PROTEIN IDENTIFICATION AND *IN VITRO* DIGESTION OF FRACTIONS FROM *TENEBRIO MOLITOR*

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Abstract – Although amino acid composition of *Tenebrio molitor* has been studied before, limited knowledge is available, on which bulk proteins it consists of, and on its digestibility, being a determinant of the nutritional value of protein. The objective of this study was to investigate in vitro protein digestibility of whole *Tenebrio molitor* larvae, a water-soluble fraction (supernatant), and water-insoluble fractions (pellet and residue), and to identify which proteins were present in the fractions studied. The digestibility of the supernatant fraction (~80%) was much higher than that of pellet (~50%) and residue (~24%) after *in vitro* gastro-duodenal digestion as was determined using the o-phthaldialdehyde (OPA) method. More proteins were digested after pepsin/pancreatin digestion than after only pepsin digestion. The most abundant proteins in the supernatant were hemolymph protein (~12 kDa) & putative allergens (*e.g.* alpha-amylase ~ 50 kDa), and muscle proteins (*e.g.* actin 30-50 kDa) in the pellet fraction as determined from LC-MS/MS and SDS-PAGE. In conclusion, the proteins in the soluble fraction that contained hemolymph proteins were more easily digestible than the insoluble, muscle protein containing fractions.

Key Words – Insect protein; *Tenebrio molitor*; *in vitro* digestion; Protein identification; LC-MS/MS

INTRODUCTION

Insects can be considered as an alternative protein source with less environmental impact [1]. [2] reported the protein content of *Tenebrio molitor* was comparable to that of conventional meat products, and the sum of essential amino acids of *T. molitor* was higher than that for the daily protein requirement.

The nutritional value of a food protein is evaluated not only in amino acid composition, but also in protein digestibility. No information from a food science point of view is available on protein digestibility of *T. molitor* proteins. The objective of this experiment was to investigate protein digestibility (*in vitro*) of the whole insects and insect fractions (supernatant, pellet and residue) obtained from an aqueous extraction designed by [2].

I. MATERIALS AND METHODS

Tenebrio molitor larvae were purchased from a commercial supplier (Kreca V.O.F, Ermelo, the Netherlands).

In vitro gastric digestion with increasing digestion time was performed followed by *in vitro* duodenal digestion, based on [3]. The obtained digested fractions were characterized in terms of molecular weight (SDS-PAGE) and protein content (Dumas). Free amino acids of all digested fractions were determined using the o-phthaldialdehyde (OPA) method.

Filter-aided sample preparation (FASP) was used to prepare protein samples from the three protein fractions obtained as described by [4] with some modifications. Peptide measurements were performed by nanoLC-LTQ-Orbitrap XL-MS/MS (Thermo electron, San Jose, CA, USA) as described by [4].

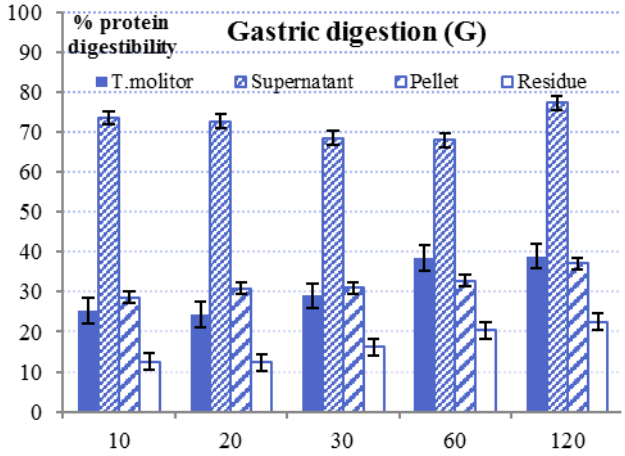
II. RESULTS AND DISCUSSION

Table 1. : Identified muscle proteins of defatted & ground whole *T. molitor*, supernatant and pellet fractions (UniProt: taxonomy 50557, *Insecta*) by LC-MS/MS; putative uncharacterized proteins identified based on family and domain databases from UniProt. Mol. Weight = molecular weight as calculated from the amino acid sequence. Log 10

(iBAQ; intensity based absolute quantitation) was used for measuring protein intensity.

	Muscle proteins	Mol. weight [kDa]	Log10 (iBAQ defatted *T. molitor*)	Log10 (iBAQ Pellet)	Log10 (iBAQ Supernatant)
1	Alpha-actinin-4	107	5.7	5.8	5.9
2	Actin like	42	6.4	7.2	4.8
3	Tropomyosin 1	75.2	6.5	7.2	5.4
4	Tropomyosin 2	32.5	6.9	8.2	5.5
5	Myosin heavy chain	262	5.8	6.8	3.5
6	Myosin-2 essential light chain	16.8		5.3	
7	Putative uncharacterized protein (Myosin_tail)	60.1	5.7	7.1	3.9
8	Calcium-transporting ATPase	72.9		4.8	
9	Calponin	20.3	6.9	6.7	7.4
10	Putative troponin C	18.4		7.0	
11	Troponin 1	23.8		6.7	
12	Troponin T	47.3	6.9	6.7	7.1

In our study, among all muscle proteins identified, protein tropomyosin was found as one of the most abundant proteins in pellet (LC-MS/MS). Corresponding to myofibril proteins found in *T. molitor*, myosin heavy chain had a molecular weight of 262 kDa, and myosin light chain of 16.8 kDa. Myosin heavy chain and light chains from sardines (Sardinella longiceps) showed molecular weights of 205, 31, 23, and 22 kDa [5]; myosin from white mackerel muscle had three light chain subunits with Mw of 26.5, 20, and 17.5 kDa [6].



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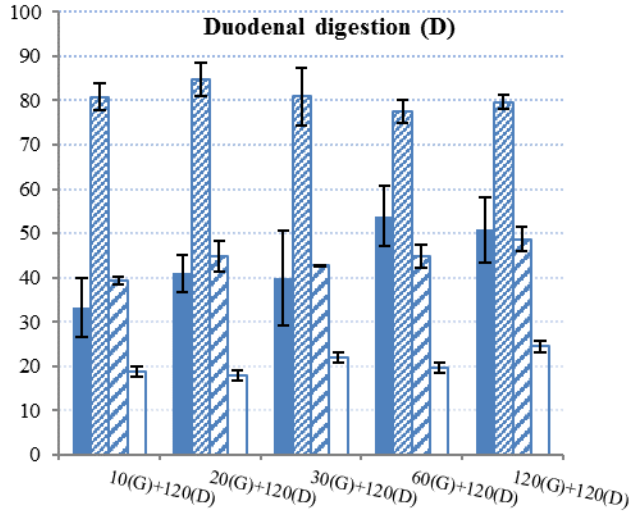

B.

Figure 1A and 1B: Protein digestibility (%) of the whole insect and insect fractions with increasing gastric digestion time (10, 20, 30, 60 and 120 min (n=2), and continue with duodenal digestion (120 min).

The digestibility of the water-soluble protein fraction (supernatant, about 80%) was higher than that of water-insoluble protein fraction (pellet 50% and residue 24%) after *in vitro* gastro-duodenal digestion as determined by the OPA essay. In comparison to water-soluble proteins of conventional meat, [7] mentioned on sarcoplasmic proteins, *e.g.* nitrogen content after digestion was 67% for chicken, 89% for beef, 88% for lamb and 87% for pork of total nitrogen after pepsin/pancreatin digestion.

III. CONCLUSION

This study gives insight in the bulk protein composition of *T. molitor* and the *in vitro* digestibility, thereby contributing to knowledge needed for future food applications of this insect species. The main conclusion is that proteins from insects do not behaviour very differently from conventional meat protein.

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