Project funder: UKRI BBSRC, FoodBioSystems DTP
Industrial sponsor: Fera Science

Optimising the Safety and Nutritional Quality of Insect Protein Ingredients

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Introduction

Insects are one of the emerging alternative sources of protein which are gaining recognition for their ability to address the environmental and nutritional challenges facing the growing global population. Insects are consumed by two billion people worldwide[1] and have comparable, and in some respects, superior nutritional quality to other animal proteins[2]. However, ensuring the safety of emerging insect consumption in Western cultures, where they are not currently habitually consumed, is critical to ensure food safety and encourage a positive perception of insect consumption. Allergenic proteins often have structural features that make them resistant to digestion, allowing intact or large fragments to reach the intestinal mucosa, where they can be absorbed and potentially trigger allergic reactions[3]. By understanding how novel proteins behave throughout the digestive system we can hypothesise as to their allergenicity, and *in vitro* models can then serve as screening tools for allergen risk assessments[4]. The overarching aim of this project is to address the allergenicity concerns associated with the cross-reaction potential of insect proteins to allergenic shellfish proteins, as well as evaluating the digestibility profile of insects as food.

In vitro digestion methods

The first stage of this research is centred around an *in vitro* digestion protocol which aims to simulate human digestion in three distinct phases: oral digestion, gastric digestion and intestinal digestion. The digestive protocol has been designed to represent a range of biological demographics and digestive conditions, at multiple time points. The protocol will be tested with four different insect samples (three species), a shellfish comparison (king prawn) and an established digestive control (skimmed milk). Results from the first two stages of digestion are presented here.

Table 1: *In vitro* digestion protocol conditions for the first two stages of digestion: oral and gastric phases. This is not inclusive of all digestive reagents but provides a summary of the variable conditions and samples.

Oral phase	Gastric phase		
Proteins	Enzyme level	pH level	Timepoints
Cricket (*Acheta domesticus*) **powder no.1** **Cricket** (*Acheta domesticus*) **powder no.2** **Mealworm** (*Tenebrio molitor*) **powder** **Black soldier fly larvae** (*Hermetia illucens*) **powder** **Freeze-dried prawn** (*Penaeus vannamei*) **powder** **Skimmed milk powder**	**High pepsin** (HP): 1000U/ml (healthy adult) **Low Pepsin** (LP): 100U/ml (healthy infant)	**2.5** (before food enters stomach) **5.5** (after food leaves stomach)	**0.3** minutes **5** minutes **10** minutes **20** minutes **120** minutes **C** (control: no enzyme)

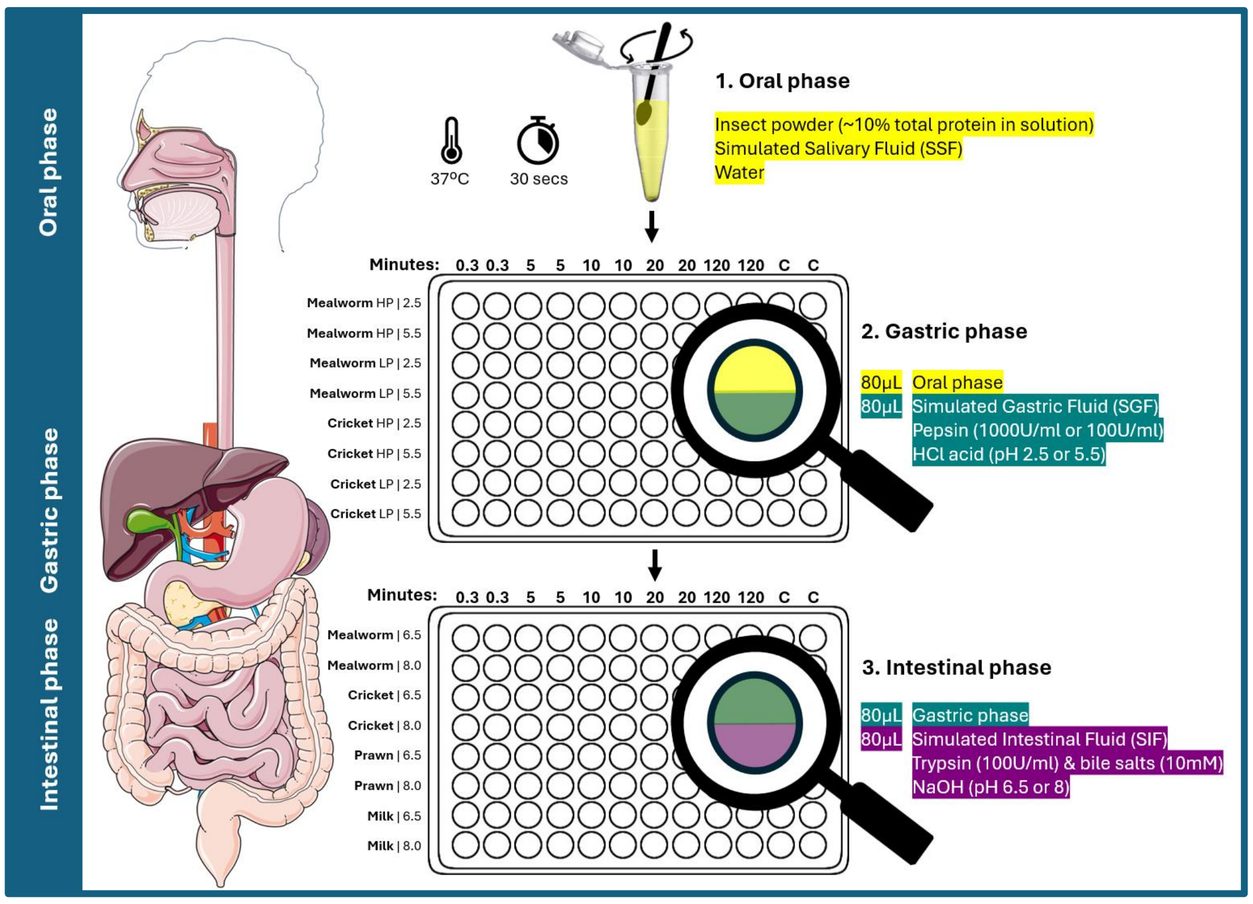
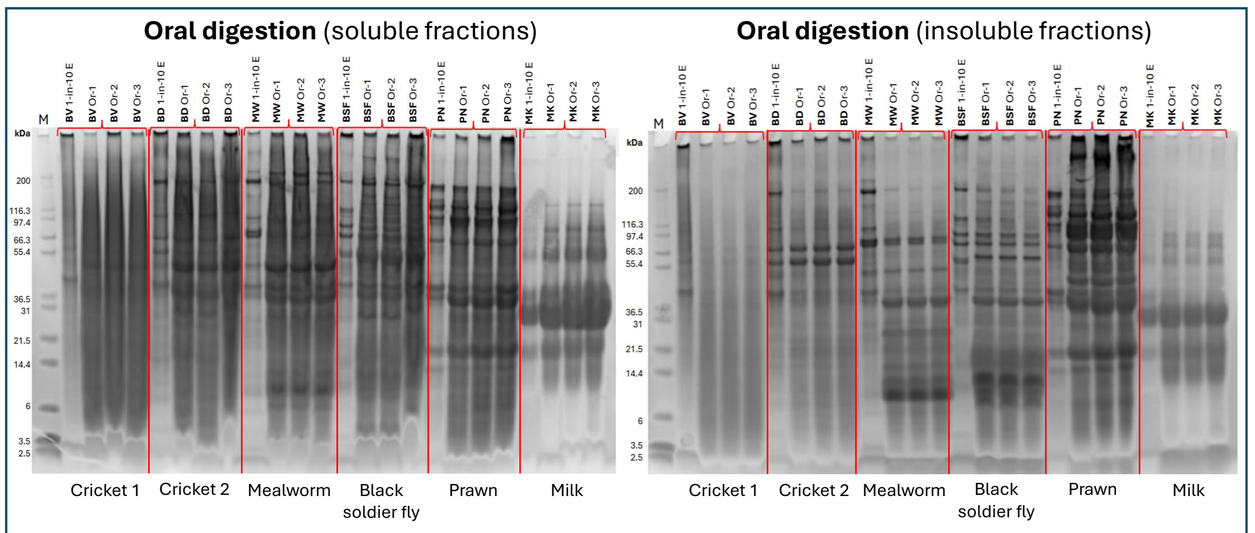


Figure 1: Schematic image depicting the multi-phase procedure of the *in vitro* digestion protocol. The insect samples included are used to exemplify what a single 96-well plate layout could look like but are not an exhaustive list of all the sample-condition combinations tested. Volumes of reagents for each phase are given for a single well.

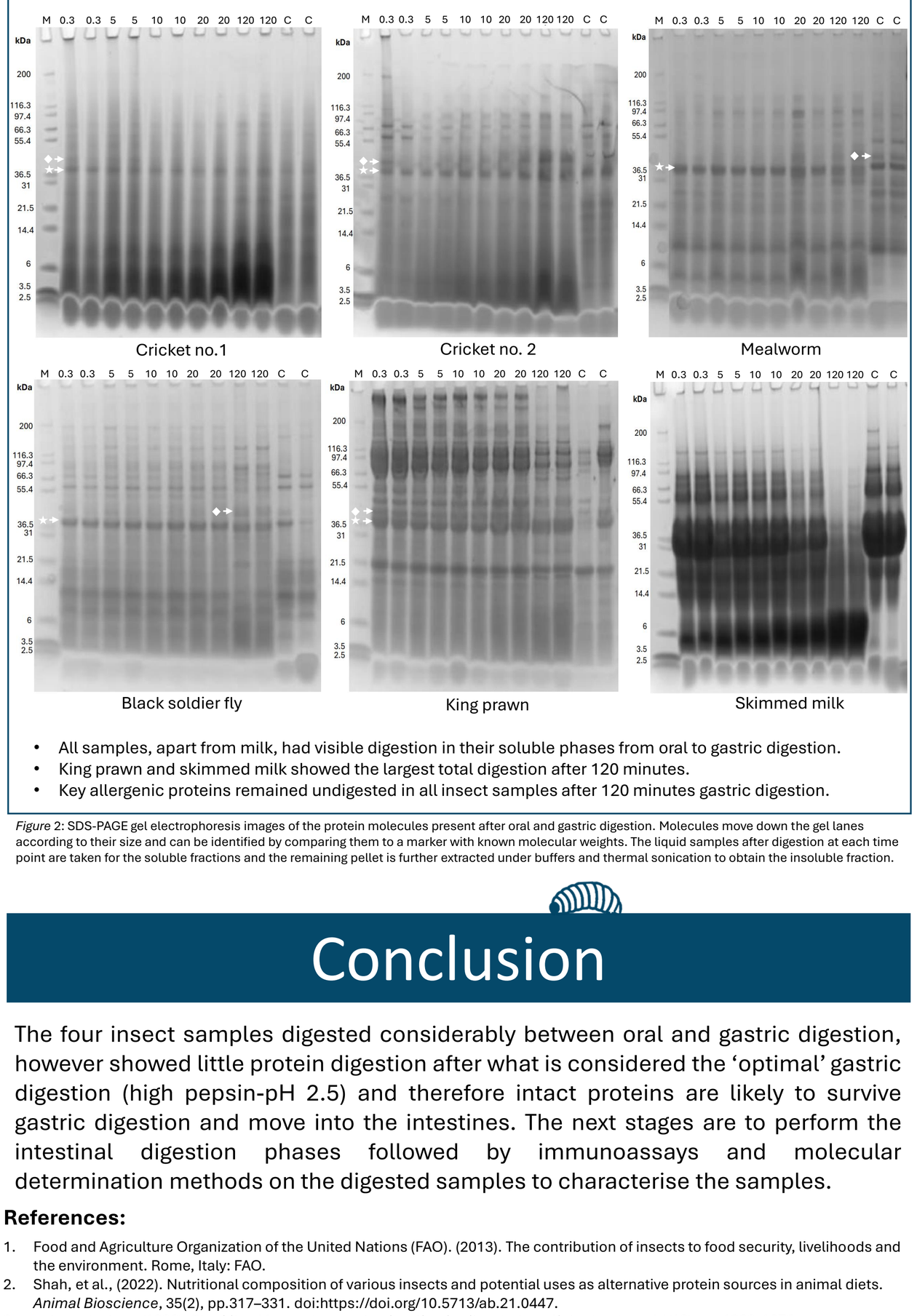
Results



- Following oral digestion, the digestive products of all insect samples were predominantly soluble molecules.
- The king prawn formed a gel during oral digestion and so had a higher amount of insoluble material.

Gastric digestion: high pepsin, pH 2.5 (soluble fractions)

Key allergenic proteins:
★ Tropomyosin (~37kDa)
◆ Arginine kinase (~40kDa)



- All samples, apart from milk, had visible digestion in their soluble phases from oral to gastric digestion.
- King prawn and skimmed milk showed the largest total digestion after 120 minutes.
- Key allergenic proteins remained undigested in all insect samples after 120 minutes gastric digestion.

Figure 2: SDS-PAGE gel electrophoresis images of the protein molecules present after oral and gastric digestion. Molecules move down the gel lanes according to their size and can be identified by comparing them to a marker with known molecular weights. The liquid samples after digestion at each time point are taken for the soluble fractions and the remaining pellet is further extracted under buffers and thermal sonication to obtain the insoluble fraction.

Conclusion

The four insect samples digested considerably between oral and gastric digestion, however showed little protein digestion after what is considered the 'optimal' gastric digestion (high pepsin-pH 2.5) and therefore intact proteins are likely to survive gastric digestion and move into the intestines. The next stages are to perform the intestinal digestion phases followed by immunoassays and molecular determination methods on the digested samples to characterise the samples.

References:

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