SUMMARY

Properties of proteins and peptides from selected species of edible insects

Entomophagy (from Greek *éntomon* – insect and *phageín* – eat), i.e. the consumption of insects by humans is popular in many countries of the world and it is becoming increasingly known also in Europe. Complete protein is the most important nutrient present in insects. Since natural protein resources may be insufficient due to the rapid population growth in the world, the search for new sources of this ingredient has contributed to the development of interest in insects in Europe. In addition, insects are rich in essential fatty acids and they are a source of vitamins – mainly B vitamins and in smaller amounts E, A, and C vitamins, as well as minerals such as iron, calcium, magnesium, zinc, potassium, phosphorus, or selenium. The consumption of edible insects has become recommended not only because of their high nutritional value, but also because of its beneficial effects on the environment. Breeding insects requires much lower consumption of drinking water and feed than livestock farming, thus emitting significantly less greenhouse gases.

The available literature is dominated by reports devoted to the nutritional value of insects. In recent years, the interest in the study of the biological potential of proteins derived from insects as a source of bioactive peptides has increased, as described in publication **I**. Peptides obtained from insect proteins have antifungal, antibacterial, antioxidant, and antidiabetic properties, but antihypertensive properties have been first and most frequently studied (angiotensin converting enzyme (ACE) inhibitors).

Bioactive food ingredients such as peptides may play an important role in the prevention of increasingly occurring civilization diseases due to their antioxidant and anti-inflammatory activity. The mechanism of action of antioxidant peptides includes neutralization of free radicals or chelation of transition metal ions, which are the substrate of Fenton's reaction. In turn, the anti-inflammatory properties are related to inhibition of lipoxygenase (LOX) and cyclooxygenase-2 (COX-2) activity. The aim of the study was to determine the health properties of peptides obtained during the enzymatic hydrolysis of proteins from selected species of edible insects. The subjects of the study were adult banana crickets (*Gryllodes sigillatus*), adult desert locusts (*Schistocerca gregaria*), and mealworm larvae (*Tenebrio molitor*). In this study, both raw and heat-treated ground insects (cooking and baking) were used as well as protein preparations thereof.

In publications **II–III**, selected species of insects (banana cricket (*Gryllodes sigillatus*), desert locusts (*Schistocerca gregaria*), and mealworm (*Tenebrio molitor*)) and protein preparations thereof were analyzed to determine their nutritional value and cytotoxicity as well as the functional properties. As part of the nutritional value, the calorific value, amino acid profile, fatty acid profile, and mineral composition were determined (**II**). In addition, the effect of insect thermal treatment on the cytotoxicity against human skin fibroblasts of hydrolysates obtained by *in vitro* digestion was evaluated (**II**).

In order to clarify the possibility of using insects in the food industry, the functional properties of proteins present in ground insects and protein preparations thereof were determined, and the assays included protein solubility, water and fat holding capacity, foaming activity and foam stability as well as emulsion activity and stability (**III**). Sensory evaluation of the examined forms of edible insects, i.e. ground and protein preparations, was also performed (**III**).

The research showed that the locust was characterized by the highest content of protein (76% d.w.), with the lowest fat content (12.97% d.w.) and the lowest caloric content (432 kcal/100g). The high protein content was also determined in the cricket (70% d.w.), while the mealworm was characterized by the lowest protein amount (52.35% d.w.) with the highest fat content (24.7% d.w.) (**II**). The *in vitro* studies of insect cytotoxicity allow a conclusion that hydrolysates obtained from raw, cooked, and baked insects are safe for human health except for locusts, in which only thermal treatment guarantees health safety (**II**). Moreover, the banana cricket protein (*Gryllodes sigillatus*) has been found to have good solubility over a wide pH range, and the meal and protein preparation thereof are characterized by the best functional properties of all tested species (**III**). As part of the organoleptic evaluation of protein preparations and meals obtained from the insects, the locust protein preparation was rated the best. The protein preparations exhibited better functional properties and were better rated in organoleptic evaluation than the insect meal, which makes them more useful from the point of view of food technology (**III**).

In the presented publications **IV–V**, an attempt was made to assess insect proteins as precursors of antioxidant peptides, taking into account the influence of insect thermal treatment. For this purpose, the analyzed species of edible insects have been subjected to cooking and baking, and protein preparations thereof were prepared. Both protein preparations as well as boiled, baked, and raw insects were digested under simulating gastrointestinal conditions and then *in vitro* absorbed using a semi-permeable membrane with

a 3.5 kDa cut-off. In the obtained hydrolysates and peptide fractions <3.5kDa, antioxidant properties including the ability to neutralize free radicals generated from ABTS and DPPH, the ability to chelate Fe^{2+} ions, the Fe^{3+} ion reduction power, and anti-inflammatory properties expressed as the ability to inhibit lipoxygenase and cyclooxygenase-2 activity were determined (**IV**). In the next stage of the research, separation of peptides with a molecular mass <3.5 kDa using chromatographic methods was carried out. Subsequently, the antioxidant properties of the fractions obtained in the chromatographic separation were determined. The fractions with the highest antioxidant properties were analyzed using mass spectrometry, which allowed determining the molecular weights and amino acid sequence of the peptides. Based on the analysis of the mass spectra of the obtained peptides, one unique peptide of each insect variant (raw, boiled, baked, and protein preparation) was selected and then chemically synthesized to unambiguously confirm which of the peptides is responsible for specific biological activity. In the last stage of the study, antioxidant and anti-inflammatory properties of chemically synthesized peptides were determined in order to confirm that the physiologically active peptides are responsible for the activity of the insect protein hydrolysates (**V**).

The conducted research indicated that the hydrolysates and peptide fractions obtained from insect protein preparations were characterized by the best antiradical and LOX and COX-2 inhibitory activity, while the weakest activity was detected in the raw insects. In the vast majority of the analyzed cases, the thermal treatment of the insects resulted in improved properties and better results were obtained in the baking treatment (**IV**–**V**).

The peptide fractions consisting of a mixture of peptides have higher antioxidant and anti-inflammatory activity than the individual synthetic peptides, and among the synthetically obtained peptides with the same amino acid sequence as the identified peptides derived from the proteins of the analysed insects, the peptide with the FDPFPK sequence has the best antioxidant and anti-inflammatory properties (**V**).

References:

(I) **Zielińska E.**, Karaś M., Jakubczyk A., Zieliński D., Baraniak B. (2018) Edible insects as source of proteins. [W]: Red. Mérillon JM., & Ramawat K. *Bioactive Molecules in Food*. Reference Series in Phytochemistry. Wyd. Springer.

(II) **Zielińska E.**, Baraniak B., Karaś M., Rybczyńska K. & Jakubczyk A., 2015, Selected species of edible insects as a source of nutrient composition, *Food Research International*, 77, 460-466.

(III) **Zielińska E.,** Karaś M., Baraniak B. (2018) Comparison of functional properties of edible insects and protein preparations thereof. *LWT – Food Science and Technology, 91*, 168-174.

(IV) **Zielińska E.**, Baraniak B., Karaś M., 2017, Antioxidant and anti-inflammatory activities of hydrolysates and peptide fractions obtained by enzymatic hydrolysis of selected heat-treated edible insects. *Nutrients*, 9(9), 970.

(V) **Zielińska E.**, Baraniak B., Karaś M., 2018, Identification of antioxidant and anti-inflammatory peptides obtained by simulated gastrointestinal digestion of three edible insects species (*Gryllodes sigillatus*, *Tenebrio molitor*, *Schistocerca gregaria*). *International Journal of Food Science and Technology*, doi:10.1111/ijfs.13848.