INSECTECH: RATIONAL ENGINEERING OF INSECT PROTEINS FOR AGE-TAILORED FUNCTIONAL FOODS

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Background – Ensuring security of edible proteins using insects

Dietary proteins are a major source of amino acids and bioactive peptides that play important roles in human health and well-being. Nowadays, health and nutrition agencies declare that one of the greatest nourishment challenges is the adequate provision of protein and/or energy[1–3]. In this respect, insect proteins are enthusiastically explored as viable, cost-effective and more sustainable alternatives to animal-based proteins, e.g. meat and poultry[2,4–7]. However, introducing insects into the Western diet is inhibited by low consumer acceptance. This may be improved by processing the insects to eliminate the insect appearance[2,8,9].

The changes in world demographics raise another nutritional challenge - the challenge of feeding growing portions of the population that have specific and unique nutritional needs, namely the ageing population. In fact, nourishing and caring for the elderly have been identified as one of the rising global challenges[10]. From the commercial perspective, personalized and age-tailored nutrition of seniors are still in their infancy, with most efforts focused on eating preferences and patterns rather than on product engineering to meet consumer physiological needs. From the research perspective, in vitro digestion (IVD) models show great promise as reliable, robust and high throughput tools of research that might facilitate such progress and tackling issues of malnutrition[11,12]. Moreover, recent studies show IVD models are well correlated with in vivo findings and might be good alternative to animal models[13].

Study goals and hypotheses

Current commercial endeavors seek to introduce insects into large-scale production for westerners through masking the insect appearance in commonly accepted products, e.g. snack bars, chips, shakes and pastry products. In many cases, the production of such products involves various processing operations, including grinding, drying and various thermal processes (for example, cooking or baking). Such processing operations are known to have diverse effects on the digestive fate of animal and plant proteins. Yet, scant scientific literature exists on the impact of such processing operations on insect proteins.

Thus, the ***long-term objective*** of this project is to systematically investigate the potential of insect proteins to serve as a rich source of dietary protein for infants, adults and the elderly. Specifically, the ***overall goal*** of this study is to link the physicochemistry of proteins from crickets, locust and silk moths to their *in vitro* digestibility and proteolytic breakdown under adult and elderly GI conditions following no processing, thermal processing and novel high pressure processing. The ***key hypotheses*** are: [I] The physicochemical differences between various insect proteins alter their colloidal stability and bioaccessibility during digestion; [II] Age-related differences in GI functions affect insect proteins' bioaccessibility and generation of bioactive peptides in the GI of adults and the elderly; [III] Thermally-induced Maillard glycation of insect proteins increases the proteins' ability to resist proteolytic degradation under adult and elderly GI conditions; [IV] High pressure processing affects the physicochemical characteristics of insect proteins which in turn alter the proteins' susceptibility to the various digestion conditions.

Summary of key results and conclusions

As a first step, a PhD student was recruited to lead the project and has intiated reseach into cricket-based proteins. To date, various chemical and biochemical analyses have established:

1. **Protein content of cricket flour (CF) is high but over-estimated in many studies** - Cricket flour contains 49.0% (w/w) protein, which allows the classification of cricket flour as a protein-rich product. This finding is contrary to past reports that have over-estimated the protein content of crickets to exceed 60% (w/w) protein. This also supports two recent publications warning from the over-estimation of protein content of various edible insects[14,15].
2. **Common processing operations alter appearance, solubility and chemistry of CF** - Cooking (70^{o}C) and baking (at 180^{o}C) of cricket proteins in the presence of fructose affects various properties of the proteins, including their solubility and appearance. This has been attributed to the cross reactivity between the proteins and the monosaccharide via the Maillard reaction.
3. **Common processing operations alter the antioxidant capacity of CF** - Another intriguing functionality of food proteins is their ability to interfere oxidative reactions, i.e. act as antioxidants [16]. Therefore, the antioxidant capacity (AOX) of processed and unprocessed cricket flour samples using FRAP and ORAC AOX assays was also determined (**Figure 1**).

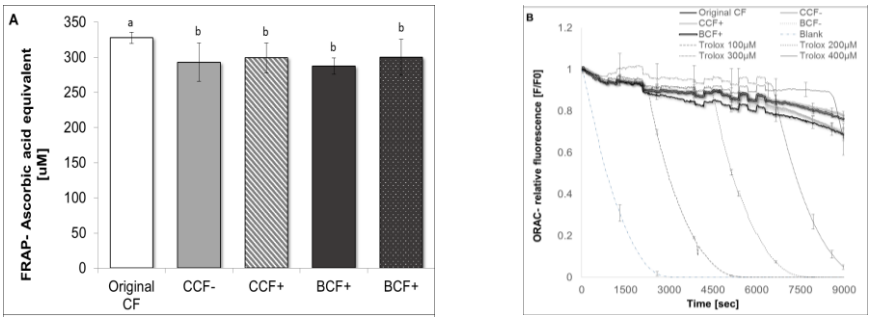

Figure 1. Antioxidant capacity of untreated cricket flour (CF), cooked cricket flour (CCF-), baked cricket flour (BCF-), cooked cricket flours with added fructose (CCF+) and baked cricket flour with added fructose (BCF+). [A] Results of FRAP analysis and [B] ORAC analysis. Letters indicate statistically different values ($p<0.05$).

The findings suggest a mixed trend in which processing both deteriorated and elevated the antioxidant capacity of the cricket flour. The obtained results imply that thermal processing of CF impaired its ability to serve as an electron donor to quench radicals (**Figure 1A**), possibly due to seclusion of some amino acids (e.g. Tryptophan or Histidine). At the same time, ORAC findings (**Figure 1B**) demonstrated an increased AOX activity of as a proton donor, attributed to the exposure of cysteine's thiol groups. The bio-relevance of the ORAC assay [17,18] stimulated a need to understand the bioaccessibility and digestive fate of the processed CF samples.

4. **Processing accelerates CF gastric proteolysis in the adult stomach** – CF control samples as well as cooked or baked CF samples have been fed into a dynamic dual-bioreactor in vitro digestion model[19] mimicking the gastro-intestinal digestion of a healthy adult to evaluate the proteolytic breakdown of the CF proteins. SDS-PAGE analyses (**Figure 2**) of digesta samples revealed that thermal treatments altered the gastric breakdown of treated CF samples (CCF-, CCF+, BCF-, BCF+) compared to the breakdown of the control unprocessed CF (**Figure 5A**). Additionally, cooking and baking in the absence of fructose (**Figure 5B** and **Figure 5C**, respectively) and in its presence (**Figure 5D** and **Figure 5E**, respectively) presented a differential impact on the proteolytic fate of CF proteins in the gastric phase but not during the intestinal phase. This concurs with prior evidence showing that early stage Maillard reaction products may exhibit increased proneness to digestive proteolysis[20–24].

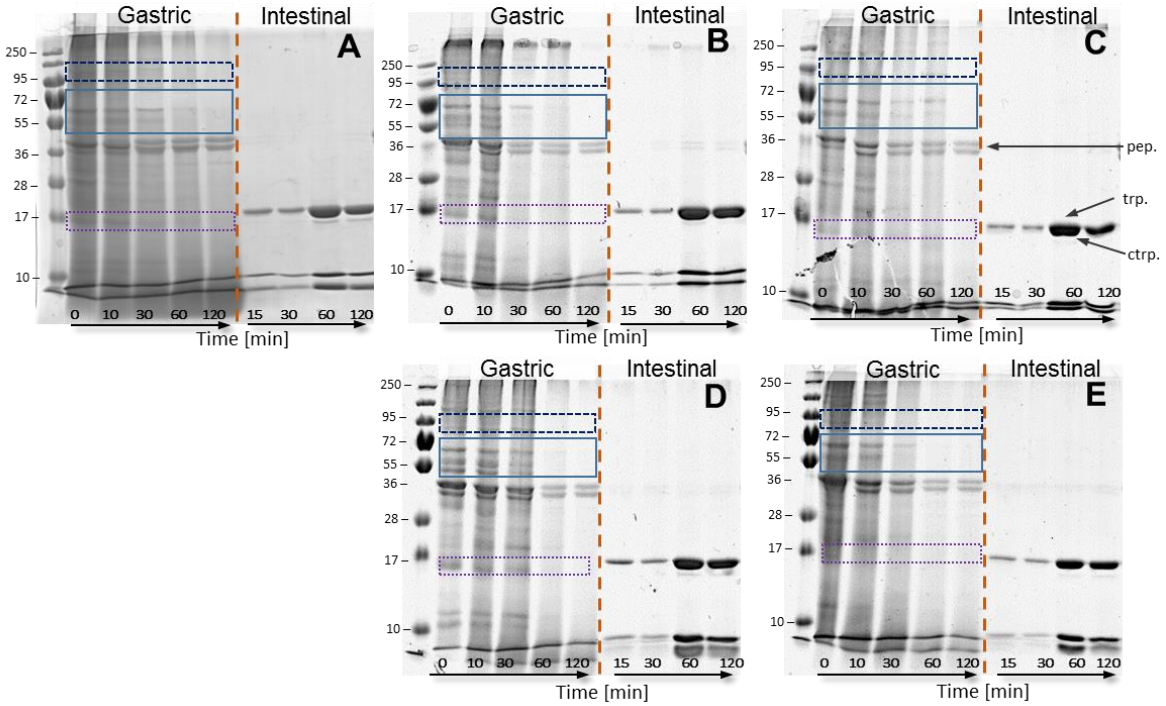

Figure 2. SDS PAGE analyses of *in-vitro* GI digestion under adult conditions of [A] untreated cricket flour (CF) [B] Cooked cricket flour (CCF-) [C] Baked cricket flour (BCF-) [D] Cooked cricket flours with added fructose (CCF+) and [E] Baked cricket flour with added fructose (BCF+). Boxes highlight protein bands that differ between samples. Proteolytic enzymes are indicated by arrows (Pepsin, Trypsin and chymotrypsin indicated as pep., trp. and ctrp.).

On going work has been interrogating the effects of high-pressure (HP) homogenization (a process similar to homogenization of cow's milk) on cricket, locust and silk moth pupae flours (CF, LOF and SMF repectively). Relevant processed and unprocessed samples were studied and subjected to a dynamic IVD model operating under adult conditions.

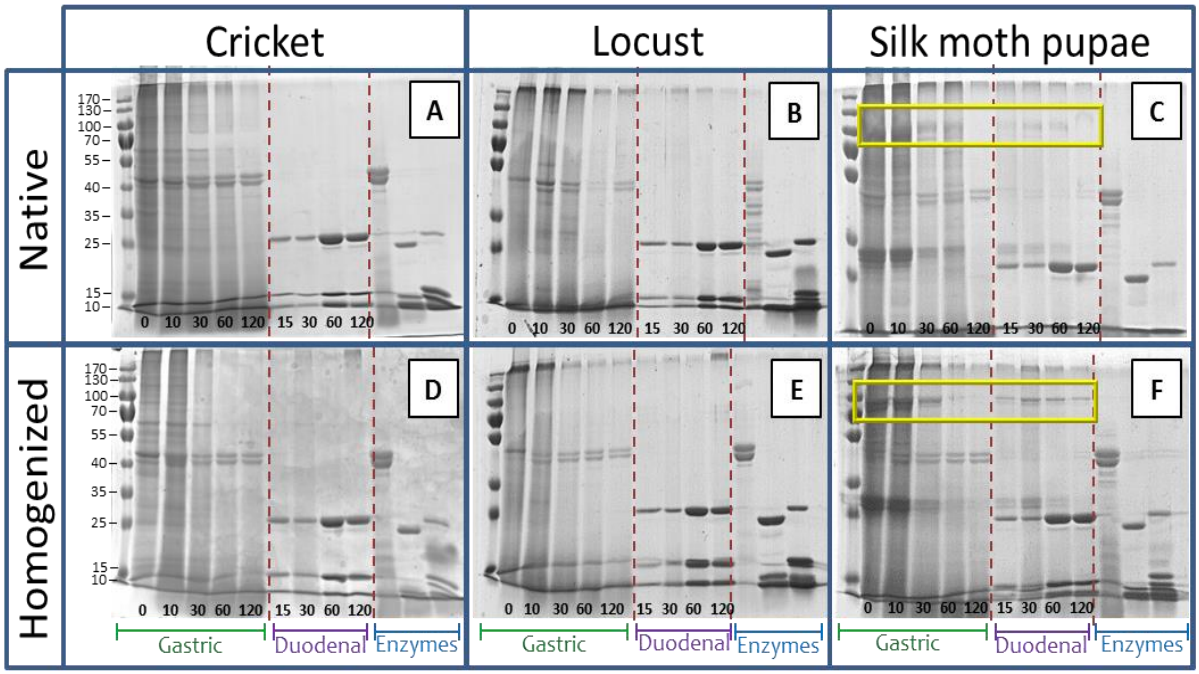

Figure 3. *SDS PAGE analyses of adult in-vitro digestion of insect flours showing that high pressure homogenization at 100MPa does not alter the digestive proteolysis of cricket and locust flours but has an effect on silk moth pupae flour. Digesta analysis of (A) native CF (B) native LOF (C) native SMF (D) homogenized CF (E) homogenized LOF and (F) homogenized SMF. Numbers on the bottom represent digestion time in minutes from beginning of digestion.*

Aliquots of digesta taken at defined time-points both from gastric (0, 10, 30, 60, 120 minutes) and intestinal (15, 30, 60, 120 minutes) bioreactors of the dynamic IVD model have been analyzed by SDS-PAGE, as shown in **Figure 3**. When comparing **Figures 3A, 3D, 3B** and **3E** it seems that HP homogenization does not alter gastrointestinal proteolysis of proteins from crickets or locust flours. This alone is a significant finding as it provides a direct indication that commercial homogenization at pressures up to 1000atm is not expected to compromise the bioaccessibility of proteins in these insect flours. Interestingly, proteins from silk moth pupae exhibit slight modification in their pattern of digestive breakdown (**Figure 3C** and **3F**). This may be linked to altered bioaccessibility and modified nutritional values, however, these are pending further investigation.

Conclusion and future plans

Part of this work has already been presented in two international conferences and collated into a manuscript submitted for peer-review and publication in the journal of Food Hydrocolloids. Moreover, the findings presented herein were elemental in the successful funding application to the Israeli Ministry of Health (grant# 3-12834). Based on the success and progress of the project, the upcoming year of research intends to dedicate efforts to two specific task:

A. **Studying adult digestive proteolysis of unprocessed and processed locust and silk moth proteins.** This part of the work will extend the investigation of cricket flour to explore the digestibility and antioxidant capacity of locust and silk moth flours. This will be done based on the established methodologies described in this report.
B. **Studying elderly digestive proteolysis of cricket, locust and silk moth proteins.** Samples will undergo simulated digestion using a dynamic IVD model mimicking elderly gastro-intestinal digestion, based on our recent publications [19,25,26]. Similar to recent work done using an adult IVD model, samples will be collected from the gastric and intestinal bioreactors at selected times and then analyzed via SDS-PAGE and proteomic analyses. These will enable monitoring and comparing proteolysis, bioaccessibility of amino acids and generation of bioactive peptides in the different IVD models, similar to our recent work[25].

Overall, this project will continue its efforts to understand the underlying scientific and technological principles guiding the digestibility of insect proteins, the impact of processing on their digestive fate and their potential nutritional values. Specific efforts will be dedicated to gather scientific evidence on the potential differential digestive fate of insect proteins in the gut of the elderly. Project closure will include collation of scientific evidence into 1-2 additional scieitific publications. This will underlie the rational processing of insect proteins to attain desired digestibility profiles in the gut of adults and seniors. This will open new opportunities for the food industry to utilize insects as nutritious yet sustainable resources. In addition, this project will provide a scientific basis for food and health care professionals as well as policy makers pertaining to the potential nutritional values of insect proteins. This could help fully exploit the potential of insects to serve humankind and beneficially affect consumer health.

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