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Development of Indirect Sandwich ELLA for Detection of Insects in Food

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Featured Application: The developed indirect sandwich ELLA offers a practical solution for the routine screening and detection of insects in food processing facilities, and holds promise for implementation by inspection authorities.

Abstract: Edible insects have been officially classified as food in the European Union since 2015. Currently, four insect species are approved for food use. However, no official method for detecting insects in food has been recognized to date. To establish a simple analytical method for insect detection in food, we developed an indirect sandwich (enzyme-linked lectin sorbent assay) ELLA specific for N-acetylglucosamine in chitin and chitosan polymers. The validation of the method demonstrated that the ELLA developed in this study is reliable for insect detection. The limit of detection (LOD) and quantification (LOQ) were 0.006 and 0.028 mg/mL, respectively. Intra-day precision ranged from 2.45% to 30.29%, and inter-day precision from 0.36% to 12.87%. Significant differences in the total amount of chitin and chitosan were observed among the insect products, processed insect products, and samples without any insect addition ($p < 0.05$).

Keywords: N-acetylglucosamine; chitin; chitosan; food adulteration; edible insect

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1. Introduction

Edible insects were officially classified as food in 2015 by Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods [1], which came into force in 2018. Under this Regulation, a list of novel foods was established, where specific insect species in specific forms were gradually included. The following species can therefore be used in foodstuffs: *Tenebrio molitor*, *Locusta migratoria*, *Acheta domesticus*, and *Alphitobius diaperinus*. Other species may also be used for feed applications, with the full list and conditions of their use laid down in Commission Regulation (EU) 2017/893 [2,3]. These species are *Hermetia illucens*, *Musca domestica*, *Tenebrio molitor*, *Alphitobius diaperinus*, *Acheta domesticus*, *Gryllodes sigillatus*, *Gryllus assimilis*, and *Bombyx mori*. Although these feed-permitted insect species cannot be used in food processing in the EU, their use in the food industry is known from other countries [4].

Though insect consumption is relatively new in European countries, it has a tradition in subtropical and tropical areas. According to Jonegma (2017), China, India, and Thailand are the world leaders in terms of the number of species consumed [5]; however, Mexico, Kenya, and other African countries also traditionally consume insects [4]. Prospectively, the FAO foresees a global population increase and new food sources are considered as a result [6]. Insect farming is considered to be more environmentally friendly compared to conventional livestock farming in several respects, while still possessing high nutritional value, especially in terms of protein content and quality [7].

In addition to the benefits that insects offer, there are several barriers. These include, for example, risks associated with anti-nutritional properties, microbial load, or allergenic potential [7]. Some studies support the hypothesis that eating insects can trigger allergic reactions in people allergic to crustaceans and mites [8]. Informing consumers about allergens is among the mandatory information based on Regulation (EU) č. 1169/2011 of the European Parliament and of the Council [9]. Thus, with the need to inform, emphasis is also placed on verifying the presence of insects, but this area is not legislatively resolved yet.

Traditionally, methods for the detection of insect pests in food have been used and are based on visual inspection, the trap method, the Berlese funnel method, the uric acid method, hidden infestation detectors, electrically conductive roller mills, SPME-GCMS, e-nose, imaging methods (optical, X-ray, thermal), NIR spectroscopy, and acoustic detection [10]. These methods are used to detect live or dead insects and cannot be applied to detect insects used as raw material for food production. Methods based on insect DNA analysis have been developed for the detection of *Tenebrio molitor*, *Locusta migratoria*, *Achaeta domesticus*, *Bombyx mori*, *Gryllus bimaculatus*, *Oxya chinensis*, *Allomyrina dichotoma*, *Teleogryllus emma*, and *Apis mellifera ligustica* [11,12]. Indirect evidence can be obtained on the basis of insect derivatives, such as some of their metabolites. Proteomic methods have been successfully used to screen *Tenebrio molitor*, *Hermetia illucens*, *Alphitobius diaperinus*, and *Gryllus assimilis* [13]. For the screening of insects and insect derivatives used as food or raw material in the food industry, methods based on the target sequences of DNA [14], multiplex polymerase chain reactions [15], ATR-FTIR spectroscopy [16], and the detection of specific peptides [17] are being developed.

Chitin and its soluble form, called chitosan, are also considered to be important components of insects, crustacean shells, mollusks, and fungi [18]. Different composite structures are formed, known as α-chitin, β-Chitin, and γ-chitin [18]. Chitin and chitosan are composed of N-acetylglucosamine where the total amount of N-acetylglucosamine coincides with the amount of chitin and chitosan [19]. The detection of N-acetylglucosamine has been verified in insects by HPLC [19]. Yet another option is to take advantage of the specific binding of the lectins of WGA (wheat germ agglutinin) Con A (Concanavalin A), DSA (*Datura stramonium* agglutinin) [20], and LEL (*Lycopersicon esculentum* lectin) [21], which are used in the enzyme-linked lectin sorbent assay of ELLA or in lectin histochemistry, where they have been successfully validated on other food polymers [22–24].

Methods that require expensive laboratory equipment or involve time-consuming sample preparation are not easily applicable for routine inspection in the food industry. Ready-to-use methods are much more appropriate. Commonly used methods include the ELISA method for allergen determination [25]. The producers are equipped and experienced for this analysis. In this case, we developed a similar method, namely, ELLA, for insect determination.

This study's aim was to develop and validate an indirect sandwich ELLA for quantifying N-acetylglucosamine as an indicator for insects used in food.

2. Materials and Methods

2.1. Materials

To include the possible variability of the samples caused by the seasonality, rearing conditions, and the means of killing, the samples were collected repeatedly over a longer period (from March 2023 until February 2024), from different breeders, and they were killed

by freezing at −20 °C or by boiling in water for 5 min. In total, nine species of insects were collected: *Acheta domesticus* (AD), *Alphitobius diaperinus* (ALD), *Blaptica dubia* (BD), *Gryllus assimillis* (GA), *Hermetia illucens* (HI), *Locusta migratoria* (LM), *Schelfordela tartara/Blatta lateralis* (ST), *Tenebrio molitor* (TM), and *Zophobas morio* (ZM). They were either reared in the insectarium at the Czech University of Life Sciences Prague or provided by 12 different farmers from the Czech Republic. To exclude bias caused by feed residuals in the intestine of insects, the insects were starved for at least 24 h before the analysis. All samples of euthanized insects were freeze-dried (L10-55 PRO lyophilizer, Gregor Instruments s.r.o., Sázava, Czech Republic) and cryogenically homogenized in the presence of liquid nitrogen using a laboratory knife mill (A11 Basic Analytical mill, IKA Labortechnik, Staufen im Breisgau, Germany).

Insect meal products, foods containing insects, and non-insect food products were obtained from Czech retail markets. The chitosan standard was obtained from Carl Roth GmbH (Karlsruhe, Germany). The chemicals for buffer preparation were obtained from Penta Chemicals in p.a. quality (Prague, Czech Republic).

2.2. Development of Indirect Sandwich ELLA

A 96-well plate was incubated overnight with 50 µL of WGA and LEL lectin (Vector Laboratories, Newark, NJ, USA). The WGA lectin was diluted 1:1000 in lectin buffer (Tris base 50 mM/L, sodium chloride 149 mM/L, magnesium chloride 2.13 mM/L, and calcium chloride 1.00 mM/L, adjusted to pH 7.6). The plate was washed with 300 µL washing buffer (Tris-buffered saline with Tween® 20, Sigma-Aldrich, St. Louis, MO, USA) and 200 µL of Carbo-free (Vector laboratories, Newark, NJ, USA) blocking buffer was added to each well and incubated at room temperature for 2 h. The plate was washed with washing buffer again. Subsequently, 50 µL of samples or a concentration of chitosan standard ranging from 0.005 to 0.25 mg/mL was added to each well and incubated in a fridge for 12 h. After washing with 300 µL wash buffer three times, 50 µL of diluted 1:1000 WGA biotinylated lectin was added to each well and incubated at room temperature for 1 h. After washing with 300 µL wash buffer three times, 50 µL of avidin–biotin complex (ABC, Vector Laboratories, Newark, NJ, USA) was added and incubated for 30 min at room temperature. After washing with 300 µL wash buffer three times, 100 µL of TMB (Vector Laboratories, Newark, NJ, USA) reagent was added and the sample was incubated in the dark for 10 min; the absorbance was measured at 650 nm.

2.3. Validation of Indirect Sandwich ELLA

Validation was conducted in accordance with Ref. [26]. Intra-day precision and inter-day precision were evaluated using chitosan standard with concentration of 0, 0.005, 0.026, 0.056 0.088, 0.125, and 0.25 mg/mL *Tenebrio molitor* powder and roasted flavored commercial product (Grig, Brno, Czech Republic) in 93% (*Acheta domesticus*) with concentrations of 25 and 50 mg/mL. The results were expressed as coefficient of variation (CV%) and relative error (%).

The sensitivity was assessed through the limit of detection (LOD), and the limit of quantification (LOQ) was calculated by the replicated determination (n = 10) of a zero blank (mean value + 3 SD for LOD and + 10 SD for LOQ) [27]. An insect-free wheat snack and protein bar were used as samples for the recovery tests conducted by spiking chitosan standards with 1 mg/g and 10 mg/g. The sensitivity of the ELLA methods was confirmed using different insect species (AD, ALD, BD, GA, HI, LM, ST, TM, ZM). Each insect sample was analyzed in eight replicates.

2.4. Validation of Indirect Sandwich ELLA in Commercial Samples

The commercial samples were prepared in concentrations of 25 and 50 mg/mL for the determination of the method's precision [28]. PBS with pH 7.4 was used as the diluent. To evaluate commercial samples from the retail market, a concentration of 50 mg/mL PBS with pH 7.4 was used. Before analysis, the samples were extracted for 30 min and filtered by

a 0.22 µm Nilon syringe filter (FiltraTech, Orleáns, France). Eight replicates of all samples were measured.

2.5. Data Analysis

Data analyses were performed using Microsoft Excel software version 1808 (Microsoft, Redmond, WA, USA), specifically, the standard deviation, coefficient of variation% and standard error%. The coefficient of variation (CV%) resulted from SD/mean × 100. The standard error resulted from SD/square root of the sample size × 100. Statistical analysis was performed by Xlstat 2024.2.0 (Adinsoft, Denver, CO, USA). The data did not follow normal distribution according to the Shapiro–Wilk test. he Kruskal–Wallis test (with post hoc multiple pairwise Dunn's procedure) was applied separately to compare various insect species, the natural chitosan content, and the chitosan content in the commercial product. It was also used to evaluate the working dilution of lectin and the effect of pH on chitosan solubility (post hoc, multiple pairwise Dunn's procedure) were used. For the standard curves of the indirect sandwich ELLA, linear regression was used. The results were considered to be significant when $p < 0.05$.

3. Results

3.1. Working Dilution of Lectin and Chitosan Solubility pH

Chitin is a non-soluble natural biopolymer. The solubility of chitosan depends on the pH. This study tested solvents with a pH of 2.4, 7.4, and 9.4. The solubility of chitosan in water is influenced by the hydrodynamic behavior of chitosan macromolecules. At pH 3, chitosan adopts a rigid rod conformation. When the pH exceeds 6, deprotonation of the amino groups begins, leading to a loss of charge on the chitosan macromolecules and the initiation of precipitation [29]. Chitosan's solubility is affected by several factors and can be modified by adding substances such as urea, acetate, PBS, or bicarbonate buffer. These additives adjust the solubility of chitosan across a pH range from 4.0 to 10 [30]. The absorbance value was higher for pH 7.4, independent from chitosan concentration. The lowest was for pH 2.4 (Figure 1).

In this study, an indirect sandwich enzyme-linked lectin sorbent assay (ELLA) was used to confirm the chitosan content in food products. In brief, purified WGA and LEL lectin were coated onto wells at dilutions of 1:1000 and 1:10,000. The absorbance values at concentrations of 10 mg/mL were all greater than 1.0 at a dilution of 1:000 at pH 7.4, confirming successful labeling and suitability for use as a detection antibody. Due to the lower absorbance value obtained at a 1:10,000 dilution, the lectin dilution was reduced, and dilution ratios were adjusted to 1:1000 for further testing.

3.2. Standard Curve of Indirect Sandwich ELLA

The indirect sandwich ELLA developed in this study utilized WGA lectin specific for N-acetylglucosamine. Purified chitosan standard was used at concentrations ranging from 0.024 to 0.25 mg/mL. ABC was employed as the detection biotinylated lectin at a dilution ratio of 1:1000. The standard curve, presented in Figure 2, was generated using linear regression, yielding an R^2 value of 0.972 ($p < 0.05$).

3.3. Validation of Indirect Sandwich ELLA in This Study

The limit of detection (LOD) and limit of quantification (LOQ) were 0.006 mg/mL (6 ppm) and 0.028 mg/mL (28 ppm), respectively. Chitosan was determined in different insect species to confirm the sensitivity of the indirect sandwich ELLA for various species (Table 1). The chitosan concentration was higher than 4.5 mg/g in the wheat products, chips, and snacks, suggesting the natural content of N-acetylglucosamine in the control samples (Table 2).

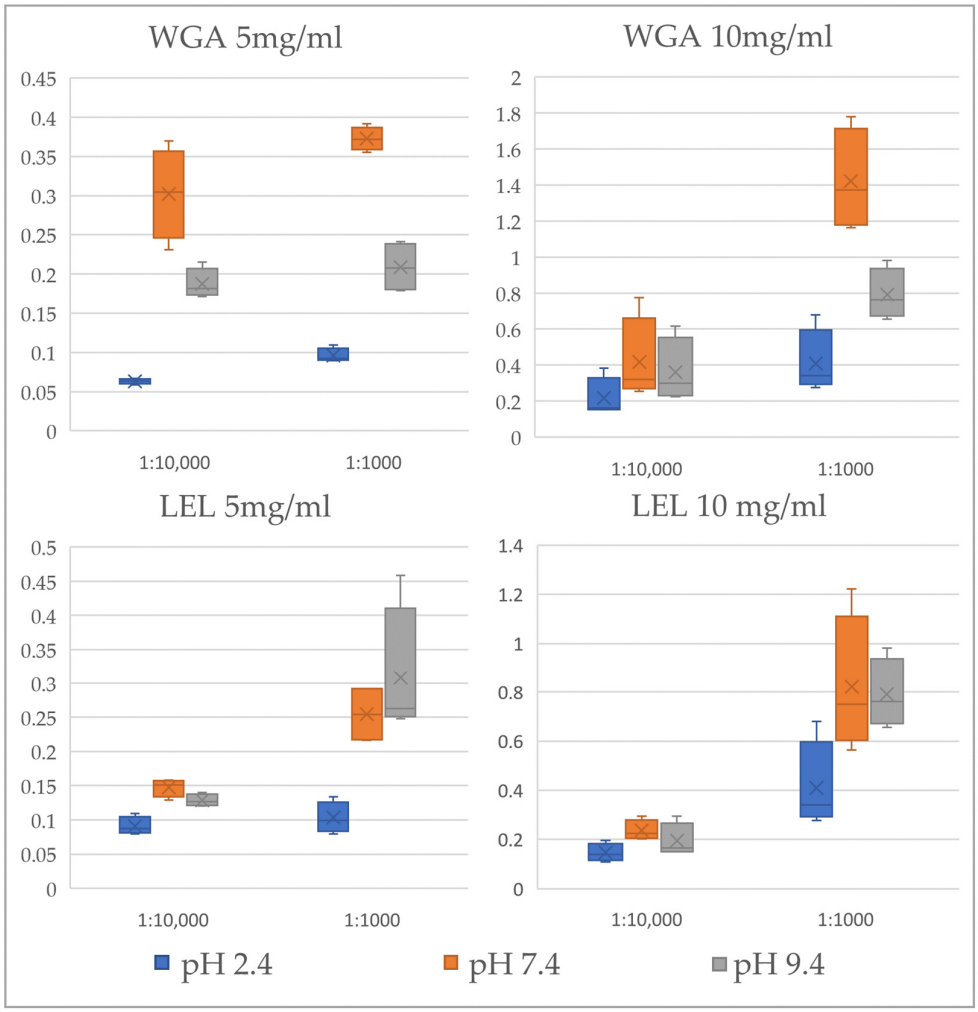

Figure 1. Box plot for WGA and LELlectin in dilutions of 1:10,000 and 1:1000 with concentrations of 5 mg/mL and 10 mg/mL.

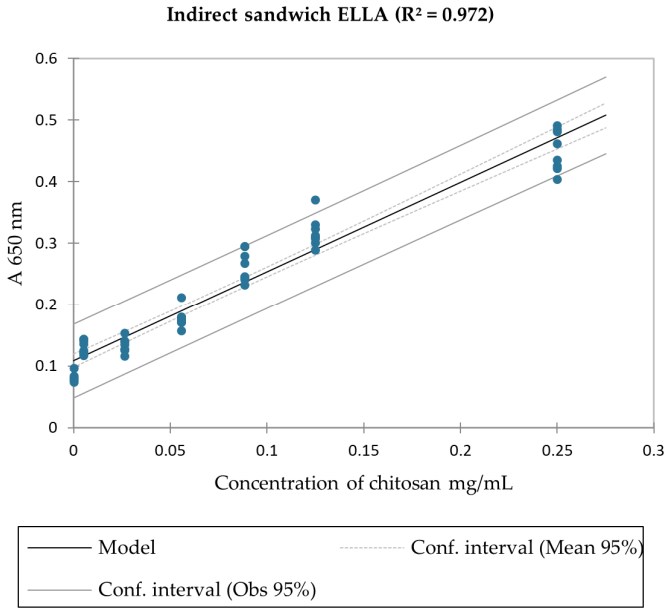

Figure 2. Standard curves of indirect sandwich ELLA for chitosan detection.

Table 1. Chitosan content in different insect species.

Sample	*n*	Chitosan (mg/g, Mean ± SD)
Acheta domesticus (AD)	18	9.83 ± 3.99 [a]
Alphitobius diaperinus (ALD)	7	4.49 ± 4.52 [e]
Blaptica dubia (BD)	12	8.06 ± 5.48 [b]
Gryllus assimilis (GA)	13	10.78 ± 6.09 [a]
Hermetia Illucens (HI)	6	9.87 ± 3.83 [a]
Locusta migratoria (LM)	4	7.09 ± 3.12 [b,c]
Schelfordella tartara (ST)	4	8.44 ± 3.42 [a,b]
Tenebrio molitor (TM)	28	5.63 ± 5.6 [d,e]
Zophobas morio (ZM)	18	5.83 ± 3.62 [c,d]

Different letters indicate statistical differences ($p < 0.05$), SD: standard deviation, *n*: number of samples analyzed.

Table 2. Natural content of N-acetylglucosamine in the samples.

Samples	*n*	Chitosan (mg/g, Mean ± SD)
Baked amaranth chips: tomato and basil (gluten free)	1	4.73 ± 0.94 [a]
Complete high-protein pasta	1	3.35 ± 0.86 [c,d]
Doritos sweet chilli flavored/hot corn	1	3.23 ± 1.07 [c,d]
Fermented chocolate granola with coconut	1	5.29 ± 1.62 [a]
Fusilli	1	4.69 ± 2.16 [a,b,c]
Durable graham sticks: Havlík	1	2.52 ± 0.99 [d]
Durable graham sticks: spicy	1	4.52 ± 0.71 [a,b]
Legume chips: garlic	1	4.87 ± 1.07 [a]
Legume chips: original	1	3.54 ± 0.67 [b,c,d]
Lentil wasabi: multigrain snack	1	5.31 ± 1.43 [a]
Protein bar: chocolate flavor	1	4.84 ± 1.65 [a]

Different letters indicate statistical differences ($p < 0.05$), SD: standard deviation.

Spiking 1 and 10 mg/g standard into the wheat snack resulted in a recovery from 80.0% to 104.0%; for the protein bar, the recovery was from 64.1% to 79.0% depending on the blank matrix concentration (Table 3). As shown in Table 4, the chitosan standards with concentrations of 0.1, 1, and 10 mg/mL, insect *Tenebrio molitor* in concentrations of 25 and 50 mg/mL, and insect product in concentrations of 25 and 50 mg/mL were used. The chitosan CV% of intra-day precision and inter-day precision ranged from 2.45% to 15.41% and from 3.61% to 3.17%, respectively. For insect product and *Tenebrio molitor*, the CV% of the intra-day precision was calculated. It ranged from 10.29 to 30.29% and was expressed as chitosan content. Additionally, the relative error of the standard sample was low for concentration 1 and 10 mg/mL and high for 0.1 mg/mL.

Table 3. Recovery test of indirect sandwich ELLA.

Statistical Parameters	Chitosan Concentration in Blank Sample—Snack		Chitosan Concentration in Blank Sample—Protein Bar	
	1 (g/g)	10 (g/g)	1 (g/g)	10 (g/g)
	Blank matrix concentration 25 mg/mL			
Recovery (%)	80.0	104.0	74.3	79.0
	Blank matrix concentration 50 mg/mL			
Recovery (%)	83.0	91.6	64.1	66.7

Table 4. Precision and relative error of the indirect sandwich ELLA developed in this study.

Chitosan	Mean ± SD	CV (%)	Relative Error (%)
	Intra-day precision		
0.1 mg/mL	0.15 ± 0.02	15.41	48.79
1.0 mg/mL	0.99 ± 0.06	5.66	0.34
10 mg/mL	11.51 ± 0.03	2.45	15.10
Insect product (AD 93%) 25 mg/mL	7.58 ± 1.38	18.19	-
Insect product (AD 93%) 50 mg/mL	15.05 ± 1.55	10.29	-
Tenebrio molitor 25 mg/mL	7.17 ± 2.17	30.29	-
Tenebrio molitor 50 mg/mL	11.45 ± 2.7	23.59	-
	Inter-day precision		
0.1 mg/mL	0.15 ± 0.02	12.87	53.75
1.0 mg/mL	0.96 ± 0.09	8.92	3.61
10 mg/mL	11.4 ± 0.04	0.36	14.37

Chitosan concentrations were detected using indirect sandwich ELLA (n = 5). SD: standard deviation, CV: coefficient of variation, AD: *Acheta domesticus*, - not relevant.

3.4. Validation of Indirect Sandwich ELLA in Commercial Products

As shown in Table 5, the quantified chitosan content using ELLA was found to be 12.32 ± 3.45 mg/g in the product prepared as a flavored and/or salted insect product. In the product prepared with a different addition of the insects, chitosan was found in the amount of 9.69 ± 2.15 mg/g, and in the product without any insect addition, chitosan was found in the amount of 4.26 ± 1.5 mg/g.

Table 5. Summarized chitosan content in commercial products.

Sample	*n*	Chitosan (mg/g, Mean ± SD)
Insect processed products	11	9.69 ± 2.15 [a]
Insect products	17	12.32 ± 3.45 [b]
Non-insect product	11	4.26 ± 1.5 [c]

Different letters indicate statistical differences ($p < 0.05$). n: number of samples analyzed.

The ELLA developed in this study can be effectively utilized for the detection of chitosan content in commercially available insect product or products with insect content.

4. Discussion

Despite the fact that insect consumption in the European Union does not have a tradition compared to other countries such as Africa, Latin America, and Asia, its use is gradually increasing, especially with regard to the environmental benefits of insect protein production [31]. The fear of insects that is often associated with insect consumption in Western countries is also gradually decreasing [32]. As such, insects are used for direct consumption after basic technological operations, but in Europe, their highest potential is associated with their use as insect-derived materials in other products. In view of the above, the use of insects in Europe is regulated by the Novel Food Regulation (EU) 2015/2283 [1]. According to Skotnicka, the production of insects and insect-based foods in Europe is expected to develop significantly. The author expects production of up to 260,000 tonnes by 2030 [33]. Significant applications are particularly expected in the field of feed production. Despite the undeniable benefits, the EU takes a moderate approach and does not underestimate the risks associated with the consumption of insects and insect products. According to the EFSA's opinion [34], insects pose risks mainly in terms of allergy potential and chemical and biological risks, but there are also concerns about the environmental impact associated with the introduction of new invasive species into the EU.

Ensuring the detection of insects in food products is crucial for consumer protection, food quality, and authenticity verification. According to Verbeke [35], the inclusion of insects as food ingredients affects consumer acceptance, as these products offer high-quality proteins, minerals, and essential fatty acids. With insect-based foods recognized

as a potential future trend to address global food needs, effective detection methods enable regulatory authorities to verify the presence or absence of insects, aligning with manufacturers' declarations. Given the allergenic potential of insect proteins, which may provoke allergic reactions in some consumers [36], reliable detection is also essential for managing allergen risks and maintaining consumer confidence. Our developed ELLA method is focused on detecting insects in food without differentiating between permitted and non-permitted species, for which PCR methods are applicable [11,12]. In contrast, the ELLA method is suitable as a screening tool, providing preliminary results before specific insect identification methods are applied.

Our developed ELLA method was validated based on ELISA. The sensitivity, specificity, recovery, LOD, and LOQ were used. The results show good performance in terms of these criteria. In food applications, these data are not known for insect determination; thus, a comparison with allergen determination was made. The LOD and LOQ in our method were higher than those for allergen determination and ranged from 0.02 to 2.5 ppm [37]. For example, for soy determination, it was 0.08 to 0.25 ppm [38]; for crustacea, it was 0.001 ppm to 0.003 ppm [39]; and for peanuts, it was 0.06 to 0.19 ppm [40]. To compare, proteomic methods for *Acheta domesticus* determination using peptide markers achieved LODs ranging from 8000 to 25,000 ppm. [17]. For multiplex real-time PCR, 1 ppm LOD was confirmed [11]. Likewise, the recovery was high for the snack product in 10 mg/g concentration of chitosan. The concentration in 1 mg/g of chitosan and spike matrix based on the protein bar was lower (Table 3). However, the values obtained correspond to AOAC requirements, which should be within the range of 50–150% [41]. These results show that there are possible interactions in the food matrix between chitin, chitosan, and proteins. The interaction between chitin and proteins was studied in insects [42], but in food, this interaction is not known.

The developed ELLA method is based on the determination of terminal carbohydrates of N-acetylglucosamine, which is contained in chitin as well as in the deacetylated chitin derivative chitosan [18]. Chitosan was used as a standard in this study. Different insects have variable contents of chitin due to the fact that the insect is collected and processed during the larval stage, before developing the exoskeleton [14]. Based on electrophoretic methods, it was determined that the chitin content in edible insects´ ranges between 43 and 84 mg/g in HI, ALD, TM, GA, and AD [43]. Using extraction methods, chitin was determined in LM, ranging from 86.4 to 123.3 mg/g [44]. Different chitosan contents were also confirmed in our study, where nine insect species were evaluated. The content of chitosan was in the range of 4.49 to 10.78 mg/g. The lowest concentration was in ALD, and the highest in GA. The results are in conformity with the chitin content determined by electrophoretic methods [43]. The absolute amount of chitosan is lower than chitin. This finding was also confirmed for LM, where the chitosan content was 58.95 to 69.75% of the total chitin [44]. The chitosan content also varies in different insect bodies: in *Schistocerca gregaria*, it was determined within the range of 8 to 10% [45]. The lower content of chitosan determined by the indirect sandwich ELLA is caused by the determination of terminal N-acetylglucosamine. Another study evaluated the total amount of chitin after long-term hydrolysis [28,46].

Intra-day CV and inter-day CV were also comparable with the ELISA method. For soy protein, the intra-day CV in a concentration of 1.0 µg/mL was 3.96%, while the inter-day CV was 12.1. For crustacea, the intra-day and inter-day CVs were 5.69 to 14.32 and 9.0 to 12.59, respectively [39]. For peanuts, the intra-day and inter-day CVs reached 0.84 to 5.21 and 0.87 to 6.12, respectively [28]. Comparing our results (Table 3), the indirect sandwich ELLA intra-day CV was higher, and the inter-day CV varied. In spite of that, the recorded values matched the repeatability $\leq$20% and reproducibility $\leq$30% values required by the AOAC [41], which implied that our indirect sandwich ELLA exhibited sufficient precision.

Numerous products based on processed insects potentially exist. In this case, it is important to validate the ELLA methods for residue or cross-reactivity analysis of commercial products [38]. Eleven commercial samples from the Czech retail market were

evaluated and compared with samples without insects (Table 2). Chitosan, or, namely, N-acetylglucosamine, was determined in all samples but with different concentrations. The samples without the addition of insects show statistically the lowest amount of N-acetylglucosamine compared with processed insect products and insect products ($p < 0.05$) (Table 4). We anticipated cross-reactivity of the ELLA method with the carbohydrate composition of the food products. The insect contamination of foods was not expected based on hygiene practices, and this was also confirmed for commercial samples by using multiplex real-time PCR [11]. Another reason for the determination of N-acetylglucosamine in non-insect samples may be the presence of mold, which contains chitin and chitosan [47]. However, such false positives are generally not anticipated due to standard hygiene practices and strict regulatory limits on mycotoxins in food products [48]. However, such cross-reactivity or cross-contamination is also expected in the ELISA method. For example, 0.16 µg/mL [38] of soy protein was observed in non-soy products.

Consequently, the indirect sandwich ELLA meets the requirements of the AOAC and could be used to detect insects in various food matrices.

5. Conclusions

An indirect sandwich ELLA was developed to the detect insects in food by targeting N-acetylglucosamine, a monomer units in chitin and chitosan polymers that make up the insect exoskelet. The ELLA method follows the requirements used in the ELISA applied for allergen determination, and it was confirmed that lectin-based assays are applicable for food analysis. Validation on commercial samples showed that the ELLA method is applicable for determination using real samples as well, but it also demonstrates that a low content of chitin or chitosan was determined in non-insect products.

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