urea buffer and stored at −20°C before further use. Protein concentration was determined using the Bradford method (Bio-Rad, Hercules, CA, USA).

2.4 SDS-PAGE gel of processed mealworm extracts

For SDS-PAGE, the Criterion system with a 10–20% Ready Gel Tris-HCl gel (Bio-Rad) was used according to the manufacturer’s instructions. All mealworm extracts (10 µg per sample) were loaded on the gel under reducing conditions (Laemmli buffer). After protein separation, the proteins were visualized using Coomassie-staining (Instant Blue, Expediton, UK).

2.5 Immunoblot with serum of shrimp allergic patients

All mealworm extracts were applied on the SDS-PAGE as described above and transferred to a polyvinylidifluoride
membrane using the Criterion Blotter system (Bio-Rad) according to the manufacturer’s instructions. The membrane was blocked overnight with 3% BSA and incubated for 1 h with serum from a shrimp allergic patient (1:50) in PBS with 0.1% Tween 20 containing 3% BSA (PBST). After thorough washing, the membranes were incubated for 1 h with Goat anti human IgE (KPL, Gaithersburg, MD, USA) 1:100 000 in PBST. After washing, the bands were visualized using a chemiluminescent peroxidase substrate kit ECL (Sigma) according to the manufacturer’s instructions. Blots were scanned using the Chemidoc XRS+ image scanner with ImageLab software (Bio-Rad).

2.6 Protein identification and quantification using Nano LC–MS/MS

2.6.1 Trypsin digestion extracts (Tris and urea)

Extracts (50 μg protein) were subjected to conventional in-solution tryptic digestion as previously described [6]. After reduction and alkylation, the proteins were digested with trypsin (enzyme:substrate ratio of 1:25 w/w) overnight at 37°C with agitation. Peptide mixtures were desalted by C18 Stage Tips, fabricated by using C18 disks (3M, Neuss, Germany), and used according to the original protocol [19]. Briefly, 1 of 10 of each tryptic digest solution was diluted fivefold in 0.1% TFA (solution A), and applied onto Stage Tips, which were previously conditioned with 10 μL of solution B (0.1% formic acid, 50% acetonitrile) followed by 10 μL of solution A. After sample loading, Stage Tips were washed with 10 μL of solution A. Peptide elution was achieved by adding 8 μL of solution B. Purified peptide eluates were diluted tenfold in mobile phase A (see below) and used for mass spectrometric analysis (0.5% of the original sample for each preparation). Three technical replicates of C18 purification and mass spectrometric analysis were injected for each sample.

2.6.2 Trypsin digestion pellets (SDS/DTT)

Pellets obtained after urea extraction and centrifugation were dissolved in 200 μL lysis buffer (100 mM Tris pH 7.6 containing 4% SDS and 0.1 M DTT), incubated for 5 min at 95°C and sonicated. Once clarified, each sample for mass spectrometric analysis was subjected to filter-aided sample preparation [20], using a 30 kDa Microcon filtration unit (Millipore). Peptides were recovered by centrifugation at 14 000 g; followed by an additional washing step to mobilize the peptides retained by the membrane in the filtration unit, using 50 μL NaCl 0.5 M. Flow-through was pooled, desalted by C18 Stage Tips (as described previously) and subsequently injected for mass spectrometric analysis (0.5% of the original sample, corresponding to 400 ng of proteins). Three technical replicates were injected for each sample.

2.6.3 Nano LC–MS/MS analysis and database search

The peptide mixture was analyzed according to Verhoeckx et al. [6], with small changes. Chromatography was performed on an Easy LC 1000 nanoscale liquid chromatography (nanoLC) system (Thermo Fisher Scientific, Odense, Denmark). The analytical nanoLC column was a pulled fused silica capillary, 75 μm id, in-house packed to a length of 10 cm with 3 μm C18 silica particles from Dr. Maisch (Entringen, Germany). Four microliters of the peptide mixtures was loaded at 500 nL/min directly onto the analytical column. A binary gradient was used for peptide elution. Mobile phase A was 0.1% formic acid, 2% acetonitrile, whereas mobile phase B was 0.1% formic acid, 80% acetonitrile. For both types of analysis, that of extracts and that of pellets, gradient elution was achieved at 330 nL/min flow rate, and ramped from 8 to 35% B in 60 min, and from 30 to 100% B in additional 8 min; after 5 min at 100% B, the column was reequilibrated at 0% B for 2 min before the following injection. MS detection was performed on a quadrupole-orbitrap mass.
spectrometer Q-Exactive (Thermo Fisher Scientific, Bremen, Germany) operating in positive-ion mode, with nanoelectrospray (nESI) potential at 1800 V applied on the column front end via a tee piece. Data-dependent acquisition was performed by using a top-12 method with resolution (FWHM), AGC target, and maximum injection time (ms) for full MS and MS/MS of, respectively, 70 000/17 500, 106/105, 50/60. Mass window for precursor ion isolation was 1.6 m/z, whereas normalized collision energy was 25. Ion threshold for triggering MS/MS events was 2 × 104. Dynamic exclusion was 30 s. Data was processed using Proteome Discoverer 1.3 (Thermo Fisher Scientific), using Sequest as search engine, and the Swiss Prot database accessed on February 2013 as sequence database (3 123 840 sequences for Metazoa taxonomy). The following search parameters were used: MS tolerance 15 ppm; MS/MS tolerance 0.02 Da; fixed modifications carbamidomethyl cysteine; enzyme trypsin; maximum missed cleavages 1; taxonomy Metazoa. Search results were filtered by q values using Percolator integrated in Proteome Discoverer, to achieve a peptide-level FDR of less than 1%.

2.6.4 Relative protein quantification

A label-free approach was adopted for relative quantification of allergens, using five unique peptides for arginine kinase and three unique peptides for tropomyosin. Peak areas for each peptide were calculated using extracted ion chromatograms (XICs) via the Xcalibur software (Thermo Fisher Scientific). Peak areas for each peptide were subsequently normalized using the total peptide-spectrum matches (TPSM) of the corresponding LC–MS/MS analysis. The Tris freeze dried sample, one with the highest TPSM, was chosen to confirm linearity between injected amount and TPSM. Triplicate measurements of peak area were averaged for each peptide and expressed as relative value compared to the average area of the same peptide in the Tris unprocessed sample. Relative quantification at the protein level was achieved for all proteins by taking the median value of all associated peptides.

2.6.5 BAT using shrimp allergic patient serum

BAT was performed as described by Meulenbroek et al. [21] with minor modifications. Cells were incubated with a dilution series (1:100–1:103) of processed and unprocessed mealworm extracts (combined TRIS and urea extracts (5 mg/mL) and SDS/DTT extracts (no concentration determined)). Shrimp extract (ALK), 2 mg/mL, and shrimp tropomyosin Pen a 1 (Indoor Biotechnologies), 1 mg/mL, were used as positive controls. CD63, CD123, and CD203c expression was analyzed by flow cytometry using FACScan Canto II and FACSDiva software (BD Bioscience, USA). The results were expressed as a percentage of CD63+ basophils. Basophils of two patients did not respond in repetition to any of the extracts, nor to the positive control. Basophils of a third patient showed spontaneous release of CD63 on the negative control. These three patients were therefore excluded.

2.6.6 SPT with processed mealworm extracts

SPT solutions of the processed and unprocessed mealworms (0.4 mg/mL) were kindly provided by ALK (ALK-Abelló, Spain). These solutions were prepared in PBS, which has more or less the same extraction characteristics as the Tris buffer mentioned above. The solutions were applied on the flexor aspect of the forearm using 1 mm tip lancets (ALK). Histamine dihydrochloride 10 mg/mL and glycerol diluent were used as positive and negative controls, respectively. SPT reactivity was recorded after 15 min and measured as the ratio of the mean of the wheal elicited by the tested extract and histamine control. When the ratio was 0.5 or greater, the reaction was regarded as positive. No statistical tests were performed due to the limited size of the group.

3 Results

3.1 Heat processing changes solubility

It can be concluded from Fig. 2 that protein profiles significantly changed after heat processing in all tested extracts (Tris, urea, and SDS/DTT). Bands of proteins from the Tris extracts with MW < 25 kDa and at ±50 kDa were more intense in all heat-processed extracts compared to the unprocessed extracts (raw and freeze dried).

In case of the urea extract, protein bands with a MW of ±40 kDa were more pronounced in unprocessed extracts, whereas bands near 45 and 50 kDa were more pronounced in all heat-processed extracts. In the SDS/DTT extract the same band at ±45 kDa diminishes after heating, while a band appears at ±37 kDa. In addition, high molecular weight proteins (70–200 kDa) were detected in the SDS/DTT buffer extracts after heating. These changes in protein profiles were the result of changes in solubility, as shown by the LC–MS analysis.

LC–MS analysis of processed and unprocessed Tris, urea, and SDS/DTT extracts identified a wide range of proteins in mealworm. Putative mealworm allergens (e.g. tropomyosin, arginine kinase, myosin light chain, and triosephosphate isomerase), identified in Tris and urea extract were previously reported by us [6]. However, in this study we also identified putative allergens in the SDS/DTT extract (Table 1). The most dominant protein in the SDS/DTT extract was arginine kinase after heat processing.

The concentrations of these putative mealworm allergens were different in the tested extracts. It can be concluded from Fig. 3 that processing causes a shift in solubility from Tris to urea and vice versa. For instance, arginine kinase, which was abundant in raw and freeze dried mealworm Tris extracts, was almost undetectable in heat processed mealworm Tris extract. However, it became detectable in urea extracts after heat processing. For tropomyosin the opposite effect was found. The
3.2 Heat processing does not obviously change IgE-binding capacity

The above-mentioned change in allergen solubility was confirmed by the immunoblot of the three shrimp allergic patients (Fig. 4). The immunoblots showed IgE binding to proteins in all tested mealworm extracts, Tris, urea, and SDS/DTT. In the lane of raw and freeze dried Tris extract, a protein band at ±40 kDa can be seen, which was previously identified as arginine kinase [6]. After processing, this band becomes more pronounced at a slightly higher MW. Comparing these results with the LC–MS analysis (Fig. 3), this band is most likely tropomyosin (±37 kDa). In the lane of raw and freeze dried urea extract, a ±37 kDa band (previously identified after in-gel digestion, as tropomyosin [6]) was detected. The intensity of this band diminished after processing. The decline of tropomyosin band intensity in the urea extract is in accordance with the LC–MS data (Fig. 3). Estimation of the overall effect of processing on IgE-binding capacity is difficult due to this shift in solubility. For this reason, a pool of Tris and urea extract was used for IgE cross-linking functionality testing.

3.3 Heat processing does not change IgE cross-linking functionality

From the 12 useful BATs, 11 showed activation after incubation with both the mixed Tris /urea extracts as well as the SDS/DTT extracts, indicated by an elevation in the percentage of CD63+ cells (Fig. 5). Basophils of one patient reacted solely to proteins in the SDS/DTT extract.

Overall, activity of basophils to processed or unprocessed mealworm proteins was not clearly different. However, basophils from three patients were somewhat more strongly activated by the processed Tris /urea mealworm extracts, than by the unprocessed mealworm extracts. All 15 patients showed a positive skin reaction to shrimp, house dust mite, and mealworm extract. In addition to unprocessed mealworm, all showed a positive skin reaction to blanched, boiled, baked, and fried extracts (Table 2).

However, some interindividual differences were seen in skin reactivity. Two patients had an increased skin reaction to processed extracts. The wheal size increased from 2+ to 3+ from unprocessed to processed. Skin reaction of one patient decreased by blanching and of one patient by frying. Overall, SPT reactions were comparable between all processed extracts in 13 of 15 patients.

4 Discussion

From the results obtained in this study it can be concluded that heat processing influences protein solubility. Some proteins became less soluble in Tris buffer due to heat-induced denaturation but these proteins could still be solubilized in a chaotropic reagent such as urea (arginine kinase). Other proteins that under natural conditions were insoluble in Tris buffer became more soluble after heating (tropomyosin). Furthermore, processing did not lower IgE-binding capacity and IgE cross-linking functionality of mealworm allergens (e.g. tropomyosin, arginine kinase). A representative panel of proteins was assessed due to the use of a sequential extraction method. To the best of our knowledge, this is the first study to assess the effect of thermal processing on mealworm allergenicity. Furthermore, the sequential protein extraction method used in this study has, as far as we know, never been used to assess the effect of processing on allergenicity.
Table 1. Proteins identified in SDS/DTT extract using LC–MS/MS

<table>
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<th>Protein (source)</th>
<th>Accession</th>
<th>Score</th>
<th>Sequence coverage (%)</th>
<th>Peptides identified</th>
<th>PSM</th>
<th>Mass (kDa)</th>
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PSM, peptide-spectrum matches, value that represents the number of MS/MS spectra that matched peptide sequences assigned to that particular protein. Score, the sum of individual Sequest scores of all the identified peptides which were assigned to the protein itself. The score is the probability that the observed match is not a random event.

Top 15 proteins identified by LC–MS/MS in the SDS/DTT fraction in the unprocessed and processed extracts. Arranged on highest mean score of three measurements. Sequence coverage, peptides identified, and PSM are given as a mean of three measurements. Identification was based on homology with metazoan proteins in the Swiss Prot database. Proteins are noted in bold when assigned as allergen by the IUIS allergen nomenclature subcommittee.

Heat processing strongly changed the solubility characteristics of mealworm proteins. Change in allergen solubility, might be caused by changes in 3D structure of the proteins after heat treatment. Some proteins will lose, irreversibly, their functional properties and solubility and form aggregates due to denaturation, while others may have increased solubility. Tropomyosin is a muscle protein, which forms a complex with the insoluble actin and troponin and is heat stable as a result of its coiled coil helical construction [22]. The improved solubility is most probably due to breakage of interactions with these other difficult to solubilize proteins. However, no evidence could be found in literature to corroborate this. Another possibility is the formation of soluble aggregates, which was also demonstrated for the Japanese cedar pollen allergen Cry j 1 by Aoki et al. [23]. Moreover, Usui et al. [24] showed that heat processing of purified tropomyosin from shrimp did not induce the formation of insoluble aggregates. However, difference in solubility in PBS buffer between heated and unheated tropomyosin was not in agreement with our results. This might be due to fact that heat processing of tropomyosin...
Figure 3. LC–MS analysis of tropomyosin and arginine kinase in processed mealworm extracts (raw, freeze dried, blanched, boiled, baked, and fried, respectively). The results are presented as mean of three LC–MS analysis and calculated as ratio relative to the amount in the raw Tris extract.

was not tested in its natural environment (complex with actin) and thus breakage of interactions with other proteins cannot be demonstrated.

In contrast, arginine kinase is a globular protein, which tends to unfold during heating, exposing hydrophobic amino acids, which are normally inside the protein. The exposed hydrophobic amino acids from different molecules will interact in such a way, that formation of larger protein aggregates will occur [23]. Cross-linking of arginine kinase may also be caused by polyphenol oxidase-mediated cross-linking. In most cases these aggregates become insoluble. Further aggregation of globular proteins during heating is favored through the formation of disulphide bridges. To solubilize these aggregates a more stringent buffer is needed, which confirms our finding that arginine kinase was not detected in the Tris buffer after heating.

Another possibility for the LC–MS detection of tropomyosin in Tris buffer after heat processing is the improved digestibility after heating. This effect was also seen in a study from Takagi et al. who showed that thermal treatment markedly increased the digestibility of ovalbumin [26]. This is because ovalbumin is a globular protein that unfolds during heating, which exposes amino acid sequences that can be hydrolyzed by trypsin. However, for mealworm tropomyosin this improved digestibility after heating was not confirmed by the immunoblot. Furthermore, enhancement of trypsin digestion is not expected for tropomyosin because of its helical structure that upon heating will not suddenly expose

Figure 4. Immunoblot of processed mealworms with serum from one of the three shrimp allergic patients. The mealworms were extracted with a Tris, urea, and SDS-DTT buffer, respectively.
Figure 5. BAT with extracts (left: pooled Tris and urea extract, right: SDS/DTT extract) from processed mealworms (freeze dried, fresh, blanched, cooked, baked, and fried, respectively). Maximum percentage of CD63+ basophils were calculated with respect to IgE positive control. Each line represents one patient.

Table 2. SPT results, expressed as a ratio of histamine control, using extracts from the different processed mealworms (raw, blanched, boiled, baked, and fried) in 15 shrimp allergic patients

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M, male; F, female. Mean SPT as a ratio of histamine control (3+).

different amino acid sequences. Therefore, it is more likely that extractability and thus solubility is the main reason for the difference between the unprocessed and heat processed samples instead of improved digestibility.

When testing only protein extracts prepared in Tris buffer, which is the routine procedure, one could wrongly conclude that IgE-binding capacity to tropomyosin would be elevated due to heat processing [17]. However, the correct explanation is that possibly important allergens are overlooked when using just one buffer type. This was demonstrated by our immunoblot data and might also be the case in some studies [16–18] where induction of IgE binding after heat processing was observed. In these studies only PBS extracts, which is a nondenaturing extraction buffer similar to the Tris buffer, were used. Solubility issues are often encountered when proteins are processed. In most cases this phenomenon is not recognized since the composition of the protein extracts is not identified [27].

IgE binding on the immunoblot was detected in all tested extracts, indicating that in mealworm there are more allergens present than the ones identified in Tris buffer. The most dominant IgE-binding proteins in the Tris extract and in the urea extract were identified as tropomyosin and arginine kinase, respectively, which confirms our previous findings [6].

In the SDS/DTT extract, arginine kinase (±40 kDa band) was also identified and in addition IgE binding to proteins with a higher molecular weight was detected. The high MW proteins that were identified in the SDS/DTT extracts by LC–MS/MS were myosin heavy chain, paramyosin, and hemocyanin. It is not clear if these are the same IgE-binding proteins as detected in the immunoblot. However, myosin heavy chain and paramyosin were recently identified as shrimp allergens [28–30]. Moreover, paramyosin and hemocyanin are included in the IUIS database as arthropod allergens. Since mealworm and shrimp are closely related it can be envisioned that paramyosin and myosin heavy chain could be potential mealworm allergens. Another option is that the high molecular weight proteins are the result of arginine kinase cross-linking. According to LC–MS identification arginine kinase
was also detected in the SDS/DTT extracts especially after heat processing.

Processing did not change IgE functionality in BAT and SPT. The advantage of BAT over SPT is that BAT allows testing of extracts prepared with stringent buffers such as urea and SDS/DTT, while due to patient safety SPT only allows protein extracts prepared according to clinical guidelines in sterile PBS buffers [31]. The results from the BAT indicate, besides some interindividual variability in basophil activation, no significant effect due to heat processing. Processing showed only induced basophil activation in three patients.

In SPT, 13 of 15 patients, did not react differently to the processed extracts. Only two patients (other than those in BAT) showed a trend of increased skin reaction. This might be caused by the increased solubility of some allergens in PBS after heat processing, which might also be the case in the study of Nowak-Wegrzyń et al. [32]. The authors reported that boiled shrimp extract induced larger skin response compared to raw shrimp extract in some shrimp allergic patients. Together these results strengthen the need for different extraction buffers to assess the allergenicity of a broad representative protein panel.

The strength of this study was the combined use of clinical, ex vivo and in vitro tests in combination with a sequential extraction method and LC–MS analysis. This allowed inclusion of a broader panel of mealworm proteins in the allergenicity assessment, than usually studied.

In conclusion, heat processing did not lower the allergenicity of mealworm proteins, but clearly changed the solubility of these proteins. A sequential extraction method allowed for inclusion of a broader protein panel in the allergenicity assessment of mealworm.

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The authors have declared no conflict of interest.

5 References


