Processing of edible insects to produce novel foaming agents

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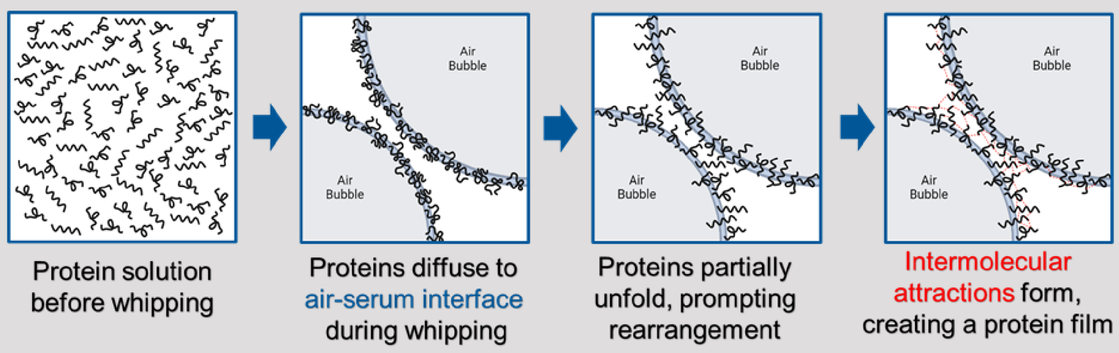
Introduction

In 2019, the United Nations (UN) projected world population would reach over 9.5 billion by 2050[4]. The UN has also projected the demand for protein to double by 2050. Food systems must incorporate alternative sources of protein to meet global needs safely and sustainably. Protein is a key component of foods for organoleptic and nutritional purposes, and one greatly assessed option to reduce the environmental impact of food systems is the utilisation of insects as a source of protein. An association of insects with disgust is prevalent in western cultures[1]. To overcome this, it is key to process insects into powders or extracts and mask the presence of insects in a food product[1].

Protein in food products also serve a functional purpose. One functionality of insect proteins that is not widely explored in literature is foaming. In protein-stabilised foams, the protein solution (termed the 'serum') is whipped to incorporate air. Soluble proteins diffuse to the air-serum interface and orient according to hydrophobicity. Interactions between proteins form a viscoelastic film around air bubbles. Proteins reduce surface tension between the air phase and liquid phase, which facilitates the formation of bubbles. Foaming is important in many food products, from a cappuccino to a meringue or a mousse, but each food product will require a certain foam stability (the rate at which a foam degrades) and foam capacity (the volume of air incorporated). To justify the use of insect protein as a novel foaming agent, insect protein foams should exhibit high foam stability and an appropriate foaming capacity given the context of the product: some products may require a dense, uniform foam where others require an airy, inhomogeneous foam.

In this study, we investigate the foaming properties of proteins extracted from two insect species, Yellow Mealworm (*Tenebrio molitor*) and Black Cricket (*Gryllus bimaculatus*), using a modified Osborne fractionation. The foaming properties of insect protein extracts, ground insect powder, and defatted insect powder were compared to a commercial foaming agent (egg white protein, EWP) to assess the potential of insect proteins as a novel foaming agent.

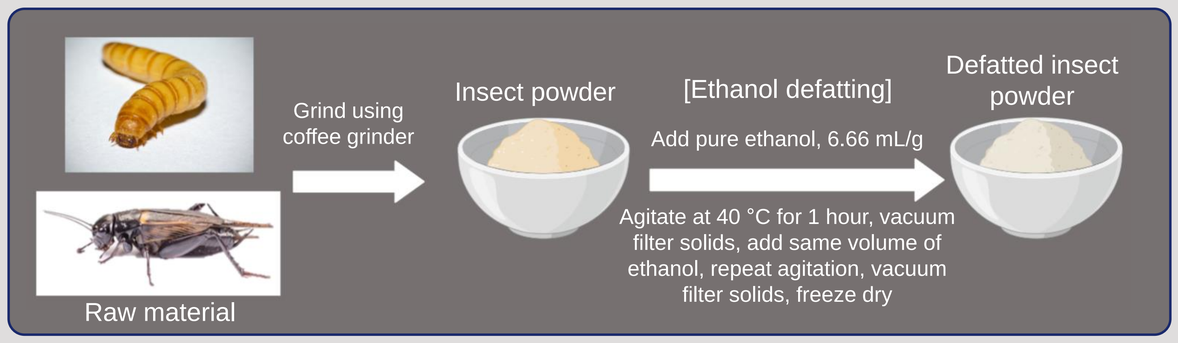
How do proteins form and stabilise foams?

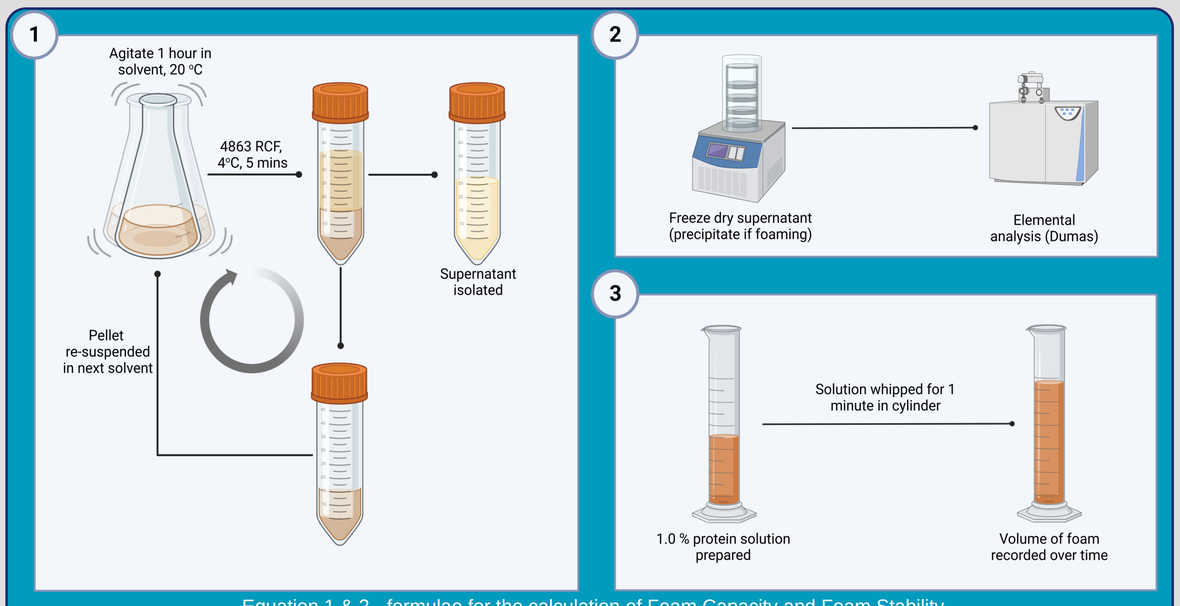


Methodology

Workflow

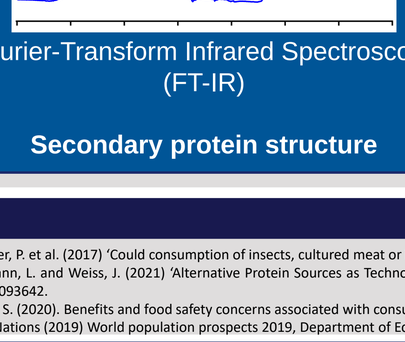
Insect powder preparation and defatting → **Protein fractionation, quantification, and foaming assay** → **Protein characterisation**





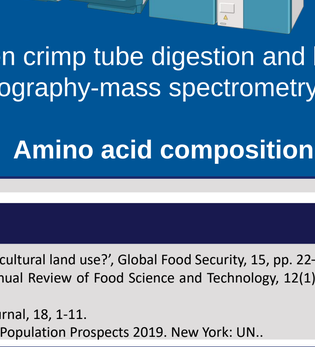
Equation 1 & 2 - formulae for the calculation of Foam Capacity and Foam Stability

$$FC = \frac{Foam\ Volume\ (0\ \mathrm{min})}{Solution\ Volume} \times 100 \qquad FS = \frac{Foam\ Volume\ (10\ \mathrm{min})}{Foam\ Volume\ (0\ min)} \times 100$$



Fourier-Transform Infrared Spectroscopy (FT-IR)

Secondary protein structure



Oven crimp tube digestion and liquid chromatography-mass spectrometry (LC-MS)

Amino acid composition

Results & Discussion

Fractional composition of insect protein

Figure 1 displays the fractional composition of both insect species. Similarities in fraction distribution are clear between insect species; alkaline-soluble glutelins presented as the major fraction; water-soluble albumins and salt-soluble globulins represented secondary fractions; alcohol-soluble prolamins and mercaptoethanol-soluble DSB-CLF presented as minor fractions. DSB-CLF was the smallest fraction extracted.

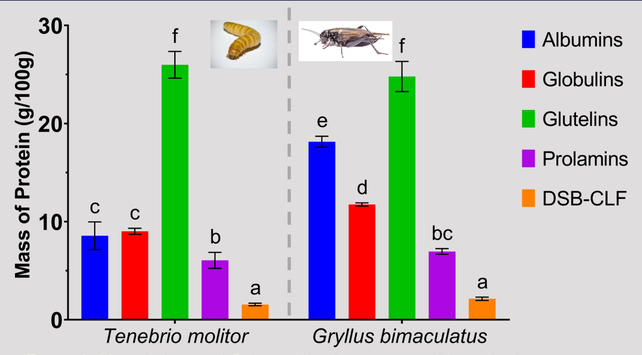


Figure 1: Mass balance of Osborne fractions extracted from the insect species. different letters above columns indicate significant difference ($p < 0.05$) according to the Bonferroni multiple comparison test, following a 2-way ANOVA. Error bars correspond to standard deviation

Glutelins are derived from connective tissue, including that of muscular protein[2], and as these tissues would be the most prevalent in the insect species, it is logical that this fraction was the major fraction. Similarly, albumins and globulins are derived from muscular protein[2] (sarcoplasmic and myofibrillar proteins, respectively) explaining why they presented as larger fractions, considering the biology of the insects.

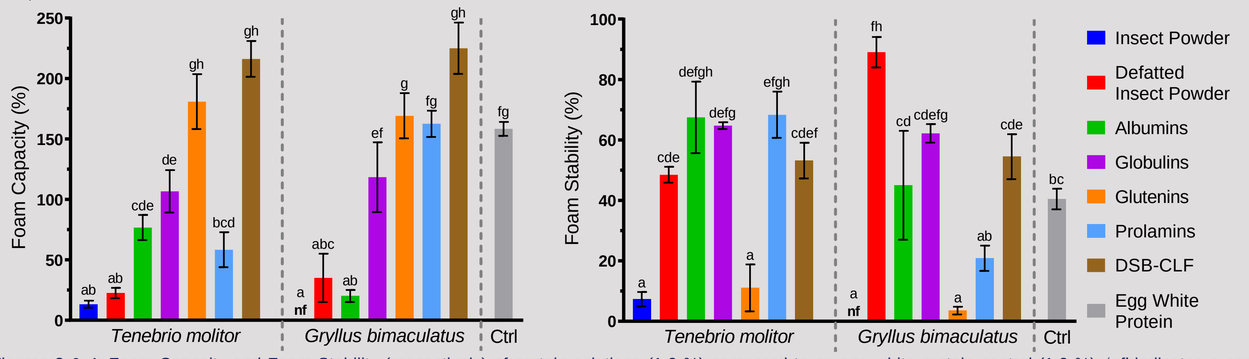
Amino acid analysis of insect powders (data not presented) showed very similar distributions between the insect species, which explains why similar fractional distributions were observed. Separation based on solubility is reliant on amino acid composition as the nature of variable R-groups determines protein charge, hydrophobicity, and acidic/basic nature, all of which affect protein solubility in different solvents. Very little cysteine and methionine was present in the insect species, and as these are the only sulphur-containing amino acids this could explain why the DSB-CLF presented as the smallest fraction, as mercaptoethanol solubilises protein by cleaving disulphide bridges.

Foaming properties

Foam stability and capacity were calculated using Equations 1 and 2. As shown in Figures 3 & 4, defatting significantly enhanced the foaming properties of insect powders. This was attributed to the lipid present in the crude powders, which is known to act as an antifoaming agent[5]. Foam capacity and stability were not significantly different between like fractions from different insect species, except for prolamins, where those from *G. bimaculatus* exhibited greater foam capacity, but those from *T. molitor* presented a greater foam stability.

FT-IR analysis showed a significant positive correlation ($p<0.05$) between the proportion of random coil protein in Osborne fractions and foam capacity. DSB-CLF from both insect species presented both the greatest foam capacity and greatest proportion of random coil structure (63.41 and 74.90 % for *T. molitor* and *G. bimaculatus* respectively). Similarly, the prolamins from both insect species presented the lowest foam capacity of the Osborne fractions and the lowest proportions of random coil structure (13.24 and 29.25 % respectively). Literature suggests that a greater proportion of random coil structure affords proteins greater flexibility, which facilitates faster unfolding at the air-water interface during whipping[6] allowing encapsulation of air bubbles within the viscoelastic protein film. No significant correlations between secondary protein structures and foam stability were found.

Considering foaming performance of Osborne fractions in comparison to the EWP control, foam capacity of glutelins and DSB-CLF extracted from *T. molitor,* and globulins, glutelins, prolamins, and DSB-CLF extracted from *G. bimaculatus* were not significantly different than the EWP control. Foam stabilities of defatted insect powder and DSB-CLF from *T. molitor*, and globulins, glutelins, and DSB-CLF from *G. bimaculatus* were not significantly different than the EWP control. Defatted *G. bimaculatus* powder exhibited greater foam stability than EWP. In the context of food foams, the protein fractions which were not different in either foam stability or capacity from EWP present the best opportunity to use insect-derived ingredients as a replacement for EWP. Those with a lower foam capacity may not be able to facilitate sufficient aeration of the food matrix, and those with a lower foam stability could result in a product which degrades rapidly and would not be suitable for products which require stability over a long period of time, such as mousse.



Figures 3 & 4: Foam Capacity and Foam Stability (respectively) of protein solutions (1.0 %) compared to an egg white protein control (1.0 %). 'nf' indicates a non-foaming sample. Error bars correspond to standard deviation, and the presence of different letters indicate significant difference between samples ($p<0.05$), according to the Bonferroni multiple comparison test, following a 2-way ANOVA. Error bars correspond to standard deviation

Conclusions & Outlook

The research presented indicates that glutelins and DSB-CLF from *T. molitor,* as well as globulins, glutelins, prolamins, and DSB-CLF from *G. bimaculatus* show potential as novel foaming agents, in food products where a high capacity is required over a high stability. DSB-CLF from both insect species could be a suitable replacement for EWP, as both the foam capacity and foam stability of this fraction were not significantly different from EWP.

Foam capacity was explained by considering the secondary structure of proteins, but further work is needed to explain observed differences in foam stability, where surface hydrophobicity and the presence of protein aggregates are likely targets of investigation

Conclusively, with suitable refinement, insects offer an under-utilised source of novel foaming agents

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