

Accepted Manuscript

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PII: S0308-8146(13)00721-8
DOI: <http://dx.doi.org/10.1016/j.foodchem.2013.05.115>
Reference: FOCH 14162

To appear in: *Food Chemistry*

Received Date: 31 December 2012
Revised Date: 18 April 2013
Accepted Date: 23 May 2013

Please cite this article as: Yi, L., M. Lakemond, C.M., C. Sagis, L.M., Eisner-Schadler, V., Huis, A.v., J. S. van Boekel, M.A., Extraction and characterization of protein fractions from five insect species, *Food Chemistry* (2013), doi: <http://dx.doi.org/10.1016/j.foodchem.2013.05.115>

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Extraction and characterization of protein fractions from five insect species

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ABSTRACT

Tenebrio molitor, *Zophobas morio*, *Alphitobius diaperinus*, *Acheta domesticus* and *Blaptica dubia* were evaluated for their potential as a future protein source. Crude protein content ranged from 19 - 22 % (Dumas analysis). Essential amino acid levels in all insect species were comparable with soybean proteins, but lower than for casein. After aqueous extraction, next to a fat fraction, a supernatant, pellet, and residue were obtained, containing 17 – 23 %,

25 33 – 39 %, 31 – 47 % of total protein, respectively. At 3 % (w/v), supernatant fractions did
26 not form stable foams and gels at pH 3, 5, 7, and 10, except for gelation for *A. domesticus* at
27 pH 7. At 30 % w/v, gels at pH 7 and pH 10 were formed, but not at pH 3 and pH 5. In
28 conclusion, the insect species studied have potential to be used in foods due to: 1) absolute
29 protein levels; 2) protein quality; 3) ability to form gels.

31 Keywords

32 Insect protein; Protein extraction; Protein characterization; Foaming; Gelation.

33

34 1. Introduction

35 1.1. Insects as a source of food

36 In most developed countries, human consumption of insects is infrequent, or even culturally
37 inappropriate, although its nutritional value is comparable to conventional meat (van Huis,
38 2013). In many regions and countries of the world, insects form part of the human diet and it
39 is a misconception to believe that this is prompted by starvation (van Huis, 2013). About
40 1900 insect species are consumed globally as human food in the world
41 (<http://www.ent.wur.nl/UK/Edible+insects/Worldwide+species+list/>).

42 With an increase in the world population, increased consumer demand for protein, and the
43 amount of available agricultural land being constrained, the sustainable production of meat
44 will represent a serious challenge for the future. Insects can be considered as an alternative
45 protein source with less environmental impact (van Huis, 2013). Insects can be consumed as
46 a whole. However, they can also be processed in less recognizable forms, which may increase
47 consumer acceptability. Insects are already used as natural food ingredients, *e.g.* the red
48 colorant carmine (E120) used in yogurt is an extract of the female cochineal insect.

49 1.2. Edible insects

50 Insects are consumed in different life stages like eggs, larvae, pupae or adults. The main
51 species consumed are, in order of importance: beetles (Coleoptera); caterpillars (Lepidoptera);
52 ants, bees and wasps (Hymenoptera); grasshoppers and locusts (Orthoptera); true bugs,
53 aphids and leafhoppers (Hemiptera); termites (Isoptera) and flies (Diptera) and some others.
54 Lepidoptera, Coleoptera, and Diptera (including flies) are commonly consumed in the larval
55 stage; while the Orthoptera, Hymenoptera, Hemiptera and Isoptera are mainly consumed in
56 the adult stage.

57 Cultivating edible insects for food consumption has several advantages: 1) Insects have a
58 high feed conversion efficiency compared with conventional livestock. For example, the feed
59 conversion ratio of house cricket (*Acheta domesticus*) can be calculated twice as efficient as
60 chickens, almost 4 times more efficient than pigs and over 12 times more than cattle (van
61 Huis, 2013); 2) Cultivating insects for protein has less environmental impact than cattle
62 ranching, due to the lower production of greenhouse gas and NH₃ emissions (van Huis, 2013);
63 3) Besides the higher production yield and less environmental impact, insect feeds can be
64 obtained from a wider range of plants than that of conventional livestock, such as cattle or
65 swine (Durst & Shono, 2010). Overall, insect farming can be introduced in terms of a
66 sustainable form of agriculture.

67 1.3. Proteins of edible insects

68 As a food source, insects are potentially nutritious, rich in protein and fat, and providing a
69 certain amount of minerals and vitamins. Studies on protein quality, nutritional value, protein
70 content, and the amino acid composition of various insects are available (Ladrón de Guevara,
71 Padilla, García, Pino, & Ramos-Elorduy, 1995); (Renault, Bouchereau, Delettre, Hervant, &
72 Vernon, 2006); (Barker, Fitzpatrick, & Dierenfeld, 1998). The protein content of common

73 edible insects was around 9 – 25 % (Finke & Winn, 2004), and the Yellow mealworm beetle
74 larvae (24 %) (Ghaly & Alkoaik, 2009), *Zophobas morio* larvae (19 %) (Finke, 2002), and
75 *Acheta domesticus* adult (19 %) (Finke & Winn, 2004), conventional meat protein sources
76 contain about 15 to 22 % protein (Ghaly & Alkoaik, 2009). In addition, some insects have not
77 only protein content comparable to meat, but also to plant protein (up to 36.5 %).

78 People may consume insect food more easily when unrecognizable insect protein (extract) is
79 incorporated in food in comparison to consuming whole insects. (Del Valle, Mena, &
80 Bourges, 1982) also indicated that the extraction of proteins from insects for further use in
81 food products is particularly relevant for countries that do not have the habit of consuming
82 insects, such as Europe and North America.

83 In this study, there are five insect species selected based on their availability (species reared
84 by companies in the Netherlands): three species of Coleoptera considered edible, including
85 the Yellow mealworm (*Tenebrio molitor*), the Superworm (*Zophobas morio*), the Lesser
86 mealworm (*Alphitobius diaperinus*) and one species of Orthoptera; the House cricket (*Acheta*
87 *domesticus*) considered edible and one of the Blattodea; the Dubia cockroach (*Blattella germanica*)
88 not edible, but can be reared in large numbers and used for animal feed.

89 1.4. Objective

90 Although researchers from entomological and zoo-biology science have studied intact edible
91 insects, still very little information from a food science point of view is available on
92 characteristics and functionality of extracted insect proteins.

93 The aim of this study was to investigate if insects could be used as a future protein source in
94 food. Therefore, insect protein characteristics and functionality were determined and
95 evaluated for each of the five insect species. The specific objectives of this study were to: (a)
96 extract proteins and characterize obtained fractions; (b) evaluate protein purity and yield of

97 the obtained fractions; (c) establish some functional properties of the protein fractions
98 focused on foaming and gelation; (d) study protein quality by analysis of protein content and
99 amino acid composition.

100 2. Materials and methods

101 2.1. Insects used

102 *Tenebrio molitor*, *Z. morio*, *A. diaperinus*, *A. domesticus* and *B. dubia* were purchased from
103 the commercial supplier Kreca V.O.F, Ermelo, the Netherlands. *Tenebrio molitor*, *Z. morio*,
104 *A. diaperinus* species were supplied in the larvae stage, *A. domesticus* and *B. dubia* in the
105 adult stage. The feed for *T. molitor*, and *Z. morio* mainly consisted of wheat, wheat bran, oats,
106 soy, rye, corn, carrot and beer yeast. The feed for *A. diaperinus*, *A. domesticus* and *B. dubia*
107 mainly consisted of carrot and chicken mash obtained from Kreca V.O.F. All insects were
108 sieved to get rid of feed and stored alive at 4 °C for about one day before processing.

109 2.2. Analysis of water content, protein, and fat content

110 All fresh insects were frozen using liquid nitrogen and subsequently grinded using a blender
111 (Braun Multiquick 5 (600 Watt), Kronberg, Germany). Frozen grinded insects were freeze-
112 dried (GRI Vriesdroger, GR Instruments B.V., Wijk bij Duurstede, the Netherlands) to
113 determine moisture and dry matter content. The freeze-drying process was stopped at a stable
114 sample weight. Next, the freeze-dried insects were used for protein content analysis. Crude
115 protein content was determined by Dumas (Thermo Quest NA 2100 Nitrogen and Protein
116 Analyser, Interscience, Breda, the Netherlands) using a protein-to-nitrogen conversion factor
117 of 6.25. D-methionine (Sigma, CAS nr. 348-67-4) was used as a standard. Furthermore, fat
118 content was determined after hexane extraction (Biosolve, CAS nr. 110-54-3) in a Soxhlet
119 apparatus for 6 hours. Afterwards, hexane was removed using a Rotary evaporator (R420,

120 Buchi, Switzerland). Defatted insect meal was stored at - 20 °C. All experiments were
 121 performed in two duplications of the same sample.

122 2.3. Determination of amino acid composition and protein quality

123 Amino acid composition of freeze-dried insect powder was analysed using ion exchange
 124 chromatography, following the International standard ISO 13903:2005. Tryptophan was
 125 determined by reversed phase C₁₈ HPLC using fluorescence detection at 280 nm, according to
 126 the procedure described by International standard ISO 13904:2005. The amino acid
 127 composition of the five insect species was compared to literature data of soybean protein and
 128 casein, representing high quality proteins among vegetable and animal proteins (Sosulski &
 129 Imafidon, 1990; Young & Pellett, 1994). Protein quality was evaluated by the essential amino
 130 acid index (EAAI), which is based on the content of all essential amino acids compared to a
 131 reference protein, being values for human requirements in this case (Smith & Nielsen, 2010).
 132 EAAI gives an estimate on the potential of using insects as a protein source for human
 133 consumption without correcting for protein digestibility (Eq.1).

$$134 \text{ EAAI} = \sqrt[9]{\left(\frac{\text{mg of lysine in 1 g of test protein}}{\text{mg of lysine in 1 g reference protein}} \right) \times (\text{etc. for the other 8 essential amino acids})}$$

135 2.4. Protein extraction procedure

136 For protein extraction, 400 g of N₂-frozen insects was used. After adding 1200 ml
 137 demineralized water, that was mixed with 2 g ascorbic acid beforehand, blending for one
 138 minute took place (Braun Multiquick 5 (600 Watt), Kronberg, Germany). Then the obtained
 139 insect suspension was sieved through a stainless steel filter sieve with a pore size of 500 µm.
 140 The filtrates and residues were collected. After centrifugation at 15,000 g for 30 min at 4 °C,
 141 three fractions were obtained from the filtrate: the supernatant, the pellet, and the fat fraction.

142 The residue, the pellet and the supernatant fractions were freeze dried for further analysis.
143 The freeze-dried supernatant and pellet fractions of all insect species studied were
144 characterized in terms of colour, protein content and molecular weight distribution using
145 SDS-PAGE. The extraction procedure was performed in duplicate starting twice with a new
146 insect batch.

147 2.5. *SDS-PAGE*

148 Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to
149 determine the molecular weight distribution of the insect protein fractions. For the detection
150 of the supernatant, pellet and residue fractions, 12.5 % acrylamide Phastgels (15 kDa to 250
151 kDa) and 20 % acrylamide Phastgels (2 kDa to 150 kDa) (GE Healthcare Bio-Sciences AB,
152 Uppsala, Sweden) were used. The applied markers were ordered from SigmaMarker (S8445,
153 wide range, molecular weight 6.5 - 200 kDa SigmaMarker). The samples were dissolved in
154 20 mM Tris/HCl, 2 mM EDTA pH 8.0 buffers with protein concentration of 7 mg/ml and
155 placed in an ultrasonic bath for 10 min. The protein concentration of the samples was
156 calculated based on protein content (Dumas) and amount of dry matter. Next, protein
157 solutions were diluted with ratio 1:1 in a sample buffer, containing 20 mM Tris/HCl, 2 mM
158 EDTA pH 8.0 (Across Organics, Cas nr. 6381-92-6), 5 % (w/v) SDS (Sigma, Cas nr. 152-21-
159 3), 0.016 % (w/v) DTT (DL- Dithiothreitol, Sigma, Cas nr. 3483-12-4), 0.02 % Bromophenol
160 Blue (Merck, Cas nr. 115-39-9). Afterwards, the samples were heated at 100 °C for 5 min and
161 centrifuged for 2 min at 10,000 rpm before applying to the gel.

162 2.6. *Foamability and foam stability*

163 The stability of foam stabilized by insect supernatant protein was determined using foam
164 tubes with a diameter of 2.0 cm, and a glass grid at the bottom (Deak, Murphy & Johnson,
165 2007). The tubes were filled with 20 ml supernatant solution with a concentration of 3 % w/v,

166 at pH 3, 5, 7, and 10. The solutions were aerated from below with nitrogen gas, at a flow rate
167 of 10.0 ml/min. Some of the samples had insufficient foamability to form stable foam at these
168 concentrations. For those samples with sufficient foamability, the samples were aerated until
169 the foam level reached 30 cm. After stopping the flow of gas, the height of the foam was
170 determined as a function of time. From these curves, the half-time of the foam (the time in
171 which foam height is reduced by 50 %) was determined. All tests were performed in
172 duplicate.

173 2.7. *Gel formation*

174 2.7.1. *Visual observation of gelation*

175 Insect supernatant solutions were heated in a water bath (86 ± 1 °C) for 10, 20 and 30 min.
176 The supernatant fractions were dissolved at concentrations of 3 % w/v and 30 % w/v at pH 3,
177 5, 7 and 10. Depending on the initial pH, the final pH was adjusted by slowly adding 1 and 5
178 M HCl/ NaOH solutions. Gel formation was determined through visual observation. If the
179 liquid was not moving upon turning the tube, it was considered a gel. This method was
180 previously used by [\(Beveridge, Jones, & Tung, 1984\)](#) for albumin gel formation. Experiments
181 were performed in duplicate.

182 2.7.2. *Strain sweeps*

183 Freeze-dried supernatant fractions from five insect species were used for this experiment.
184 Protein solutions were prepared as followed: freeze-dried supernatant fractions were
185 dissolved in demineralised water at a concentration of 15 % w/v, stirred for 30 minutes at
186 room temperature and adjusted to pH 7 using 1 M NaOH.

187 To determine the rheological properties of the supernatant protein solutions and gels made
188 from them, oscillatory strain tests were performed on a stress-controlled rheometer (Physica

189 MCR 501, Anton Paar, Graz, Austria) with stainless steel and titanium CC-10 concentric
190 cylinder geometry (diameter inner cylinder: 9.997 mm; diameter cup: 10.845 mm). After
191 filling the geometry with supernatant solution, all samples were covered with a thin layer of
192 silicone oil to prevent sample evaporation. Samples were first heated from 20 to 90 °C at a
193 heating rate of 1 °C/min (phase 1), kept at 90 °C for 5 min (phase 2), and cooled to 20 °C at a
194 rate of 3 °C/min (phase 3). During the temperature ramp, the storage modulus G' and loss
195 modulus G'' were determined by applying oscillatory deformations with a strain amplitude of
196 0.005 and a frequency of 0.1 Hz. The point at which G' started to increase and became
197 greater than the background noise, was designated as the gelation temperature (Renkema,
198 Knabben, & van Vliet, 2001).

199 After formation of the gel, an oscillatory strain sweep was performed on the samples, with
200 strains ranging from 10^{-4} to 10, and a frequency of 0.1 Hz. Strain sweeps were also performed
201 to confirm whether this strain was in the linear response regime. All samples were tested at a
202 supernatant fraction concentration of 15 % (protein content of around 8 % for five types of
203 insects) w/v. *Tenerio molitor* was also tested at concentrations of 7 % (protein content of
204 4.1 %), and 30 % (protein content of 16.6 %) w/v. Values for G' for this fraction from the
205 linear response regime were plotted against protein concentration C , and the exponent n , in
206 the relation $G' \sim C^n$, was determined using linear regression to obtain information on the
207 structure of the gels. For all fractions the maximum linear strain, where G' starts to decrease
208 as a function of increasing strain, was also determined. This was done by separately fitting
209 the data points in the linear region and the fully nonlinear region, and extrapolating both
210 curves to their point of intersection (see Figure 2C). This method of determining the
211 maximum linear strain is only approximate, but since we are not interested in the absolute
212 value of this strain, but rather in the differences in this strain for the various protein samples,

213 this approximation was considered sufficiently accurate. All tests were performed in
214 duplicate.

215 3. Results and discussion

216 3.1. Chemical composition of five insect species

217 The proximate composition of five insect species with regard to moisture, fat, protein was
218 determined on live weight basis (Table 1). The moisture content of the five insect species
219 ranged from 60 % to 71 %, fat content ranged from 3.6 % to 16 %, and crude protein from
220 19 % to 22 % (including chitin nitrogen). Other components, calculated by difference, ranged
221 from 3.4 % to 7.5 %.

222 The proximate composition of *T. molitor* was comparable to the results of (Barker,
223 Fitzpatrick, & Dierenfeld, 1998); (Finke, 2002); (Jones, Cooper, & Harding, 1972); (Ghaly &
224 Alkoaik, 2009). In addition, the crude protein content measured for *A. domesticus* and *Z.*
225 *morio*, 19.3 % and 20.6 % respectively, was comparable to the range described in literature,
226 namely 17.3 % to 20.5 % (Barker, Fitzpatrick, & Dierenfeld, 1998; Finke, 2002). For *A.*
227 *diaperinus* and *B. dubia*, no crude protein data are available in literature. The measured crude
228 protein contents of the five insect species might be relatively higher than their actual protein
229 content, since amounts of nitrogen are also bound in the exoskeletons as chitin. (Barker,
230 Fitzpatrick, & Dierenfeld, 1998) reported that 5 - 6 % of total nitrogen was measured as
231 chitin-bound nitrogen in *T. molitor*. This would lead to an overestimation in protein content
232 of 1.1 – 1.3 % on a fresh weight basis. It is a reasonable estimate for true protein content in
233 most insect species. However, no detailed study on this issue is available.

234 The measured protein content of the tested insect species (around 20 %) in this study is
235 comparable with that of beef (18.4 %), chicken (22.0 %) and fish (18.3 %) (Ghaly, 2009b).
236 Further, measured insect protein content was higher than that of lamb (15.4 %), pork (14.6 %)

237 (Ghaly, 2009), eggs (13 %), and milk (3.5 %), but lower in comparison to soy (36.5%)
238 (Young & Pellett, 1994).

239 3.2. Amino acid composition and protein quality of five insect species

240 The insect protein quality of the insect species was estimated by the amino acid composition
241 (Table 2). The larvae of *A. diaperinus*, *T. molitor* and *Z. morio* contained all the essential
242 amino acids in quantities that are necessary for humans (FAO/WHO/UNU, 1985).

243 Also, the sum of the amount of total essential amino acids (EAA) for *A. diaperinus*, *T.*
244 *molitor* and *Z. morio* was comparable to that of soybean protein, but slightly lower than that
245 of casein, as reported by (Young & Pellett, 1991). Furthermore, the sum of EAA for *A.*
246 *domesticus* and *B. dubia* was lower than in casein and soybean protein, but EAA were
247 available in quantities that are necessary for human requirement (sum of 277 mg/g crude
248 protein). The amino acid profiles found for *T. molitor* were similar to the profiles that were
249 reported by (Ghaly, 2009b); (Finke, 2002) and (Jones, Cooper, & Harding, 1972). The amino
250 acid profiles of *Z. morio* reported by (Finke, 2002) and those of *A. domesticus* reported by
251 (DeFoliart & Benevenga, 1989) were similar to ours. To our knowledge, no literature is
252 reported on the amino acid profiles for *A. diaperinus* and *B. dubia* before.

253 The sum of total amount of amino acids (TAA) per g crude protein of *A. diaperinus* (927
254 mg/g), *T. molitor* (910 mg/g) and *Z. morio* (931 mg/g) was higher than that in *A. domesticus*
255 (864 mg/g) and *B. dubia* (776 mg/g). The fact that the sum of the total amount of amino acids
256 did not add up to 1000 mg/g crude protein is mainly explained by the presence of non-protein
257 nitrogen in the form of chitin. *Acheta domesticus* and *B. dubia* are used in adult form and are
258 known to contain a higher level of chitin as compared to *T. molitor*, *A. diaperinus* and *Z.*
259 *morio*.

260 The calculated essential amino acid index (EAAI) of *A. diaperinus*, *T. molitor* and *Z. morio*
261 was somewhat higher than that of soybean, but lower than that of casein, also indicating that
262 the quality of the insect protein for these three insect species was comparable to conventional
263 food protein sources. The EAAI of *A. domesticus* and *B. dubia* was the lowest in comparison
264 to other insects, and lower than the EAAI for casein and soybean. For a more detailed insight
265 in insect protein quality, digestibility data need to be taken into account in future studies,
266 since digestibility is not included as a factor in determining EAAI. (Ramos-Elorduy, Moreno,
267 Prado, Perez, Otero, & De Guevara, 1997) found that protein digestibility, calculated from a
268 vitro study, ranged from 76 to 98 % for seventy-eight species of edible insects, representing
269 twenty-three insect families in Mexico. Their study indicated that insect proteins might have
270 a high nutritional value.

271 3.3. Protein distribution in obtained fractions and colour of supernatant fractions

272 A mass balance was built up based on protein content in the residue, pellet and supernatant
273 fractions (Fig. 1). The amount of protein in the fractions was calculated based on protein
274 content determined by Dumas, in combination with weight of the fractions (dry matter based).
275 The protein recoveries ranged from 86.5 % to 103 % (Fig. 1). The losses did occur during the
276 extraction procedure, especially for *B. dubia*. The pellet contained 32.6 % to 39.4 % of total
277 protein and the residue 31.4 % to 46.6 % of total protein (Fig. 1). The obtained pellet and
278 residue fractions were higher in protein content than that in the supernatant (17 % to 23.1 %)
279 for all five types of insects. The amount of proteins in the residue was higher than that in the
280 pellet, except for *Z. morio* (31.4 %).

281 In addition, the protein content on dry matter basis of each fraction ranged from 50 % to
282 61 % in the supernatant, from 65 % to 75 % in the pellet, from 58 % to 69 % in the residue
283 and around 0.1 % in the fat fraction. All chitin-bound nitrogen is expected to be present only

284 in the pellet and residue fractions, because chitin is insoluble in aqueous solvents (Goycoolea,
285 Argüelles-Monal, Peniche, Higuera-Ciapara, Doxastakis, & Kiosseoglou, 2000). Except for
286 the presence of chitin-bound nitrogen, there is also uncertainty in the protein-to-nitrogen
287 conversion factor of 6.25 leading to inaccuracy in the absolute protein content reported.

288 After aqueous extraction, the *B. dubia* had the lightest (light yellow), and the *T. molitor* the
289 darkest, colour (dark brown) among all insect supernatant solutions. The colour of *A.*
290 *diaperinus*, *Z. morio* and *A. domesticus* supernatant solutions was comparable. This visual
291 observation indicated that chemical reactions took place during processing. Preliminary
292 experiments showed that colour formation was most likely due to enzymatic browning
293 reactions. In addition, the colour of residue and pellet fractions was similar to that of the
294 supernatant fractions.

295 3.4. SDS-PAGE

296 The reduced SDS-PAGE using 12.5 % acrylamide gels results show a range of protein bands
297 of the supernatant fractions < 95 kDa, and that of the pellet fractions < 200 kDa for all five
298 insect species (Fig. 2). Five major groups of protein bands could be distinguished in Fig. 2,
299 namely bands ≤ 14 kDa, 14 - 32 kDa, 32 - 95 kDa and > 95 kDa. Due to insolubility in
300 sample buffer, protein bands of the residue fractions were absent on the gels used in this
301 experiment.

302 Based on intensity, the bands ≤ 14 kDa were abundant, especially for *T. molitor*. SDS-PAGE
303 analysis using 20 % acrylamide gels showed that the band ≤ 14 kDa consisted of a range of
304 protein bands from 6.5 kDa to 14 kDa for all insect species studied (results not shown). For *T.*
305 *molitor*, the bands ≤ 14 kDa could possibly originate from anti-freeze type of proteins
306 ranging from 8.5 - 13 kDa, including hemolymph proteins having a molecular weight ~ 12
307 kDa (Graham, Liou, Walker, & Davies, 1997); (Liou, Thibault, Walker, Davies, & Graham,

308 1999); (Graham, Tang, Baust, Liou, Reid, & Davies, 2001). For the other insect species
309 studied, no literature is available for specific proteins, not for those ≤ 14 kDa but also not for
310 those > 14 kDa.

311 Next, the bands observed ranging from 14 to 32 kDa could possibly originate from *T. molitor*
312 cuticle proteins with molecular weights predominantly between 14 and 30 kDa (Andersen,
313 Rafn, Krogh, Hojrup, & Roepstorff, 1995), *e.g.* chymotrypsin-like proteinase (24 kDa)
314 (Elpidina, Tsybina, Dunaevsky, Belozersky, Zhuzhikov, & Oppert, 2005),.

315 The bands observed ranging from 32 to 95 kDa in the *T. molitor* supernatant fractions could
316 possibly be linked to enzymes and other proteins, *e.g.* melanization-inhibiting protein (43
317 kDa), β -glycosidase (59 kDa), trypsin-like proteinases (59 kDa), and melanization-engaging
318 types of protein (85 kDa) (Ferreira, Marana, Terra, & Ferreira, 2001); (Zhao, Soderhall, Park,
319 Ma, Osaki, Ha, et al., 2005); (Prabhakar, Chen, Elpidina, Vinokurov, Smith, Marshall, et al.,
320 2007); and (Cho, Choi, Moon, Kim, Kwon, Homma, et al., 1999).

321 Above 95 kDa, no bands were observed in the supernatant fractions of *T. molitor*. Compared
322 to *T. molitor*, the pattern of protein bands from supernatant fractions in *A. diaperinus* and *A.*
323 *domesticus* were similar, but not identical. For *Z. morio* and *B. dubia*, more bands were found
324 in the range of 30 to 95 kDa.

325 The observed bands with molecular weight > 95 kDa in the pellet fractions of *T. molitor*
326 possibly originate from vitellogenin-like protein with a molecular weight of 160 kDa (Lee,
327 Lee, Choi, Cho, Kwon, Kawabata, et al., 2000). No subunit structures of the proteins
328 mentioned were found using UniProt: Universal Protein Resource Knowledgebase (UniProt
329 ID: Q9H0H5), so that actual molecular weight reported in literature is similar to apparent
330 molecular weight on gel.

331 Besides the proteins mentioned before, proteins incorporated in the exoskeleton and muscle
332 proteins are present in the five types of insects and in the fractions obtained. For the adult
333 stage of *A. domesticus* and *B. dubia* muscle proteins include insect flight and leg muscles,
334 which mainly consist of large size proteins, *e.g.* M-line protein, (flight and leg muscle, 400
335 kDa); kettin (leg muscle isoform, 500 kDa); kettin (flight muscle isoform, 700 kDa) (Bullard
336 & Leonard, 1996); (Lahey, Ferguson, Labeit, Reedy, Larkins, Butcher, et al., 1990). For the
337 larval stage of *T. molitor*, *A. diaperinus* and *Z. morio* skeletal muscles, which likely consist of
338 large size proteins, are present.

339 3.5. Protein functionality measurements

340 Due to the insolubility of the pellet and residue fractions, only the supernatant fraction of the
341 protein was tested for its functionality with respect to foamability, foam stability, and
342 gelation.

343 3.5.1. Foamability and foam stability

344 As a reference for the foam stability measurements, albumin from chicken egg white was
345 used at a concentration of 1.5 % w/v. The reference sample is a good stabilizer for foam, and
346 was capable of producing foam with a half-time of 17 minutes. *Zophobas morio* formed foam
347 at pH 3, 7 and 10 with a half-time of 6 minutes, *A. domesticus* at pH 3 with a half-time of 4
348 minutes, and *B. dubia* produced foam at pH 5 with a half-time of 5 minutes. Foams with half-
349 time of < 6 minutes are not considered to be stable foams. All other supernatant fractions had
350 negligible foam ability at a concentration of 3 % w/v, at pH 3, 5, 7, and 10. This may be due
351 to the protein concentration in the supernatant fraction solution (around 1.7 % w/v) being too
352 low to generate stable foam. The stability of the foam can be influenced by protein structure,
353 protein concentration, and ionic strength. In addition, the stability of the foam can be also
354 influenced by presence of oil. As mentioned by (Lomakina & Mikova, 2006), the effect of oil

355 at levels above 0.5 % reduced the volume of egg white foam. In our case, the supernatant
356 fractions obtained from five insect species also contained some amount of oil in
357 concentration of around 0.1 %, which may also influence foamability of proteins in
358 supernatant fractions.

359 3.5.2. Gelation

360 3.5.2.1 Visual observation of gelation

361 The visual appearance was determined of gels of five supernatant fraction solutions, with
362 fraction concentrations of 3 and 30 % w/v, at pH 3, 5, 7, and 10, after heating for 10 minutes
363 in a water bath at 86 ± 1 °C (Table 3). A heating time of 20 and 30 minutes was also tested,
364 but no differences were seen in gel formation (not shown). Factors affecting the gel
365 properties in general are pH, protein concentration, and thermal treatment. The protein
366 concentrations selected for gelation are in the range from 0.5 to 25 % concentration that are
367 used in general to make gels. At a concentration of 3 % w/v, none of the protein fractions
368 showed gel formation, except for *A. domesticus* at pH 7. At pH 5 and pH 7, for all samples
369 (except *A. domesticus* at pH 7) heating induced the formation of visible large aggregates
370 rather than gel formation.

371 All 30 % w/v supernatant fractions formed a gel at pH 7 and 10, but not at pH 3. At pH 5,
372 very weak gels were formed, that yielded when turned upside down. In table 3, these samples
373 are designated as “V” (viscous fluid). All samples at pH 7 and 10 were turbid, indicating that
374 the characteristic size of the structures forming the gel was larger than the wavelength of
375 visible light. All gels were already formed after 10 minutes and longer heating times had no
376 influence on the appearance of the gel.

377 Some insect proteins have an isoelectric point of about 5. For instance, the pI of proteins from
378 silkworm (*Bombyx mori*) and spider (*Nephila edulis*) are 4.37 - 5.05, and 6.47, respectively

379 (Foo, Bini, Hensman, Knight, Lewis, & Kaplan, 2006). If our protein fractions also have a pI
380 of around pH 5, this may explain why all fractions at this pH formed aggregates at a
381 concentration of 3 % w/v, and very weak gels at concentrations of 30 % w/v. Close to the pI,
382 the electrostatic interactions between the proteins are very weak, which, upon denaturation,
383 tends to lead to the formation of dense aggregates. These dense aggregates have a much
384 higher gelling concentration than aggregates formed at a pH above or below the isoelectric
385 point. To form a firm gel at this pH, higher protein concentrations are needed.

386 Samples at pH 3 and 10 at 3 % w/v were more transparent than samples heated at pH 5 and 7.
387 The increased charge on the protein at pH 3 may prevent the proteins from aggregating, since
388 even at 30 % w/v these fractions did not form a gel or even a viscous fluid. The decrease in
389 turbidity observed at pH 10 suggests that the aggregates formed at this pH were less dense
390 and/or smaller than the ones formed at pH 5 and 7.

391 3.5.2.2. *Rheological properties of gels*

392 According to the visual observation of gelation, at a pH of 7 and a concentration of 3 % w/v a
393 weak gel was formed, and at 30 % w/v a strong gel was formed. Therefore, for studying gel
394 strength, fraction concentrations in between these two values (7.5 and 15 % w/v) were
395 chosen. For all five fractions, we determined the evolution of the storage modulus G' and loss
396 modulus G'' during the temperature ramp at a concentration of 15 % w/v and a pH of 7. The
397 storage modulus is a measure for the elastic energy stored reversibly in a gel during
398 deformation, and characterizes its stiffness; the loss modulus is a measure for the energy
399 dissipated during deformation as a result of viscous friction. As an example, the results for
400 the mealworm supernatant fraction (the other fractions showed similar results) are provided
401 (Fig. 3A). G' gradually increased during the heating phase of the ramp. During the second
402 phase, when the temperature was kept constant at 90 °C, G' kept on increasing gradually.
403 This observation showed that the gel structure did not yet reach an equilibrium state. During

404 the cooling phase, both G' and G'' increased sharply. This is typical for gels in which
405 hydrogen bonds are formed between structural elements (Ould Eleya, Ko, & Gunasekaran,
406 2004). The gelation temperature observed ranged from about 51 °C to 63 °C (*T. molitor* 61.7
407 $\pm 1.1^\circ\text{C}$, *A. diaperinus* 58.2 $\pm 2.1^\circ\text{C}$, *Z. morio* 51.2 $\pm 1.5^\circ\text{C}$, *A. domesticus* 56.2 $\pm 0.7^\circ\text{C}$, *B.*
408 *dubia* 63.2 $\pm 0^\circ\text{C}$, from which the lowest and the highest temperature were from *Z. morio*
409 and *B. dubia* supernatant fractions respectively (results not shown).

410 To obtain more information on the gel structure, the value of $\log G'$ of *T. molitor* supernatants
411 was determined as a function of $\log C$ (concentration) with fraction concentrations of 7.5 %
412 w/v, 15 % w/v and 30 % w/v (corresponding to actual protein concentrations of 4.1 %, 8.3 %
413 and 16.6 %) at 90 °C and 20 °C (Figure 3B). Values for G' at 90 °C were taken from end of
414 phase 2 from the ramp, and values at 20 °C were taken from the end of phase 3, which is
415 similar to the procedure of (Ould Eleya, Ko, & Gunasekaran, 2004). The values of the power-
416 law exponent n in the scaling relation $G' \propto C^n$, were used for evaluation of gel structure
417 (Shih, Shih, Kim, Liu, & Aksay, 1990). The parameter n had a value equal to 3.0 ± 0.4 at the
418 end of the isothermal stage at 90 °C, and a value of 2.8 ± 0.6 from the end of the cooling
419 stage at 20 °C. These two values are comparable, so there were no significant structural
420 rearrangements in the gel network upon cooling of the samples. An exponent n of about 2.8 is
421 typical for fractal protein gels and points to a fractal dimension d_f which is close to 2 (Ould
422 Eleya, Ko, & Gunasekaran, 2004).

423 Fig. 3C shows G' at the end of phase 3 of the temperature ramp as a function of strain, for
424 insect supernatant gels at 20 °C and a concentration of 15 % w/v. The value for G' in the
425 linear response region of *A. domesticus* supernatant gels was around 2500 Pa, which was
426 almost 1.5 times stronger than that of *B. dubia* (around 1600 Pa), 6 times stronger than that of
427 *Z. morio* (around 390 Pa), and 25 times stronger than that of *T. molitor* (around 100 Pa) and
428 *A. diaperinus* (around 140 Pa). In interpreting these results, we must be careful, since the

429 actual protein concentrations in the fractions was lower than 15 % w/v, and differed slightly
430 from fraction to fraction. As seen before, the actual protein contents were for *T. molitor* 8.3
431 %; *A. diaperinus* 9.2 %; *Z. morio* 7.6 %; *A. domesticus* 9.2 % and *B. dubia* 7.4 %.

432 Several conclusions can be drawn from these results. Although the *B. dubia* supernatant
433 sample had the lowest actual protein content, it formed the strongest gels among all other
434 three insect species, except *A. domesticus*. Supernatants from *A. diaperinus* and *A.*
435 *domesticus* had similar protein concentration, but they showed significant differences in gel
436 strength. In addition, supernatants from *B. dubia* and *A. domesticus* that were in the adult
437 stage formed relatively stronger gels than the other three insect species that were in the larvae
438 stage. Apparently, the insect growth stage influences the body protein composition, and
439 different species differ in protein type and structure (Wilson, 2010).

440 All insect gels had a comparable maximum linear strain at supernatant fraction concentration
441 of 15 % w/v, with a value of around 50 %. An example is shown for *Z. Morio* (Fig. 3C). The
442 maximum linear strain is, of course, dependent on heating rate and protein concentration, and
443 it would therefore be interesting to investigate the concentration dependence of this property,
444 since it can provide additional information on the fractal dimension of the gels.

445 These detailed rheological results show that insect proteins can form gels that have similar
446 properties as those formed from conventional food proteins. It therefore shows that insect
447 proteins have indeed functionalities that are desirable for food application.

448 **4. Conclusions**

449 Proteins were extracted from five insect species and protein purity and yield of the obtained
450 fractions was evaluated: Around 20 % of total protein was found back in the supernatant, the
451 rest of the protein was divided about equally over the residue and the pellet fraction for all
452 five insect species after aqueous extraction. The extraction method is easy and feasible to

453 apply, but the yield of extracted supernatant fractions is relatively low. The purity of
454 measured protein content expressed as percentage of dry matter ranged from 50 % to 61 % of
455 supernatant fractions, from 65 % to 75 % of pellet fractions and from 58 % to 69 % of residue
456 fractions depending on the insect species.

457 We established some functional properties of the protein fractions, focusing on foaming and
458 gelation: The soluble protein fractions of all five types of insects had poor foaming capacity
459 at pH 3, 5, 7, and 10, but could form gels at a concentration of 30 % w/v. At a concentration
460 of 15 % w/v at pH 7 and 10, *A. domesticus* supernatant formed the strongest gels among all
461 insect species. The gelation temperature ranged from about 51 °C to 63 °C for all insect
462 species at pH 7. In addition, all insect gels had a comparable maximum linear strain at this
463 concentration, with a value of around 50 %.

464 We studied the protein quality of whole insects by analysis of protein content and amino acid
465 composition. The protein content of the five insect species was comparable to conventional
466 meat products in terms of protein quantification. The sum of EAA per g protein for all insect
467 species was comparable with the sum of EAA for soybean protein, lower than that for casein,
468 but higher than that for the daily protein requirement of an adult (FAO/WHO/UNU, 1985).
469 Differences in calculated EAAI were similar.

470 Although differences are observed in protein content, amino acid composition, protein
471 distribution of the fractions obtained, SDS-PAGE data, foaming and gelation properties, the
472 similarities between the insect species are more apparent than the differences. The fact that
473 gels could be formed for all five insect species, using the soluble fractions obtained by a
474 simple aqueous extraction procedure, is promising in terms of future food applications. More
475 research is needed for developing further extraction and purification procedures, and for more
476 detailed insight into functional properties.

477 **Acknowledgments**

478 This project in part of SUPRO2 project (Sustainable production of insect proteins for human
479 consumption) was supported by a grant from the Dutch Ministry of Economic Affairs.

480 **References**

- 481 Andersen, S. O., Rafn, K., Krogh, T. N., Hojrup, P., & Roepstorff, P. (1995). Comparison of
482 larval and pupal cuticular proteins in *Tenebrio molitor*. *Insect Biochemistry and*
483 *Molecular Biology*, 25(2), 177-187.
- 484 Barker, D., Fitzpatrick, M. P., & Dierenfeld, E. S. (1998). Nutrient composition of selected
485 whole invertebrates. *Zoo Biology*, 17(2), 123-134.
- 486 Beveridge, T., Jones, L., & Tung, M. A. (1984). Progel and gel formation and reversibility of
487 gelation of whey, soybean, and albumen protein gels. *Journal of Agricultural and*
488 *Food Chemistry*, 32(2), 307-313.
- 489 Bullard, B., & Leonard, K. (1996). Modular proteins of insect muscle. *Advances in*
490 *Biophysics*, 33(0), 211-221.
- 491 Cho, M. Y., Choi, H. W., Moon, G. Y., Kim, M. H., Kwon, T. H., Homma, K.-i., Natori, S.,
492 & Lee, B. L. (1999). An 86 kDa diapause protein 1-like protein is a component of
493 early-staged encapsulation-relating proteins in coleopteran insect, *Tenebrio molitor*
494 larvae. *FEBS Letters*, 451(3), 303-307.
- 495 Deak, N. A., Murphy, P. A. & Johnson, L. A. (2007). Characterization of Fractionated Soy
496 Proteins Produced by a New Simplified Procedure. *Journal of American Oil*
497 *Chemistry Society*, 84 (2), 137-149.
- 498 DeFoliart, G. R., & Benevenga, N. J. (1989). Use of a four-parameter logistic model to
499 evaluate the quality of the protein from three insect species when fed to rats. *Journal*
500 *of nutrition*, 119(6), 864-871.
- 501 Del Valle, F. R., Mena, M. H., & Bourges, H. (1982). An investigation into insect protein.
502 *Journal of Food Processing and Preservation*, 6(2), 99-110.
- 503 Durst, P. B., & Shono, K. (2010). Edible forest insects: exploring new horizons and
504 traditional practices. In *In Proceedings of a workshop on Asia-Pacific resources and*
505 *their potential for development: Forest insects as food: humans bite back*, (pp. 1-4).
506 Bangkok, Thailand.
- 507 Elpidina, E. N., Tsybina, T. A., Dunaevsky, Y. E., Belozersky, M. A., Zhuzhikov, D. P., &
508 Oppert, B. (2005). A chymotrypsin-like proteinase from the midgut of *Tenebrio*
509 *molitor* larvae. *Biochimie*, 87(8), 771-779.
- 510 FAO/WHO/UNU. (1985). Energy and protein requirements. In *Report of a Joint*
511 *FAO/WHO/UNU Expert Consultation*, (pp. 206). Geneva, Switzerland.: Food and
512 Agriculture Organization, World Health Organization and the United Nations
513 University.
- 514 Ferreira, A. H., Marana, S. R., Terra, W. R., & Ferreira, C. (2001). Purification, molecular
515 cloning, and properties of a beta-glycosidase isolated from midgut lumen of *Tenebrio*
516 *molitor* (Coleoptera) larvae. *Insect Biochemistry and Molecular Biology*, 31(11),
517 1065-1076.
- 518 Finke, M. D. (2002). Complete nutrient composition of commercially raised invertebrates
519 used as food for insectivores. *Zoo Biology*, 21(3), 269-285.
- 520 Finke, M. D., & Winn, D. (2004). Insects and related arthropods: a nutritional primer for
521 rehabilitators. *Journal of Wildlife Rehabilitation*, 27(3/4), 14.

- 522 Finke, M. D. (2007). Estimate of chitin in raw whole insects. *Zoo Biology*, 26(2), 105-115.
- 523 Foo, C. W. P., Bini, E., Hensman, J., Knight, D. P., Lewis, R. V., & Kaplan, D. L. (2006).
- 524 Role of pH and charge on silk protein assembly in insects and spiders. *Applied*
- 525 *Physics A: Materials Science & Processing*, 82(2), 223-233.
- 526 Ghaly, A. E. (2009a). The black cutworm as a potential human food. *American Journal of*
- 527 *Biochemistry and Biotechnology*, 5(4), 210-220.
- 528 Ghaly, A. E. (2009b). The use of insects as human food in Zambia. *OnLine Journal of*
- 529 *Biological Sciences*, 9(4), 93-104.
- 530 Ghaly, A. E., & Alkoaik, F. N. (2009). The Yellow Mealworm as a Novel Source of Protein.
- 531 *American Journal of Agricultural and Biological Sciences*, 4(4), 319-331.
- 532 Goycoolea, F. M., Argüelles-Monal, W., Peniche, C., Higuera-Ciapara, I., Doxastakis, G., &
- 533 Kiosseoglou, V. (2000). Chitin and chitosan. In *Developments in Food Science*, vol.
- 534 Volume 41 (pp. 265-308): Elsevier.
- 535 Graham, L. A., Liou, Y.-C., Walker, V. K., & Davies, P. L. (1997). Hyperactive antifreeze
- 536 protein from beetles. *Nature*, 388(6644), 727-728.
- 537 Graham, L. A., Tang, W., Baust, J. G., Liou, Y.-C., Reid, T. S., & Davies, P. L. (2001).
- 538 Characterization and cloning of a *Tenebrio molitor* hemolymph protein with sequence
- 539 similarity to insect odorant-binding proteins. *Insect Biochemistry and Molecular*
- 540 *Biology*, 31(6-7), 691-702.
- 541 Jones, L. D., Cooper, R. W., & Harding, R. S. (1972). Composition of Mealworm *Tenebrio*
- 542 *molitor* Larvae. *The Journal of Zoo Animal Medicine*, 3(4), 34-41.
- 543 Ladrón de Guevara, O., Padilla, P., García, L., Pino, J. M., & Ramos-Elorduy, J. (1995).
- 544 Amino acid determination in some edible Mexican insects. *Amino Acids*, 9(2), 161-
- 545 173.
- 546 Lakey, A., Ferguson, C., Labeit, S., Reedy, M., Larkins, A., Butcher, G., Leonard, K., &
- 547 Bullard, B. (1990). Identification and localization of high molecular weight proteins
- 548 in insect flight and leg muscle. *European Molecular Biology Organization journal*,
- 549 9(11), 3459-3467.
- 550 Lee, K. M., Lee, K. Y., Choi, H. W., Cho, M. Y., Kwon, T. H., Kawabata, S.-i., & Lee, B. L.
- 551 (2000). Activated phenoloxidase from *Tenebrio molitor* larvae enhances the synthesis
- 552 of melanin by using a vitellogenin-like protein in the presence of dopamine. *European*
- 553 *Journal of Biochemistry*, 267(12), 3695-3703.
- 554 Liou, Y.-C., Thibault, P., Walker, V. K., Davies, P. L., & Graham, L. A. (1999). A Complex
- 555 Family of Highly Heterogeneous and Internally Repetitive Hyperactive Antifreeze
- 556 Proteins from the Beetle *Tenebrio molitor*. *Biochemistry*, 38(35), 11415-11424.
- 557 Lomakina, K., & Mikova, K. (2006). A study of the factors affecting the foaming properties
- 558 of egg white – a review. *Czech Journal of Food Sciences*, 24(3), 110-118.
- 559 Oonincx, D. G. A. B., van Itterbeeck, J., Heetkamp, M. J. W., van den Brand, H., van Loon, J. J.
- 560 A., & van Huis, A. (2010). An exploration on greenhouse gas and ammonia
- 561 production by insect species suitable for animal or human consumption. *PLoS ONE*,
- 562 5(12).
- 563 Ould Eleya, M. M., Ko, S., & Gunasekaran, S. (2004). Scaling and fractal analysis of
- 564 viscoelastic properties of heat-induced protein gels. *Food Hydrocolloids*, 18(2), 315-
- 565 323.
- 566 Prabhakar, S., Chen, M. S., Elpidina, E. N., Vinokurov, K. S., Smith, C. M., Marshall, J., &
- 567 Oppert, B. (2007). Sequence analysis and molecular characterization of larval midgut
- 568 cDNA transcripts encoding peptidases from the yellow mealworm, *Tenebrio molitor*
- 569 L. *Insect Molecular Biology*, 16(4), 455-468.

- 570 Ramos-Elorduy, J., Moreno, J. M. P., Prado, E. E., Perez, M. A., Otero, J. L., & De Guevara,
571 O. L. (1997). Nutritional value of edible insects from the state of Oaxaca, Mexico.
572 *Journal of Food Composition and Analysis*, 10(2), 142-157.
- 573 Renault, D., Bouchereau, A., Delettre, Y. R., Hervant, F., & Vernon, P. (2006). Changes in
574 free amino acids in *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) during
575 thermal and food stress. *Comparative Biochemistry and Physiology Part A:
576 Molecular & Integrative Physiology*, 143(3), 279-285.
- 577 Renkema, J. M. S., Knabben, J. H. M., & van Vliet, T. (2001). Gel formation by β -
578 conglycinin and glycinin and their mixtures. *Food Hydrocolloids*, 15(4-6), 407-414.
- 579 Renkema, J. M. S., Gruppen, H., & van Vliet, T. (2002). Influence of pH and Ionic Strength
580 on Heat-Induced Formation and Rheological Properties of Soy Protein Gels in
581 Relation to Denaturation and Their Protein Compositions. *Journal of Agricultural and
582 Food Chemistry*, 50(21), 6064-6071.
- 583 Schwenke, K. D. (1997). J. F. Zayas:
584 Functionality of Proteins in Food. *Food / Nahrung*, 41(5), 319-319.
- 584 Shih, W.-H., Shih, W. Y., Kim, S.-I., Liu, J., & Aksay, I. A. (1990). Scaling behavior of the
585 elastic properties of colloidal gels. *Physical Review A*, 42(8), 4772-4779.
- 586 Smith, D. M., & Nielsen, S. S. (2010). Protein Separation and Characterization Procedures.
587 Food Analysis. In D. R. Heldman (Ed.), (pp. 261-281): Springer US.
- 588 Sosulski, F. W., & Imafidon, G. I. (1990). Amino acid composition and nitrogen-to-protein
589 conversion factors for animal and plant foods. *Journal of Agricultural and Food
590 Chemistry*, 38(6), 1351-1356.
- 591 van Huis, A. (2013). Potential of Insects as Food and Feed in Assuring Food Security. *Annual
592 Review of Entomology*, 58(1).
- 593 Wilson, R. (2010). P. J. Gullan and P. S. Cranston: The insects: an outline of entomology (4th
594 edition). *Journal of Insect Conservation*, 14(6), 745-746.
- 595 Young, V. R., & Pellett, P. L. (1991). Protein evaluation, amino acid scoring and the food
596 and drug administration's proposed food labelling regulations. *Journal of nutrition*,
597 121, 145-150.
- 598 Young, V. R., & Pellett, P. L. (1994). Plant proteins in relation to human protein and amino
599 acid nutrition. *The American Journal of Clinical Nutrition*, 59(5), 1203S-1212S.
- 600 Zhao, M., Soderhall, I., Park, J. W., Ma, Y. G., Osaki, T., Ha, N. C., Wu, C. F., Soderhall, K.,
601 & Lee, B. L. (2005). A novel 43-kDa protein as a negative regulatory component of
602 phenoloxidase-induced melanin synthesis. *Journal of Biological Chemistry*, 280(26),
603 24744-24751.

604

605

Table 1. Proximate composition of five insect species on live weight basis (mean \pm S.D., n=2).

Table 2. Amino acid pattern of five insect species, casein, soybean protein, recommendation for adult and calculated essential amino acid index of five insect species and casein & soybean protein (FAO/WHO/UNU, 1985) and (Young & Pellett, 1991).

Table 3. Gel formation of supernatant fractions from five insect species (X: no gel formation; A: aggregation; V: viscous fluid; O: gel formation).

Table 1.

Insects	Moisture (%)	Fat (%)	Crude protein (%) (including chitin nitrogen)	Other components (%) (e.g. carbohydrates, minerals and vitamins)
<i>T. molitor</i>	63.5 \pm 1.8	9.9 \pm 1.0	19.1 \pm 1.3	7.5 \pm 2.2
<i>A. diaperinus</i>	64.5 \pm 1.0	8.5 \pm 0.2	20.6 \pm 0.1	6.4 \pm 1.0
<i>Z. morio</i>	59.9 \pm 5.4	16.0 \pm 0.7	20.7 \pm 0.3	3.4 \pm 5.5
<i>A. domesticus</i>	70.8 \pm 2.0	3.6 \pm 0.4	21.5 \pm 0.5	4.1 \pm 2.1
<i>B. dubia</i>	67.4 \pm 2.1	7.7 \pm 0.1	19.3 \pm 0.9	5.6 \pm 2.3

¹

¹ Table 1. Proximate composition of five insect species on live weight basis (mean \pm S.D., n=2).

Table 2.

unit (mg/g crude protein)	<i>A.diaperinus</i>	<i>T.molitor</i>	<i>Z.morio</i>	<i>A.domesticus</i>	<i>B. dubia</i>	Casein	Soybean	1985 FAO/WHO/U NU
Essential amino acid (EAA)								
Histidine	34	29	31	21	23	32	25	15
Isoleucine	43	43	46	36	31	54	47	30
Leucine	66	73	71	66	56	95	85	59
Lysine	61	54	54	53	43	85	63	45
Methionine	26	26	24	25	23	35	24	22
+Cysteine								
Phenyl-alanine	120	100	111	92	93	111	97	38
+ tyrosine								
Threonine	39	39	40	35	32	42	38	23
Tryptophan	12	12	14	9	8	14	11	6
Valine	58	61	63	55	52	63	49	39
Sum of EAA	459	437	454	392	361	531	439	277
Non-essential amino acid								
Alanine	66	70	68	81	71			
Arginine	54	54	54	65	46			

Aspartic acid	83	80	82	73	67		
Glutamic acid	123	109	127	110	96		
Glycine	46	50	48	51	53		
Proline	56	66	56	54	48		
Serine	40	44	42	38	34		
Sum of total AA	927	910	931	864	776		
EAAI	1.65	1.60	1.66	1.39	1.28	1.93	1.56

2

²Table 2. Amino acid pattern of five insect species, casein, soybean protein, recommendation for adult and calculated essential amino acid index of five insect species and casein & soybean protein (FAO/WHO/UNU, 1985) and (Young & Pellett, 1991).

Table 3.

	pH 3	pH 5	pH 7	pH 10
3 %				
<i>T.molitor</i> supernatant	X	A	A	X
<i>A.diaperinus</i> supernatant	X	A	A	X
<i>Z.morio</i> supernatant	X	A	A	X
<i>A.domesticus</i> supernatant	X	A	O	X
<i>B.dubia</i> supernatant	X	A	A	X
30 %				
<i>T.molitor</i> supernatant	X	V	O	O
<i>A.diaperinus</i> supernatant	X	V	O	O
<i>Z.morio</i> supernatant	X	V	O	O
<i>A.domesticus</i> supernatant	X	V	O	O
<i>B.dubia</i> supernatant	X	V	O	O

3

³ Table 3. Gel formation of supernatant fractions from five insect species (X: no gel formation; A: aggregation; V: viscous fluid; O: gel formation).

Figure Captions and Tables

Fig. 1. Protein content of supernatant, pellet and residue fractions expressed as percentage of total protein and total recovery (n=2).

Fig. 2. Molecular weight distribution of *T.molitor* protein fractions, determined by SDS-PAGE using 12.5% homogeneous phastgel and (Samples from left to right: supernatant, pellet and marker); marker is ranging from 6.5 kDa to 200 kDa. Mw is molecular weight.

Fig. 3. A: Dynamic moduli G' and G'' of *T. molitor* supernatant solution as a function of time. Heating and cooling phases are plotted as a secondary axis. B: Plots of the storage modulus G' as a function of protein concentration of mealworm supernatant fractions on a logarithmic scale at pH 7 (heating period 90 °C and cooling period 20 °C). C: Storage modulus G' (Pa) as a function of strain γ % for insect supernatant gelation at 20 °C at a supernatant fraction concentration of 15 % w/v.

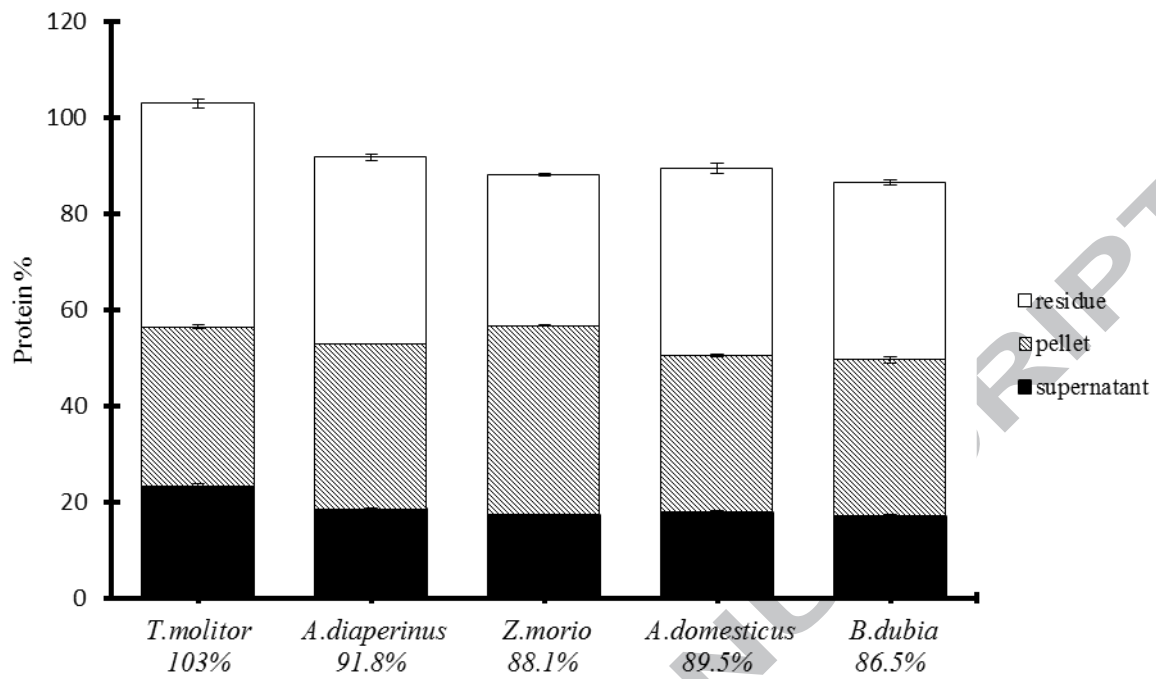


Fig. 1.

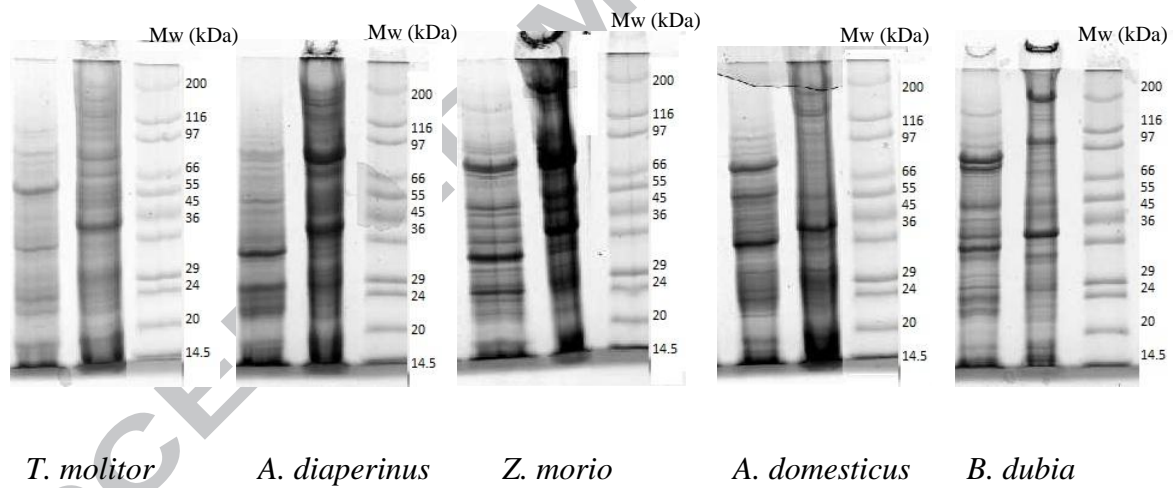
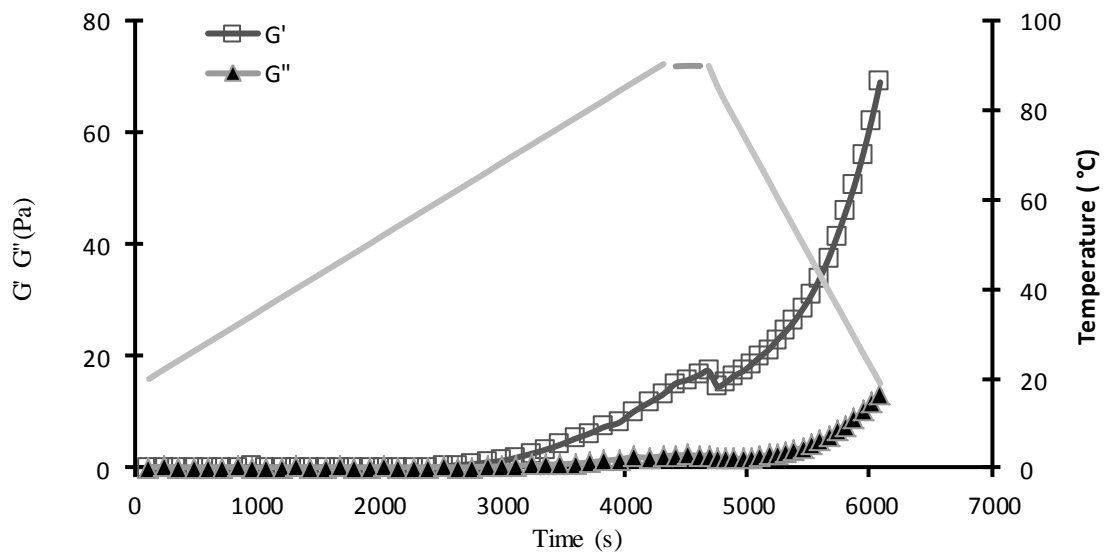
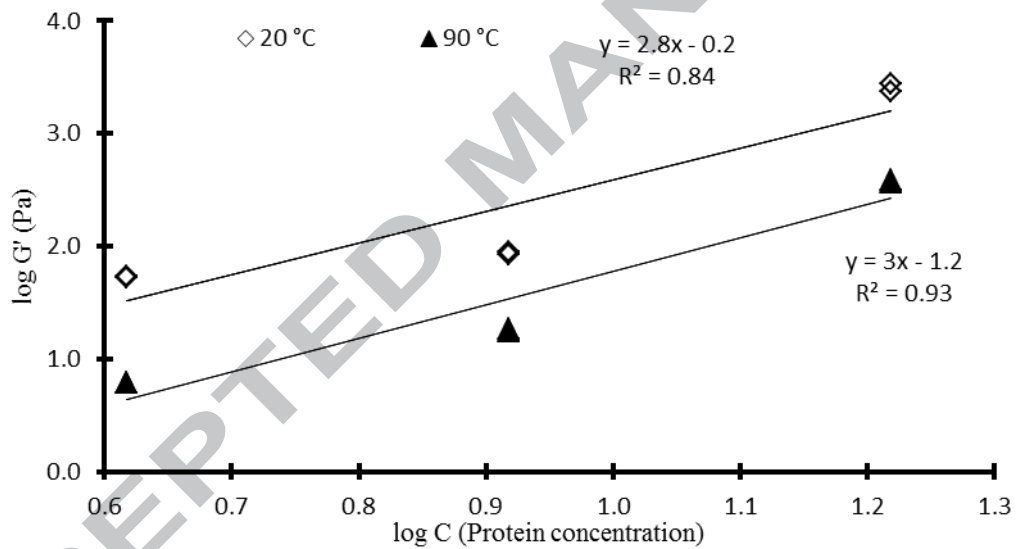


Fig. 2.



A



B

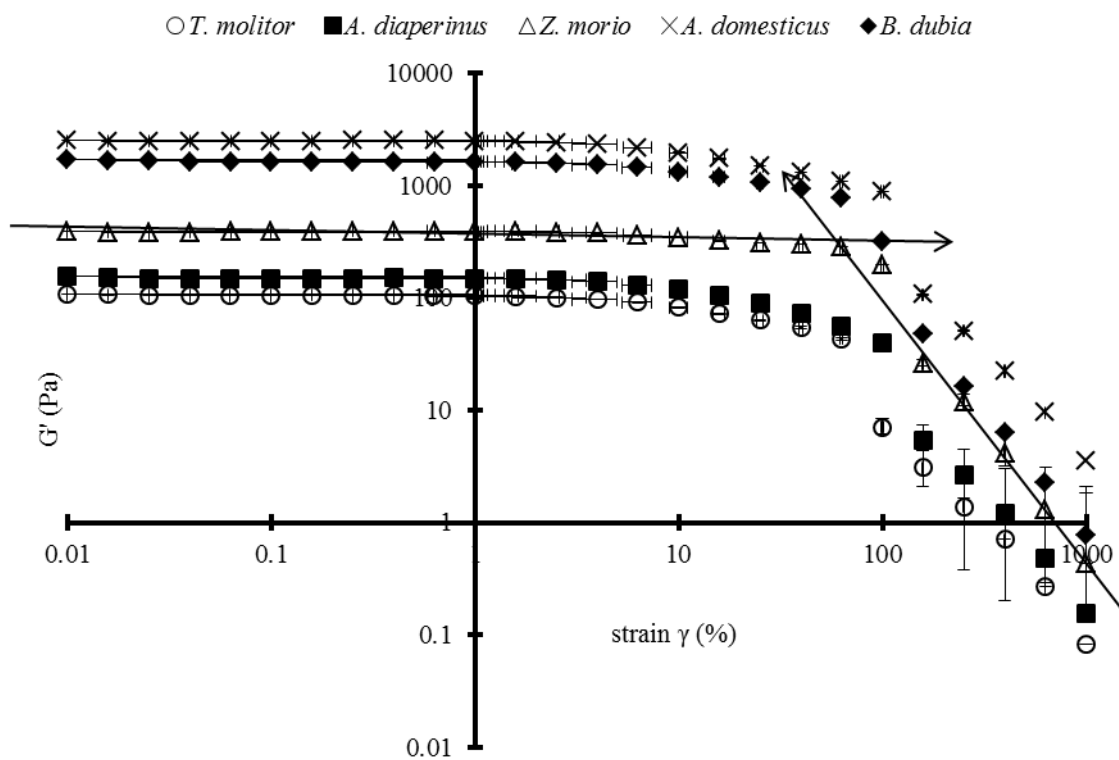


Fig. 3.

ACCEPTED MANUSCRIPT
C

Highlights

Crude protein content of insects was similar to conventional meat products.

The amount of EAA of insects was higher than daily protein requirement of an adult.

The supernatant, pellet, fat and residue fractions were obtained after an aqueous extraction.

Protein bands were < 95 kDa for supernatant fractions and < 200 kDa for pellet fractions.

Most supernatant fractions did not foam, but could form gels depending on protein concentration and the pH.