



# Evaluation of ACE, $\alpha$ -glucosidase, and lipase inhibitory activities of peptides obtained by in vitro digestion of selected species of edible insects

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Received: 16 January 2020 / Revised: 30 March 2020 / Accepted: 4 April 2020 / Published online: 23 April 2020  
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## Abstract

The objective of this study was to examine the inhibition of the activity of enzymes associated with development of the metabolic syndrome by peptide fractions received from simulated gastrointestinal digestion and absorption of heat-treated edible insects. The inhibitory activities of insect-derived peptides were determined against key enzymes relevant to the metabolic syndrome such as the angiotensin-converting enzyme (ACE), pancreatic lipase, and  $\alpha$ -glucosidase. After the in vitro absorption process, all hydrolysates showed high inhibitory activity; however, the most effective metabolic syndrome-inhibitory peptides were received after separation on Sephadex G10. The best results were found for peptide fractions obtained from *Schistocerca gregaria*. The highest enzymes inhibitory activities were obtained for peptide fractions from *S. gregaria*: boiled for ACE ( $IC_{50}$  3.95  $\mu\text{g mL}^{-1}$ ), baked for lipase ( $IC_{50}$  9.84  $\mu\text{g mL}^{-1}$ ), and raw for  $\alpha$ -glucosidase ( $IC_{50}$  1.89  $\mu\text{g mL}^{-1}$ ). Twelve sequences of peptides from the edible insects were identified and their chemical synthesis was carried out as well. Among the synthesized peptides, the KVEGDLK, YETGNGIK, AIGVGAIR, IIAPPER, and FDPFPK sequences of peptides exhibited the highest inhibitory activity. Generally, the heat treatment process applied to edible insects has a positive effect on the properties of the peptide fractions studied.

**Keywords** Edible insects · Entomophagy · Metabolic syndrome · Bioactive peptides · In vitro digestion

## Introduction

Insects not only have high levels of good-quality protein but also minerals, vitamins, and essential fatty acids. This is evidenced by the large number of publications issued in recent years [1]. However, the latest research complements the list of the benefits of insect consumption with a pro-healthy aspect i.e. their bioactivity. Several studies indicate that insects, due to their high protein content ranging from 7 to 91%, are a valuable source of biologically active peptides [2–6].

The best studied species of edible insects for bioactive peptides is *Bombyx mori*. In the protein of this species peptides with in vitro inhibitory activity against enzymes such

as the angiotensin-converting enzyme (ACE) (EC 3.4.15.1) and  $\alpha$ -glucosidase (EC 3.2.1.20) have been identified [6–9]. The investigations into bioactive peptides from insects are rather new and many activities remain to be investigated. Following the results of recent work, we hypothesized that insect-derived peptides would inhibit the activity of enzymes associated with development of the metabolic syndrome. This disease includes a range of cardiovascular disease or type 2 diabetes risk factors including abnormalities in blood glucose levels, increased blood pressure, increased level of triglycerides in the blood, and obesity [10]. According to American Heart Association/National Heart, Lung, and Blood Institute scientific statement to diagnose this syndrome three of the above-mentioned risk factors must be occurred in a patient [11]. This is related to the activity of enzymes that participate in disease entities such as glucose intolerance, hypertension or abdominal obesity, e.g.  $\alpha$ -glucosidase (EC 3.2.1.20), the angiotensin-converting enzyme (ACE) (EC 3.4.15.1), and pancreatic lipase (EC 3.1.1.3) [12].

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ACE is the important metabolic enzyme in the renin–angiotensin–aldosterone system (RAA). In turn, this hormonal system is used for regulating of blood pressure and cardiovascular functions of organism. Hence, attenuation of ACE activity plays an important role in reducing hypertension or increased blood pressure, the most important risk factor for the occurrence of stroke, myocardial infarction, cardiac insufficiency and renal diseases [6, 12]. Many synthetic inhibitors of ACE have been used to prevent hypertension but ACE inhibitors occurring in the natural products are sought, which justifies this research [6].

$\alpha$ -Glucosidase is an enzyme associated with the decomposition of oligosaccharides to monosaccharides in the intestine in the postprandial phase [13]. Inhibition of  $\alpha$ -glucosidase is one of the therapeutic targets for reducing intestinal uptake of glucose and suppressing postprandial hyperglycemia in type 2 diabetics [9].

Pancreatic lipase is a metabolic enzyme involved in decomposition of triacylglycerols in the intestine and their absorption [14]. Inhibition of lipase is one of the methods for the treatment of obesity by attenuation of digestion and thereby absorption of lipids in the digestive tract [15].

Inhibition activity of enzymes associated with development of the metabolic syndrome may be a useful method to supporting treatment of diseases classified to them. However, it should be noted that such action may also have side effects. Insufficient activity of pancreatic lipase may cause steatorrhea (e.g. oily, loose stools with excessive intestinal putrefaction and flatus due to unabsorbed fats reaching the large intestine), fecal incontinence, frequent bowel movements and consequently reduced absorption of fat-soluble compounds such as vitamins [16]. In turn, inhibition activity of  $\alpha$ -glucosidase also may cause side effects including gastrointestinal disorders, flatulence and abdominal pain [17]. Such side effects are caused by the fermentation of undigested carbohydrate by the microbiota in the large intestine [18]. Nevertheless, in the treatment of type 2 diabetics and obesity, the commercial inhibitors of  $\alpha$ -glucosidase (e.g. voglibose and acarbose) and pancreatic lipase (e.g. orlistat) are used as a clinical drugs [16, 17].

Our previous study [4] has confirmed that we could obtain antioxidant and anti-inflammatory peptides from *Tenebrio molitor*, *Schistocerca gregaria*, and *Gryllobates sigillatus* protein. Based on our research, it has been proven that peptides from edible insects have great antiradical activity, chelating iron ions activity, and ability to inhibit lipoxigenase (EC 1.13.11.12) and cyclooxygenase-2 (EC 1.14.99.1) activity. The aim of the present investigation was to evaluate the impact of the thermal processing of edible insects (raw, boiled, baked) on formation of selected metabolic syndrome-inhibitory peptides and comparison of their activities with that of chemically synthesized peptides. Identification and chemical synthesis of the sequences of peptides was

performed previously as part of another study concerning antioxidant activity of insects [19], but the full methodology is also included in this work.

## Materials and methods

### Raw materials

The research material consisted of three insect species: mealworms *Tenebrio molitor* (Linnaeus, Coleoptera: Tenebrionidae) (larvae), locusts *Schistocerca gregaria* (Forsk., Orthoptera: Acrididae) (adult) and crickets *Gryllobates sigillatus* (Fabricius, Orthoptera: Gryllidae) (adult). The insects were obtained from a local breeder (Fabryka Owadów, Warsaw, Poland).

### Chemicals

Hippuryl-L-histidyl-L-leucine (HHL),  $\alpha$ -amylase from hog pancreas (50 U mg<sup>-1</sup>), pepstatin A, PMSF (phenylmethanesulfonyl fluoride), pepsin from porcine gastric mucosa (250 U mg<sup>-1</sup>), pancreatin from porcine pancreas, bile extract, pNPG (*p*-nitrophenyl- $\alpha$ -D-galactopyranoside),  $\alpha$ -glucosidase from *Saccharomyces cerevisiae*, lipase from porcine pancreas, pNPA (*p*-nitrophenyl acetate), DMSO (dimethyl sulfoxide) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). All other chemicals were of analytical grade.

### Insect preparation

Insects were fasted for 2 days to empty their digestive tract. Then, insects were heat treated by two methods. Some of them were boiled for 10 min at 100 °C in the water while others were baked in a heated oven at 150 °C for 10 min. Afterwards, insects were frozen, lyophilized and kept at – 18 °C until further analysis.

### Production of protein preparation

Protein preparations were produced through the protein isolation method of Girón-Calle Alaiz, and Vioque [20] with modifications as mentioned previously [4]. Briefly, grounded insects were stirred with 0.2% NaOH in a ratio of 1:10 (*w/v*) for 1 h at room temperature. Afterwards, the samples were centrifuged at 4 °C for 20 min at 8000×g. Precipitation of protein was performed at isoelectric point. Next proteins were centrifuged at 4 °C for 20 min at 8000×g and washed with distilled water. Protein preparations obtained from the insects were lyophilized and kept at – 18 °C to further analysis.

## In vitro hydrolysis under gastrointestinal conditions and simulated absorption process

Simulated digestion of lyophilizates from heat-treated insects and insect protein was conducted in accordance with Jakubczyk et al. [21] using the method mentioned previously [4]. Briefly, grounded insects were suspended (4%, w/v) in a stimulated saliva solution (7 mM NaHCO<sub>3</sub> and 0.35 mM NaCl, pH 6.75) and stirred for 10 min. For the gastric digestion, the supernatants were adjusted to pH 2.5 with 1 M HCl and the reaction was carried out for 2 h with the addition of pepsin (250 U/mg). For the simulated intestinal digestion, the solution was neutralized with 1 M NaOH and a mixture containing 0.7% pancreatin and 2.5% bile extract (1:2.5, v/v) was added and samples were incubated for 1 h. The whole process of enzymatic hydrolysis was carried out at 37 °C in darkness and the reaction was stopped by heating at 100 °C for 5 min. The hydrolysates were clarified by centrifugation at 8000 × g for 10 min.

For the simulated absorption process, the hydrolysates were dialyzed with a membrane tube (molecular weight cut-off 3.5 kDa Serva, Heidelberg, Germany). The process was carried out without light for 1 h at 37 °C. In the last step, the samples were lyophilized and stored at – 18 °C until further use.

## The peptide concentration assay

Adler-Nissen method with the trinitrobenzenesulphonic acid (TNBS) was used to the determination of soluble peptides concentration [22].

## Gel-filtration chromatography

The gel filtration chromatography on Sephadex G10 (Sigma-Aldrich, USA) was used to the separation of peptide fractions obtained from the studied species of insects according to the method mentioned in the previous work [19]. Briefly, peptide fractions (10 mg) were separated by gel-filtration chromatography (column: 1.5 × 30 cm; eluent: distilled water; flow rate: 0.8 mL/min). One milliliter of each fraction was collected, and the absorbance was monitored at 220 nm. Fractions with the highest absorbance were combined and enzymes inhibitory activity was determined for them. Fractions with the highest inhibitory activity were lyophilized and used in further analysis.

## Identification of peptides

Liquid chromatography coupled to the mass spectrometer was used to the peptide identification. Investigation was performed by the Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences

(Warsaw, Poland) [19]. The samples were concentrated and desalted on a RP-C18 pre-column (Waters), and further peptide separation was achieved on a nano-Ultra Performance Liquid Chromatography (UPLC) RP-C18 column (Waters, BEH130 C18 column, 75 μm i.d., 250 mm long) of a nano-ACQUITY UPLC system, using a 100 min linear acetonitrile gradient. The column outlet was directly coupled to the Electrospray ionization (ESI) ion source of the Orbitrap Velos/Elit type mass spectrometer (Thermo) working in the regime of data-dependent MS to MS/MS switch. An electrospray voltage of 1.5 kV was used.

Raw data files were pre-processed with Mascot Distiller software (version 2.4.2.0, MatrixScience). The obtained peptide masses and fragmentation spectra were matched to the National Center Biotechnology Information (NCBI) non-redundant database (57412064 sequences/20591031683 residues), with an Orthoptera filter (7705 sequences) or Insecta filter (1038531 sequences) using the Mascot search engine (Mascot Daemon v. 2.4.0, Mascot Server v. 2.4.1, MatrixScience). The following search parameters were applied: enzyme specificity—none, peptide mass tolerance to ± 30 ppm, and fragment mass tolerance to ± 0.1 Da. The protein mass was left as unrestricted, and mass values as monoisotopic with one missed cleavage allowed. Methylation of cysteine and oxidation of methionine were set as a fixed and variable modification, respectively.

Peptide identification was performed using the Mascot search engine (MatrixScience), with a probability-based algorithm. An expected value threshold of 0.05 was used for the analysis, which means that all peptide identifications had less than 1 in 20 chance of being a random match.

## Chemical synthesis of peptides

The chemical synthesis of identified sequences was realized by Novazym with purity (HPLC) > 97.0% [19].

## Preparation of ACE from pig lung

The angiotensin-converting enzyme was prepared in accordance with the method of Jakubczyk et al. [21]. Pig lungs were purchased in a local market as a starting material. Lung tissues were homogenized in 0.1 M borate buffer pH = 8.3 containing pepstatin A (0.1 mM) and PMSF (0.1 mM) at 4 °C in the ratio 1:2 (w/v); next, the homogenate was centrifuged at 8000 × g at 4 °C for 20 min. The purification of ACE was initiated by addition of solid ammonium sulfate at 80% saturation. Afterwards, the sample was dialyzed (MW cut-off 12 kDa) for 24 h at 4 °C against 20 volumes of 0.1 M borate buffer pH = 8.3. The dialysate was centrifuged at 8000 × g at 4 °C for 20 min and the supernatant containing the active enzyme was frozen and used for further analysis.

### Assay of ACE inhibitor activity

The spectrophotometric technique was used to the ACE inhibitory activity indication with 5 mM HHL as a substrate described by Jakubczyk and Baraniak [23]. Briefly, 50  $\mu\text{L}$  of peptide sample was added to 50  $\mu\text{L}$  of 5 mM HHL solution and 50  $\mu\text{L}$  of 3  $\text{mU mL}^{-1}$  ACE (one unit of ACE activity was defined as an increase absorbance of 0.001 per minute at 390 nm). The reaction mixture was incubated at 37 °C for 60 min. The reaction was terminated by adding 0.7 mL of the 0.1 M borate buffer with 0.2 M NaOH. The absorbance was measured at 390 nm and the ACE inhibition was determined as follows:

$$\text{ACE inhibition (\%)} = [1 - ((A1 - A2)/A3)] \times 100,$$

where A1 is the absorbance of a sample with ACE and the inhibitor, A2 is the absorbance of a sample with the inhibitor but without ACE (with buffer), A3 is the absorbance of a sample with ACE and without the inhibitor (with buffer).

The results were presented as the  $\text{IC}_{50}$  value (inhibitory concentration) ( $\mu\text{g mL}^{-1}$ ).

### Assay of $\alpha$ -glucosidase inhibitory activity

The inhibitory activity of the samples on  $\alpha$ -glucosidase activity was performed in accordance to the procedure proposed by Kim et al. [24] with slight modifications regarding to the activity of the enzyme ( $1.0 \text{ U mL}^{-1}$ ) and substrate concentration (35.0 mM solution of the *p*-nitrophenyl glucopyranoside (pNPG)). The substrate solution *p*-nitrophenyl glucopyranoside (pNPG) (35.0 mM) was prepared in 20 mM phosphate buffer, pH 6.9. 10  $\mu\text{L}$  of the enzyme ( $1.0 \text{ U mL}^{-1}$ ), 10  $\mu\text{L}$  of the sample, 20  $\mu\text{L}$  of the substrate, and 500  $\mu\text{L}$  of the phosphate buffer were mixed and incubated at 37 °C for 20 min. After the incubation period, 2 mL of 0.1 M  $\text{Na}_2\text{CO}_3$  was added to the reaction mixture to terminate the reaction. The absorbance of the mixtures was measured at 405 nm. The final results were compared with the activity of the same amount of the enzyme without the inhibitor (buffer instead of inhibitor). The inhibitory effect was calculated using the formula:

$$\text{Inhibition (\%)} = [(A \text{ control} - A \text{ sample})/A \text{ control}] \times 100,$$

where A sample is the absorbance of the reaction mixture; A control is the absorbance of control sample.

The results were presented as the  $\text{IC}_{50}$  value (inhibitory concentration) ( $\mu\text{g mL}^{-1}$ ).

### Assay of lipase inhibitory activity

Procedure proposed by Wang et al. [25] was used to examine the lipase inhibitory activity with minor modifications

detailed by Jakubczyk et al. [12]. Briefly, 20  $\mu\text{L}$  of lipase ( $100 \text{ mg mL}^{-1}$ ) were mixed with 50  $\mu\text{L}$  of the sample and 1.42 mL of 100 mM potassium phosphate buffer, pH 7.5. After preincubation at 30 °C for 3 min, the reaction was initiated by adding 10  $\mu\text{L}$  of 100 mM pNPA solution in dimethyl sulfoxide (DMSO). The variation of absorbance at 405 nm was measured for 3 min. The final results were compared with the activity of the same amount of the enzyme without the inhibitor (buffer instead of inhibitor). The inhibitory effect was calculated using the formula:

$$\text{Inhibition (\%)} = [(A \text{ control} - A \text{ sample})/A \text{ control}] \times 100,$$

where A sample is the absorbance of the reaction mixture; A control is the absorbance of control sample.

The results were presented as the  $\text{IC}_{50}$  value (inhibitory concentration) ( $\mu\text{g mL}^{-1}$ ).

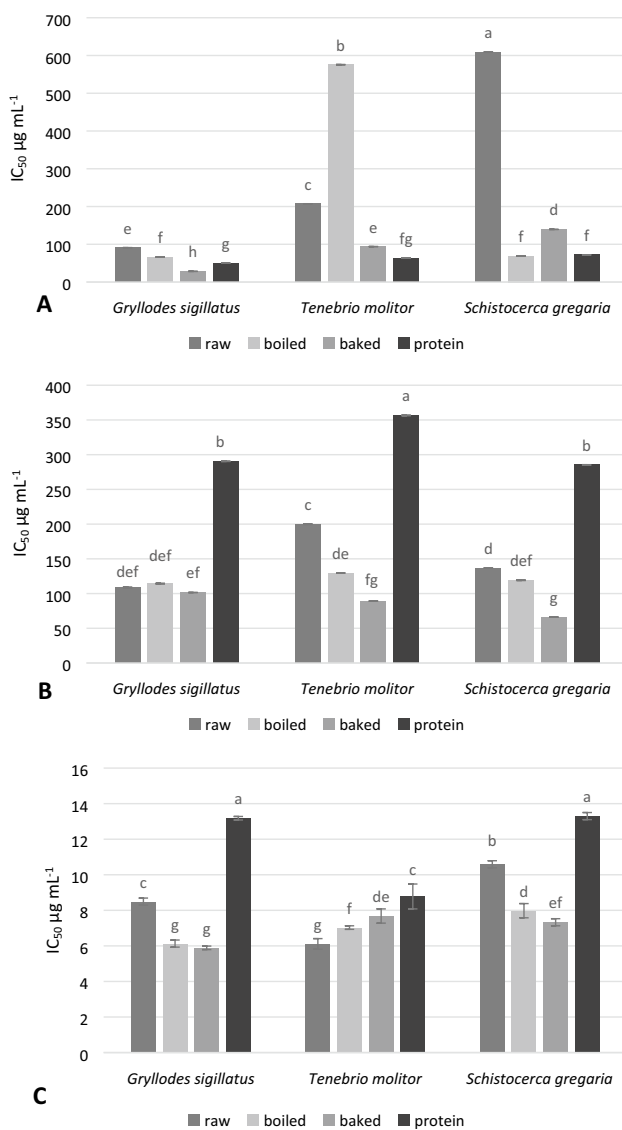
### Statistical analysis

All data are expressed as means  $\pm$  standard deviation for triplicate of experiments. Statistical analyses were performed using the Statistica (13.1) (StatSoft, Krakow, Poland) for comparison of means using ANOVA with post hoc Tukey's Honestly Significant Difference (HSD) test at the significance level  $p < 0.05$ .

## Results and discussion

### Evaluation of the activity of hydrolysates after the simulated absorption process

Raw, heat-treated (boiled and baked), and protein preparations of all the tested insects species were in vitro digested; subsequently, the hydrolysates were separated using a membrane tube with molecular weight cut-off 3.5 kD for the simulated absorption process. All the peptide fractions obtained exhibited a metabolic syndrome enzyme-inhibitory effect, as shown in Fig. 1; furthermore, the thermal treatment had a major influence on improvement of the enzyme inhibition with a minor exception for  $\alpha$ -glucosidase activity in hydrolysates obtained from *T. molitor* and *S. gregaria*. Several studies have shown that peptides obtained from in vitro digested insects, for example cotton leafworm *Spodoptera littoralis* or silkworm *Bombyx mori*, exhibit ACE inhibitory activity [6, 7, 26, 27]. In our study, all the hydrolysates obtained after the in vitro absorption process showed ACE inhibitory activity and  $\text{IC}_{50}$  values ranging from 28.2 to 608.85  $\mu\text{g mL}^{-1}$  (Fig. 1) In turn, Verduyck et al. [7] determined the  $\text{IC}_{50}$  value of the ACE inhibitory activity of *S. littoralis* after gastrointestinal digestion at 320  $\mu\text{g mL}^{-1}$ , which is within the range of values estimated in the present study.



**Fig. 1** Inhibitory activity of hydrolysates after the in vitro absorption process. Inhibition of ACE (a), lipase (b), and  $\alpha$ -glucosidase (c). Values with different letters are significantly different ( $p < 0.05$ )

Moreover, a slightly higher value was reported by Wu et al. [6] for *Bombyx mori* hydrolysate ( $IC_{50}$  596.11  $\mu\text{g mL}^{-1}$ ). Overall, the hydrolysates obtained from protein preparations showed high levels of  $IC_{50}$  in all the species tested. Furthermore, the thermal treatment of the insects elevated the ACE inhibitory activity and the greatest ACE inhibition was observed for the hydrolysate from the baked *Gryllobes sigillatus* ( $IC_{50}$  28.2  $\mu\text{g mL}^{-1}$ ). Our results correspond well with those presented by Barbana and Boye [28] for lentil hydrolysates ( $IC_{50}$  0.053 and 0.09  $\text{mg mL}^{-1}$ , respectively), and legumes are known as a rich resource of ACE inhibitory peptides.

In the case of lipase inhibitory activity, the hydrolysates obtained from the baked insects yielded particularly better

results (Fig. 1). The highest lipase inhibitory activity was observed for the hydrolysate from the baked *Schistocerca gregaria* ( $IC_{50}$  66.11  $\mu\text{g mL}^{-1}$ ), although its activity was weaker than that of orlistat ( $IC_{50}$  0.75  $\mu\text{g mL}^{-1}$ ) which was used as a pancreatic lipase inhibitor. It should be noted that the mechanism of lipase inhibition by peptides is still poorly understood. Orlistat, a well-known anti-obesity agent, has shown to decrease the systemic absorption of dietary fat by interacting with the catalytic sites of the enzyme. There are synthetic soybean peptides (EITPEKN-PQLR and RKQEEDDEEEQQRE) that also showed a similar manner to block the catalytic domain of the enzyme [29]. Moreover, results of study indicated that also cumin seed peptides hindered the catalytic activity of lipase through direct contacts with the enzyme residues located in the active site [30].

For  $\alpha$ -glucosidase inhibition, the hydrolysate obtained from baked *G. sigillatus* had the highest inhibition activity— $IC_{50}$  5.89  $\mu\text{g mL}^{-1}$  (Fig. 1). Actually, just a handful commercial inhibitors of  $\alpha$ -glucosidase have been confirmed for diabetes therapy with rather complex and multi-step production processes; hence, natural compounds with  $\alpha$ -glucosidase inhibitory activities and with a non-sugar core structure are still being sought [15]. There are studies about peptide inhibitory  $\alpha$ -glucosidase where carried out with molecular docking models. According to the Mollica et al. [31], the crystallographic ligand acarbose interacts with  $\alpha$ -glucosidase in the enzymatic cavity by establishing several hydrogen-bond (H-bond) interactions. In particular, the key interactions involve Asp1157, Asp1279, Lys1460, Arg1510, Asp1526, His1584. The peptide inhibitors may contain several hydrophilic groups, such as the amide and amine groups, which may mimicry the polar interactions with the polar region of the binding site in a similar way as acarbose.

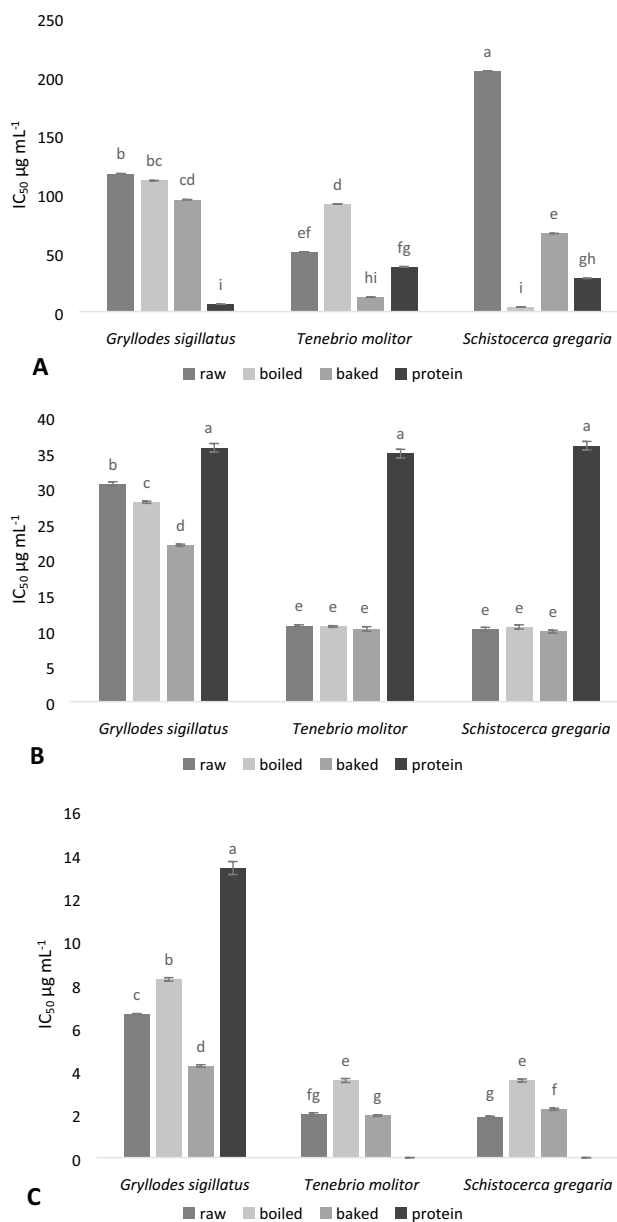
Other authors described that the  $\alpha$ -glucosidase inhibition by the peptides could be mainly attributed to the formation of five strong hydrogen bonds between Glu-Ala-Lys and His-674, Asp-518, Arg-600, Asp-616, and Asp-282 in  $\alpha$ -glucosidase, and to four hydrogen bonds between Gly-Ser-Arg and residues Asp-282, Asp-518, and Asp-616. The results indicated that an  $\alpha$ -glucosidase inhibitory peptide may have hypoglycemic efficacy when used as an ingredient in novel functional foods [32]. For example, among natural compounds, a very good  $\alpha$ -glucosidase inhibitor is the grape peel extract— $IC_{50}$  10.5  $\mu\text{g mL}^{-1}$  [33], whereas most tested insect hydrolysates exhibited better  $\alpha$ -glucosidase inhibitory properties. Concluding, the edible insect species studied are a source of biologically active peptides obtained as a result of protein digestion in gastrointestinal conditions and their bioactivity is manifested in significant inhibition of enzymes that contribute to the development of the metabolic syndrome (ACE, lipase,  $\alpha$ -glucosidase).

## Evaluation of the activity of peptide fractions

Size exclusion chromatography on Sephadex G10 was used for separation of peptides with molecular weight up to 3.5 kDa obtained a result of the simulated absorption process. The elution profiles are a part of previous study [19]. As a result of this proceeding, a few peaks were detected in each sample and named in the order in which they appear—fractions F1–F8 as you can see in the previous work [19] and the same designations have been used in this work. All obtained fractions were assessed for antioxidant properties as a part of previous study [19] and for inhibitory activity against enzymes related to development of the metabolic syndrome. The numbers of the fractions with the strongest inhibiting properties (one fraction from each hydrolysate), which were used in further analysis and the results of their inhibiting properties are presented in Fig. 2. Majority of the fractions were reduced meaning the increase of activity. Moreover, the thermal treatment of the insects differentiated their inhibition of the analyzed enzymes and baking yielded predominantly better results. The peptide fractions from *Schistocerca gregaria* were characterized by the strongest enzyme inhibitory activity. The highest ACE, lipase, and  $\alpha$ -glucosidase inhibiting effect was obtained for peptide fractions from boiled ( $IC_{50}$  3.95  $\mu\text{g mL}^{-1}$ ), baked ( $IC_{50}$  9.84  $\mu\text{g mL}^{-1}$ ), and raw ( $IC_{50}$  1.89  $\mu\text{g mL}^{-1}$ ) *S. gregaria*, respectively. Wu et al. [6] purified ACE inhibitory peptides of a gastrointestinal protease hydrolysate from silkworm pupa protein. After separation on Sephadex G10, the sample with an  $IC_{50}$  value of 118.27  $\mu\text{g mL}^{-1}$  exhibited similar ACE inhibiting effect to that of our peptide fractions from raw *G. sigillatus* or raw *S. gregaria* ( $IC_{50}$  117.32 and 204.65  $\mu\text{g mL}^{-1}$ , respectively).

To date, the effect of insect-derived peptides on the inhibition of lipase activity has not been studied; however, other natural lipase inhibitors are known. Ercan and El [34] showed a strong lipase inhibiting activity of chick-pea and *Tribulus terrestris* with  $IC_{50}$  values of 9.74 and 15.3  $\mu\text{g mL}^{-1}$ , respectively. These results correspond well with the activities of the peptide fractions in this study, especially those obtained from *T. molitor* and *S. gregaria* with  $IC_{50}$  ranged from 9.84 to 10.67  $\mu\text{g mL}^{-1}$ .

Generally, divided peptide fractions from insect hydrolysates have higher inhibitory activity than hydrolysates obtained as a result of simulated absorption process but with some exceptions for example ACE inhibitory effect of the peptide fractions obtained from *G. sigillatus*. This implies that it can be the result of the synergistic effect of the peptides present in the hydrolysate. Similar effect was also presented in relation to the activity of ACE inhibitory peptides obtained from lentil [35], and tilapia [36]. The whole hydrolysate obtained as a result of simulated absorption process contain peptides of all molecular



**Fig. 2** Inhibitory activity of selected fractions separated by gel filtration on Sephadex G-10. Inhibition of ACE (a), lipase (b), and  $\alpha$ -glucosidase (c). Values with different letters are significantly different ( $p < 0.05$ )

weights < 3.5 kDa, while the fractionates are composed of a limited range of molecular mass peptides. The difference in ACE inhibition between peptide fractions separated on Sephadex G10 and the whole hydrolysates after simulated absorption process could be due to the absence of peptides with high activity in the peptide fractions while they occur in hydrolysates. The activity of bioactive peptides could be higher when present together in the whole hydrolysate [36].

**Table 1** Inhibitory activity of chemically synthesized peptides

Studied species	Treatment	Sequence of peptide	Inhibition of		
			ACE (IC <sub>50</sub> µg mL <sup>-1</sup> )	Lipase (IC <sub>50</sub> µg mL <sup>-1</sup> )	α-Glucosidase (IC <sub>50</sub> µg mL <sup>-1</sup> )
<i>Grylodes sigillatus</i>	Raw	IIAPPER	6.93 ± 0.05 <sup>g</sup>	49.44 ± 0.2 <sup>h</sup>	22.86 ± 0.5 <sup>b</sup>
	Boiled	LAPSTIK	11.14 ± 0.08 <sup>e</sup>	104.95 ± 0.6 <sup>b</sup>	45.60 ± 0.7 <sup>a</sup>
	Baked	VAPEEHPV	18.85 ± 0.05 <sup>b</sup>	100.13 ± 0.1 <sup>c</sup>	n.d.
	Protein	KVEGDLK	3.67 ± 0.02 <sup>i</sup>	115.44 ± 0.3 <sup>a</sup>	18.37 ± 0.4 <sup>d</sup>
<i>Tenebrio molitor</i>	Raw	NYVADGLG	12.09 ± 0.08 <sup>d</sup>	115.59 ± 0.1 <sup>a</sup>	20.37 ± 0.2 <sup>c</sup>
	Boiled	AAAPVAVAK	8.31 ± 0.02 <sup>f</sup>	57.69 ± 0.4 <sup>f</sup>	10.92 ± 0.2 <sup>g</sup>
	Baked	YDDGSYKPH	5.81 ± 0.02 <sup>h</sup>	117.94 ± 0.2 <sup>a</sup>	n.d.
	Protein	AGDDAPR	8.34 ± 0.02 <sup>f</sup>	77.46 ± 0.8 <sup>e</sup>	19.47 ± 0.2 <sup>cd</sup>
<i>Schistocerca gregaria</i>	Raw	GKDAVIV	12.82 ± 0.02 <sup>d</sup>	53.17 ± 0.2 <sup>g</sup>	15.94 ± 0.1 <sup>e</sup>
	Boiled	AIGVGAIER	14.43 ± 0.02 <sup>c</sup>	49.95 ± 0.2 <sup>h</sup>	13.04 ± 0.2 <sup>f</sup>
	Baked	FDPFPK	79.25 ± 0.04 <sup>a</sup>	96.75 ± 0.2 <sup>d</sup>	5.95 ± 0.1 <sup>h</sup>
	Protein	YETGNGIK	3.25 ± 0.09 <sup>i</sup>	94.91 ± 0.1 <sup>d</sup>	n.d.

Means ± standard deviation (SD) in triplicate. Values with different letters in the same column are significantly different ( $p < 0.05$ )

n.d. not detected

## Identification and chemical synthesis of inhibitory peptides

All fractions listed in Fig. 2 were used for further analysis of amino acid sequences. Identification of peptides present in the most effective fractions was carried out and then one unique peptide from each of them was synthesized (Table 1) [19]. The biological activity of peptides, e.g. metabolic syndrome enzymes inhibitory activity, depends on several factors such as absorption, bioavailability in the human body, and chemical structure [12]. The most popular bioactive peptides isolated from insects were ACE and α-glucosidase inhibitory peptides containing from three to ten amino acids and mainly obtained from *B. mori* [8, 9, 26, 37]. In this study, YETGNGIK and KVEGDLK were the most effective ACE inhibitors (IC<sub>50</sub> 3.25 and 3.67 µg mL<sup>-1</sup>, respectively). There is a connection between the structure and activity of ACE peptide inhibitors but the number of amino acids is not the only factor influencing the activity of the peptide. The strongest ACE inhibitors include hydrophobic amino acid residues at the C-terminal [23]. The desirable amino acid is proline, but also aliphatic amino acids e.g. lysine are responsible for the ACE-inhibitory activity [32], which explains the activity of the peptides identified in this study. Wu et al. [6] identified an ACE inhibitory peptide ASL from *B. mori*, Wang et al. [37] identified hexapeptide APPPKK, and Tao et al. [27] identified another peptide GNPWM. Furthermore, even more examples have been reported [2].

The highest lipase inhibitory effect was found for IIAPPER and AIGVGAIER with IC<sub>50</sub> of 49.44 and 49.95 µg mL<sup>-1</sup>, respectively. Analyzing the structure of these peptides, it is important to note that both peptides are

comprised of the same residue Glu-Arg (ER) which could indicate that it might be responsible for this activity. Moreover, the only peptide containing phenylalanine (F) FDPFPK was identified as the strongest α-glucosidase inhibitor (IC<sub>50</sub> 5.95 µg mL<sup>-1</sup>). Lee et al. [8] isolated α-glucosidase inhibitory peptides from silk cocoon hydrolysate. They identified two peptides (GEY and GYG) but their inhibitory activity was much lower than the activity of the peptides presented in our study, as their IC<sub>50</sub> values were 1.5 mg mL<sup>-1</sup> and 2.7 mg mL<sup>-1</sup>, respectively. In turn, Zhang et al. [9], screened α-glucosidase inhibiting peptides from the silkworm protein peptide database and QPGR, SQSPA, QPPT, and NSPR were selected as peptides with the highest α-glucosidase inhibition with IC<sub>50</sub> values in the range from 20 to 560 µM L<sup>-1</sup>.

## Conclusions

It is important to note that studied species of insects, *Grylodes sigillatus*, *Tenebrio molitor*, and *Schistocerca gregaria* are good source of bioactive peptides with in vitro inhibitory activity against selected enzymes that may be involved in the pathogenesis of the metabolic syndrome. Generally, the heat treatment process has a substantial impact on improvement of these properties. The sequences of the most active insect-derived peptides were KVEGDLK, YETGNGIK, AIGVGAIER, IIAPPER, and FDPFPK. It is worth emphasizing that insects, apart from their nutritional value, may potentially have a nutraceutical value and as part of our diet can increase its pro-health potential.

**Acknowledgement** The research was partly financed by the Polish National Science Centre [grant number 2014/15/N/NZ9/04045].

**Funding** This work was partially supported by National Science Centre [grant number 2014/15/N/NZ9/04045].

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Compliance with ethics requirements** This article does not contain any studies with human participants or animals performed by any of the authors.

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