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ORIGINAL RESEARCH PAPER

THE PHYSICOCHEMICAL PROPERTIES OF A COMMERCIAL CRICKET (*ACHETA DOMESTICUS*) PROTEIN POWDER AS A BASIS FOR ITS USE IN FOOD PRODUCTS

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Abstract: The present work investigates the stability over time and the possibility of using commercial cricket (*Acheta domesticus*) protein powder as an ingredient in various food products. The results indicated that the protein powder is hygroscopic, but it can be kept for 7 months in a package, even if it is unsealed. After one week of storage in an open package, in contact with atmospheric air, the water activity increases from 0.150 to 0.514 and the moisture value increased almost three times. Differential Scanning Calorimetry (DSC) analysis showed that the proteins are completely denatured. The values for water holding capacity increase as the *p*H of the environment increases between 6 and 8, and the swelling index has higher values for *p*H between 4 and 6. In the case of liquid products, commercial cricket (*Acheta domesticus*) protein powder can be add if the liquids have an ionic strength as low as possible and a *p*H around 4.

Keywords: *additives, commercial, DSC, food, hygroscopicity, protein, swelling index, water holding capacity*

INTRODUCTION

In recent times, the use of insects in certain food products has become an interest for large organizations that analyze the safety of insect consumption, researchers, and manufacturers. Although, Europeans are still skeptical about eating insects, but businesses in this field are starting to develop in ever greater numbers [1].
European Food Safety Authority (EFSA) concluded that frozen, dried, or powdered *Acheta domesticus* can be added to foodstuffs. From a nutritional point of view, it mainly contains proteins, lipids, fibers (especially chitin which is an allergen), inorganic substances and water depending on the way the insects are processed [2]. The proteins are of good quality with most of the essential amino acids above the values recommended by FAO, while their digestibility is lower than that of casein [3, 4]. Stone *et al*. found a value of 76.2 % for protein digestibility *in vitro* [5].
To improve the sensory and nutritional properties, various processing methods are used such as: steaming, roasting, smoking, frying [6]. Thermal treatments aim at microbial reduction and lead to biochemical changes such as: modification of protein structure, their denaturation and interaction with lipids or carbohydrates [7, 8].
Currently, there are articles presenting nutritional aspects of the use of insects in various food products. There are fewer articles that study physicochemical aspects of the possible use of insects, and in these cases the analyses started directly from the insects. In the present study, the research was done on a commercial product and aims to establish the physicochemical characteristics of food products in which commercially cricket protein powder can be used.

MATERIALS AND METHODS

Materials

The domestic cricket (*Acheta domesticus*) protein powder used in the analysis was purchased from Sens Foods (Czech Republic). The nutritional value of the powder can be found in Table 1.

Table 1. *Nutritional value of protein powder*

Nutritional values	**per 100 g**
Declared energy value [kJ/kcal]	1939 / 463
Fats [g]	20
- from which saturated fats [g]	5.2
Carbohydrates [g]	0.5
- from which sugars [g]	0
Fiber [g]	9.5
Protein [g]	70
Iron [mg]	5.67
Vitamin B12 [µg]	5.76

To study the influence of ionic strength (0, 1, 2, 3, 4) and *p*H (2, 4, 6, 8, 12) in the environment, KCl solutions, respectively acetic acid-sodium acetate buffer solutions were used.

Differential Scanning Calorimetry analysis

To study the degree of protein denaturation in the commercial sample, DSC analysis was used using Thermogravimetric Analyzer (TGA) & Differential Scanning Calorimeter (DSC) SDT Q600 (TA Instruments, USA) calibrated with zinc and indium. For the analysis, 5 ± 0.5 mg of protein powder, located in a hermetically sealed cup, was heated up to 120 °C at a rate of 5 °C·min^{-1}. The DSC curve and its derivative were obtained using TA Universal Analysis software.

Hygroscopicity

Hygroscopicity was expressed as g of water gained per 100 g dry solids [9]. This was determined for three different situations when samples were maintained: seven days in the desiccator (as control), seven days in the air, and seven months in unsealed original packaging. The blank sample was made by keeping 2 g of protein powder for seven days in a desiccator, at an air temperature of 22 °C and an air humidity of 56 % in a crucible with a lid and with a diameter of 5 cm. At the same time 2 g of protein powder was kept for seven days in air at room temperature in a crucible with the same diameter but without a lid. The last sample was also kept at room temperature, because the instructions on the package allowed it, and the seven-month period was approaching its expiration date.

Water activity and water content

Water activity (aw) of the cricket protein powder was determined by the Novasina LabMASTER (Switzerland). Water content was determined by the infrared drying (A&D, Japan) method at 103 °C until constant weight [10].

Water holding capacity

To determine the water holding capacity, the method described by Igual *et al.* was used with some modifications [11]. Briefly, 2.5 g of insect powder were weighed, over which 25 g of distilled water was added in the case of the control sample or KCl solutions with different ionic strength (1, 2, 3, 4), respectively with different *p*H (2, 4, 6, 8, 12). The samples were stirred using Vortex Mixer "MIXTUB-P" (RAYPA) for 30 min and centrifuged (Nüve NF 1200R, Turkey) for 20 min at 6000 rpm, at 4 °C, for a better separation of the supernatant from sediment. After centrifugation, the supernatant was separated from the sediment. Water holding capacity is calculated with equation (1).

$$WHC = \frac{V_a - V_d}{m} \quad \textbf{(1)}$$

where:
WHC - water holding capacity [mL·g^{-1}];
V_a - the volume of the added solution [mL];
V_d - the volume of the decanted [mL];
m - mass of the sample [g].

Determination of the swelling index

The swelling index was measured by the volume increase method described by Robertson *et al.* [12]. One gram of insect powder was weighed, transferred to a graded cylinder over which was added 10 mL of distilled water or solutions of known concentrations of KCl and solutions of variable *p*H. Samples were left for 18 h at 4 °C. Results were expressed as mL swollen/g dry solid.

Determination of soluble protein

Soluble protein was determined by the biuret method [13].

Statistical analysis

All analyses were made in triplicate. The results were presented as mean and the standard deviations are indicated on the graphs. One-way analysis of variance (ANOVA) was used to evaluate the results.

RESULTS AND DISCUSSION

DSC analysis

Proteins are biopolymers with a specific three-dimensional structure that is critical for their biological function. The physicochemical properties of native proteins are different from those of denatured proteins. DSC analysis allows the characterization of the thermal properties of proteins. For these reasons the DSC analysis was done. The process of thermal denaturation of proteins is an endothermic phenomenon, and if unfolded conformers aggregate, then an exothermic process is observed [14, 15]. Examining the DSC curves and its derivative, as shown in Figure 1, no thermal phenomenon could be observed, indicating that the proteins in the commercial powder are irreversibly denatured.

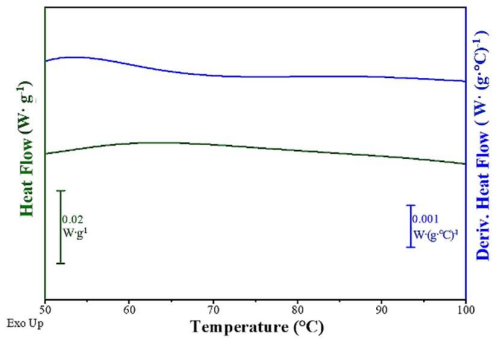

Figure 1. *The DSC curve and its derivative obtained for commercial cricket flour*

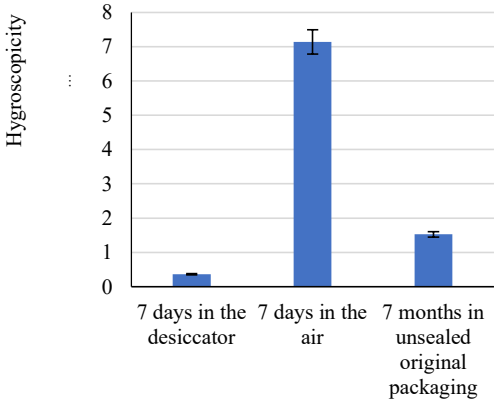

Figure 2. *Hygroscopicity expressed as g of water gained per 100 g dry solids in three storage situations*

Hygroscopicity

The hygroscopicity of a powder refers to its ability to attract and absorb moisture from the surrounding environment. It is a measure of the powder's affinity for water and its tendency to undergo changes in moisture content. The obtained results are presented in the Figure 2.

The sample kept in the unsealed package gained 1.53 g water/100 g dry matter in the 7-month period, while the sample kept in atmospheric air for 7 days gained water the most, reaching 7.14 g water/100g dry matter. Cai and Corke and later Murikipudi *et al.* showed that according to hygroscopicity the products can be classified into three groups. In the case of a water gain below 2 %, the product is slightly hygroscopic, for the range 2 - 15 % the products are hygroscopic and above 15 % are considered very hygroscopic [9, 16].

From the obtained results it is obvious that cricket protein powder is hygroscopic and attention must be paid to the manner in which the storage is done and time of storage because it adsorbs water from air humidity.

The hygroscopicity of a powder is influenced by several factors, including its chemical composition and their hygroscopic effect [17, 18]. Xue *et al.* showed that the interaction with water molecules depends on the amino acid side groups. Thus, for example carboxyl groups interact more strongly with water molecules and have stronger hydrophilicity compared to methyl groups [19].

Water activity and water content

The preservation of the protein powder also depends on the water activity. At the time of opening the package, the water activity for the protein powder was 0.15 and after 7 days of storage in the desiccator it increased to 0.186. This difference can be explained by the adsorption of air moisture during handling. A water activity value of 0.514, much higher, was obtained in the case of the sample maintained for 7 days in atmospheric air. This high value can be explained by the fact that the protein from crickets (*Acheta domesticus*) contains many hydrophilic groups.

However, the development of microorganisms does not occur at the recorded values, but there are numerous studies that show that pathogenic bacteria can survive on food products that have low water content [20]. For example, *Salmonella* and *L. monocytogenes* survive in food products with a low water content stored at 16 °C. The resistance of pathogens also depends on the composition of the food product, and in high-protein matrices *Salmonella* resists better than in high-fat matrices [21, 22]. On the other hand, most commercial products contain edible insects that have been blanched to reduce the microbial load [6].

From the obtained results it can be stated that the cricket protein powder, especially since it was initially thermally treated, is safe from a microbiological point of view during storage even if it accidentally takes moisture from the air.

Water activity is influenced by the presence of free water in the food product, while its moisture depends on free and bound water. Models of sorption isotherms for different materials show that there is not always a linear variation between moisture content and water activity [23, 24]. Figure 3 shows the results obtained for moisture percentage in

three storage situations compared to the first day. The moisture percentage variation is similar to the water activity variation, the correlation coefficient being 0.99.

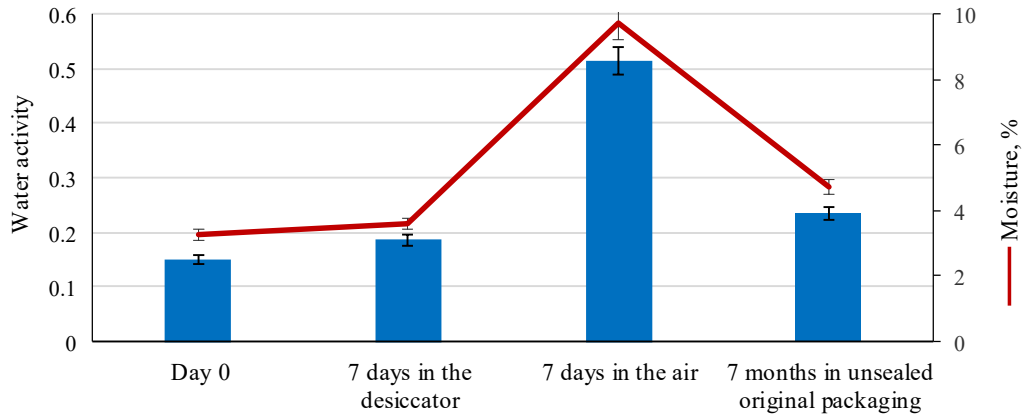

Figure 3. *Water activity and moisture percentage in three storage situations compared to the first day*

The influence of ionic strength and *p*H of the environment on the water holding capacity

Water holding capacity is important in certain branches of the food industry, such as in the bakery or meat industry, as it is necessary for the own and/or added water to be maintained in the product during processing [25]. Water holding capacity is negatively influenced by the increase in fat concentration (20 % for the cricket flour taken in the analysis) and positively by the presence of proteins in large quantity (70 % in this case). In turn, proteins can influence water holding capacity by changing the ionic strength of the medium or its *p*H [26].
The obtained results are presented in Figure 4.

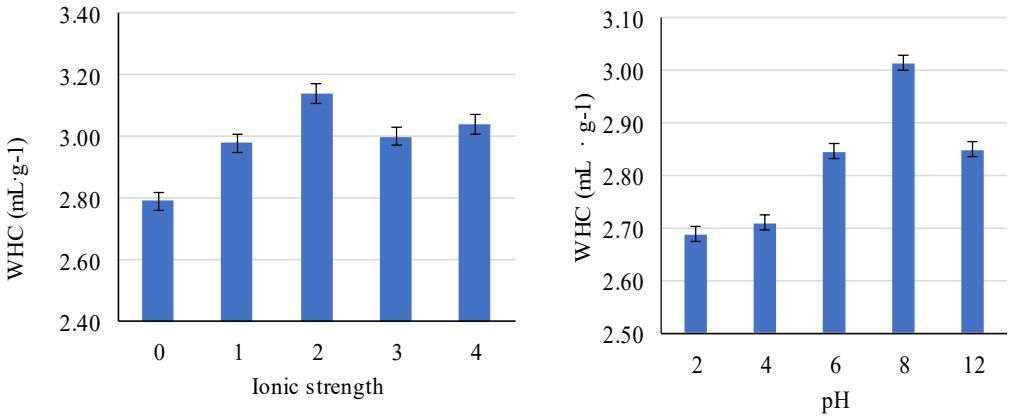

Figure 4*. The influence of ionic strength and pH of the environment on the water holding capacity (WHC)*

Analyzing the results from the Figure 4, it can be seen that the highest values for water holding capacity were obtained at an ionic strength equal to 2 and at *p*H 8. The minimum values were reached at an ionic strength equal to 0 and *p*H 2. The water holding capacity values varied between 2.79 $mL \cdot g^{-1}$ and 3.14 $mL \cdot g^{-1}$ in the case of solutions with different ionic strength, respectively between 2.69 and 3.01 for media with different *p*H. Similar results have been reported by other authors. Ndiritu *et al.*, for proteins extracted from crickets (*Acheta domesticus*) by different methods, obtained values of the water retention capacity between 2.03 $mL \cdot g^{-1}$ and 2.74 $mL \cdot g^{-1}$ depending on the method of protein extraction [27], while Lawal, for African locust bean (*Parkia biglobossa*) protein isolate, obtained values between 1.8 and 3.7 $mL \cdot g^{-1}$ [28]. These authors used sodium chloride solutions. The potassium ion, used in this research, has a greater penetrating capacity than the sodium ion, and for this reason, for the same ionic strength, the water holding capacity values may differ. Also, the use of KCl can have the effect of changing the *p*H of the analyzed sample [29].

Swelling index

The matrix of some foods can absorb or retain water molecules, leading to an increase in volume. This phenomenon can have a significant impact on various aspects of food processing and the final product.

In the case of proteins, their swelling depends on their affinity for water, which in turn depends on the presence of groups that can interact with water, but also on environmental conditions [30]. The results obtained in the case of changing the ionic strength and *p*H are shown in the Figure 5.

For different values of the ionic strength, the swelling index was between 3.5 and 4.5 mL swollen/g dry solid, the differences being significant ($p < 0.05$). When the *p*H changed, the swelling index varied between 4.0 and 4.96 mL swollen/g dry solid, but this time no significant differences were recorded ($p > 0.05$). Also, in both cases, no significantly different values were obtained after 18 hours.

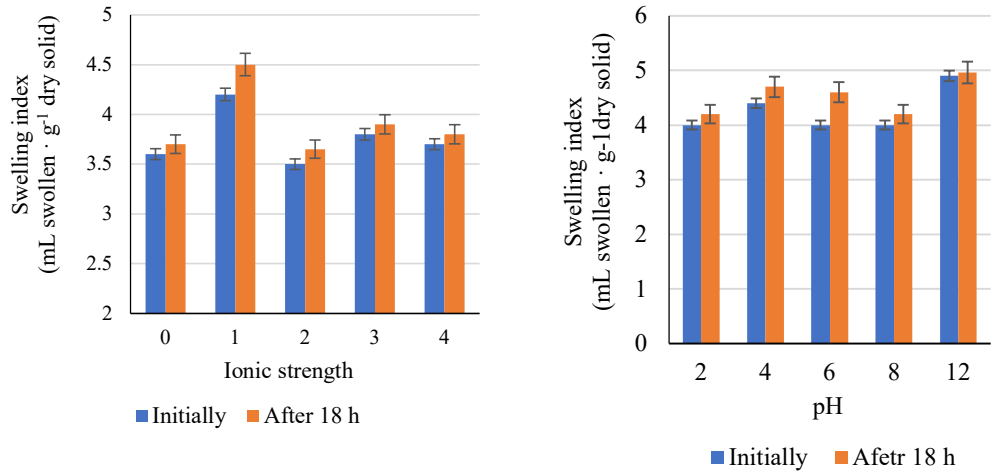

***Figure 5**. The influence of ionic strength and pH of the environment on swelling index*

In general, foods with a high swelling index absorb more water and increase their volume. These changes are sometimes desired and various additions are used to achieve this goal. This is why several authors have studied the swelling index in the case of various mixtures containing different percentages of protein flour. Ehoche *et al.* studied corn flour supplemented with cricket (*Acheta gossypii*) flour and obtained the highest swelling index (g/g) at a 10 % addition [31]. Igual *et al*. studied the addition of *Acheta domesticus* flour to extruded products and observed significant differences for mixtures with 5 - 10 % protein flour [11].

The influence of ionic strength and *p*H of the environment on the protein solubility

The solubility of proteins depends on their structure and the presence of hydrophilic groups on the protein surface. They are dependent on the ionic strength and *p*H of the medium.

From the results presented in Figure 6, it can be seen that the solubility decreases from 9.62 to 3.01 mg·mL^{-1} by increasing the ionic strength from 0 to 3. On the other hand, a high solubility was obtained for *p*H 4 and 12.

Kim *et al.* studied a freeze-dried cricket meal and observed a maximum solubility for a 1.4 M NaCl solution and *p*H 10. They concluded that *p*H 4 represents the majority of proteins, while Hall *et al.* found for cricket protein from the *Gryllidae* family the isoelectric *p*H was 3 [25, 32]. The same authors showed that by enzymatic hydrolysis of proteins there is an increase in repulsive interactions between peptide chains and thus new hydrogen bonds with water molecules can appear [32]. Quinteros *et al.* using freeze-dried and then defatted cricket flour (*Gryllus assimilis*), showed that the best solubility is obtained in an acidic and especially basic environment (*p*H 10.0 and *p*H 12.0) [33]. David-Birman *et al.* showed that by cooking or baking the solubility of proteins depending on *p*H is different compared to untreated flour. At *p*H 4 they obtained for the cooked sample a much better solubility than in the control sample, while for the baked sample the solubility is somewhat lower. Previously it was shown that water - soluble from *Tenebrio molitor* have a much better digestibility than water-insoluble [34, 35].

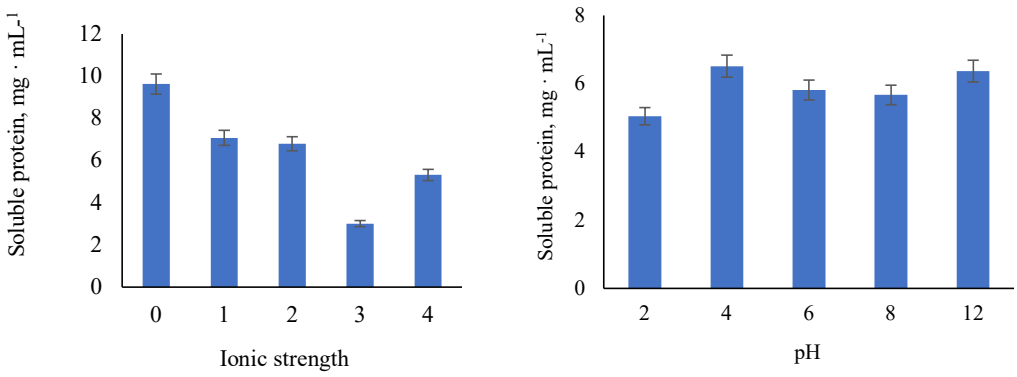

Figure 6. *The influence of ionic strength and pH of the environment on protein solubility in cricket powder*

CONCLUSIONS

The results obtained showed that the commercial cricket powder can be stored for 7 months in the original closed packaging, even if it has been unsealed. During this period, the water activity increased up to 0.235, a value that does not raise problems from a microbiological point of view. In the case of storage in open packages, in contact with atmospheric air, the water activity increased to 0.514 in 7 days, which proves that the powder is hygroscopic.

In contrast to other researches, in the present work, the cricket protein in the commercial powder was denatured, as revealed by DSC analysis.

In the case of adding this commercial cricket (*Acheta domesticus*) protein powder as an ingredient in food products where the water retention capacity is of interest, it would be indicated that they have an ionic strength equal to 2 and a neutral or even slightly basic *p*H. For the swelling index, the highest value was obtained for an ionic strength equal to 1 and an environment with *p*H 4.

If commercial protein powder is added to liquid products, for the best possible solubility of the added proteins, the liquid product should have as low an ionic strength as possible and a *p*H of 4.

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