Promoting entomophagy as a response to food insecurity through the development of suitable food products

Key words: Entomophagy, Cricket Powder, Production, Nutrition, Safety, Consumer Acceptance, Product Development

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Abstract

Utilisation of insects as food is a rapidly expanding global market due to insect's viability as a sustainable and economical source of high-quality protein. While they are traditionally consumed in many countries, in the Western world it is still unclear how best to exploit this new resource, while conforming to speculative safety regulations. Although literature demonstrates the highly malleable and varied nature of insects as a food source, a powder, produced from crickets, is seen by many as a versatile and nutritious form in which insect-derived food may be accepted in Western culture. The primary aim of this study was to take four cricket species (Gryllus bimaculatus, Gryllodes sigillatus, Gryllus assimilis, Acheta domesticus) and Desert locust (Schistocerca gregaria) from farm to the fork through exploring the impact of different methods of powder production on the nutritional value and safety of the powder before considering barriers to consumer acceptance and how products containing cricket powder may be produced that are acceptable to such consumers. Some differences were seen in nutritional composition between cricket species, and drying methods impacted on the fatty acid and protein composition. Drying methods also had some impact on the microbial content of the powders. However, in general, all insect species and methods of production yielded a potential food ingredient rich in high quality protein and a range of minerals which, when stored appropriately, represented a low biological risk to the consumer. Further analysis of commercial cricket powder demonstrated the presence of hard to remove pathogenic microorganisms (Bacillus cereus & Bacillus lichenformis), an insecticidal microorganism (Brevibacillus Laterosporus), that may pose an issue for insect farmers, and two organisms with limited previous study (Rummeliibacillus stabekisii & Lysinibacillus Pakistanesis). A survey on consumer perspectives demonstrated clear expectations for insect-based food, as well as biases towards what types of products may be suitable for the UK market and key areas that would be unacceptable to consumers at this stage. Combining all of the information gathered throughout the study, allowed for the development of an alkaline noodle product which provided insight into how the utilisation of insects in food development resulted in a decrease of product quality that could be overcome through adjustments to the formulation. Overall, this project demonstrates that products containing insects can be produced which are safe, and nutritious and potentially suitable for both the promotion of entomophagy in the Western world and combatting a key area of protein malnutrition in South-East Asia.

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Chapter 1: Introduction and Literature review

Population is predicted to continue rapid growth throughout this century resulting in expanding food scarcity and even more difficulties for the populations that already struggle with this issue (Gerland et al., 2014; Kummu et al., 2017). Further exacerbating this issue is the impact of climate change. Part of the difficulty associated with climate change is that food production contributes to the factors that cause climate change and most methods of increasing necessary food production are a detriment to the goal of reducing climate change (Frank *et al.*, 2017). A major contributing factor of the climate change dilemma is protein production as the majority of this industry is unsustainable but also required to continue expanding so that the world can be adequately fed (Hartmann and Siegrist, 2017). As the need for sustainable protein sources increases due to these pressures, insects are being increasingly proposed as a potential solution to a future crisis (Durst, et al., 2008). This is primarily due to their ethical and sustainability credentials when compared to conventional protein sources, particularly meat (van Huis et al., 2013). Additionally, insects are by far the most diverse class of animals our planet has to offer but one of the least explored as far as their potential for food resources goes (Van Huis, 2016).

Currently there are approximately 2000 species of insects that are recorded as being edible, spread across 113 countries, being consumed by over 3000 unique ethnic groups (Yen, 2009). However, rates of consumption in some areas are decreasing, and the Western culture is a focal point for blame. Bias towards farming methods and cultural food acceptance has led to the promotion that insects are a "primitive food source that serves little purpose" (Looy, et al., 2014). This has led to some indigenous populations to turn away from traditional sources of food and revere the westernised diet (DeFoliart, 1999). The rejection of entomophagy is preventing the consumption of a food source that could reduce nutritional deficiencies, as insects can supply a large portion of protein, micronutrients and calories to the diet, especially when there is little available alternative.

The promotion of Westernised intensive farming processes, relying heavily on pesticides, has generated a nutrient deficiency in some cultures by killing off insect protein sources that were harvested as a by-product of farming other crops (Durst and FAO Regional Office for Asia and the Pacific., 2010). This was the initial spark of interest in entomophagy, generated through studies by the FAO (van Huis, et al., 2013). Through this work It is now believed that promoting entomophagy in Western cultures could reduce this damage, by developing an understanding of the value of insect protein and creating an acceptance of insects as a food source. The simplified theory is that a reduction in cultural bias in the Western world would in turn allow for the re-introduction of traditional foods, where insects make a significant contribution to avoiding malnutrition. This thesis focuses on the first part of the cycle, considering ways to utilise insects within the Western diet in order to overcome the bias we have towards consuming them.

1.1 Insect consumption around the world

Entomophagy in areas of Asia and South America is a relatively common practice but consumption of insect's spreads to areas of India, Africa and the Middle East too (Durst and FAO Regional Office for Asia and the Pacific., 2010; van Huis *et al.*, 2013). A few countries, such as Thailand and Mexico, popularise insects as an important part of their diet (Tan *et al.*, 2015). Contrary to most other populations, they do not simply believe in their nutritional necessity, but they believe them to have good hedonic properties as well (Taylor and Wang, 2018). Outside of these countries, where insect consumption is popularised almost ubiquitously, the majority of insects are consumed by small rural communities and tribal populations in developing tropical countries (DeFoliart, 1997; Lautenschläger *et al.*, 2017; Gahukar, 2018; Stull *et al.*, 2018). Insects often form their primary protein source due to meat being either too costly, or livestock serving other primary purposes, such as providing necessary labour in the rearing of crops or important products such as milk and eggs.

The type of insect consumed varies greatly by what is available, but consumption in these areas is almost entirely from wild harvest. Harvesting insects from the wild in these tribal areas usually provides multiple benefits. In addition to the nutrition when they are eaten, they can be harvested as a by-product of crop production, lending benefit to the farmers as they suffer less from pests if they are removed. Other insects harvested from the wild either nest in large numbers or swarm, making them an attractive option for people looking for food because the level of effort for the reward is low (Paoletti, 2005; Meyer-Rochow and Hakko, 2018). Few exceptions exist to those generalisations on the wild harvest, such as tarantula's that are commonly eaten in rural Cambodia or the hornets of Japan which are theorised to meet the criteria for being worthwhile due to their individual size (Yen, 2015).

The wild harvest potentially poses some problems though, as some insects harvested from the wild are linked with health risks. Some species such as stink bugs, produce toxins as a defence mechanism but these are still consumed in India (Gahukar, 2018; Murefu *et al.*, 2019). Other potential risks come in the form of antinutrients and heavy metals. There have been cases of ataxia syndrome from the consumption of silkworms which can contain thiaminases (van Huis *et al.*, 2013). A few cases of heavy metal toxicity from insects have been reported including lead toxicity in California resulting from the consumption of imported chapulines (crickets) from Mexico (Handley *et al.*, 2007). Heavy metal toxicity can occur due to insect's ability to bioaccumulate chemicals from their environment which also makes chemicals such as pesticides and fertilisers a potential risk too (Fernandez-Cassi *et al.*, 2018).

There are a few examples of insect farming from around the world. Notably, Thailand has developed farming methods, as the wild harvest is not enough to keep up with their growing consumption (Paoletti, 2005). In addition, there is the consumption of silkworm pupae as a by-product from silk production (Mishra *et al.*, 2003). Due to providing multiple functions, silkworms have been suggested to be one of the insect species with the greatest potential, even for the future of endeavours such as extended space travel (Yang *et al.*, 2009). This dual functionality makes silkworms an attractive proposition as there is already a surplus of them available, but this surplus is not reared for human consumption and as such has none of the regulations required to ensure them safe to consume (Zhou and Han, 2006). The changing of the standards for an entire established industry is no small task. In more recent years, farming has begun to be established in the US, Canada and parts of Europe for use in both animal feed and for the production of powder to be used in food production.

1.2 Why the Western World does not consume insects

According to anthropologists, insects were consumed globally during the huntergatherer period of human development and this practice continued into ancient civilisations, demonstrated by hieroglyphics and cave paintings (Kohl, 2016). In addition, reference to the consumption of some swarming insects by cultures with restrictive diets have been noted in the bible suggesting that these could have been an important food source when they were destroying crops (van Huis *et al.*, 2013). However, the practice faded out over time in temperate and cool regions. This is believed to have been due to the effort vs reward being less than other sources of protein (van Huis, 2017). Warm climates allowed insects to evolve larger or in such large numbers that harvesting them remained viable, but not so in the Western World where hunting, animal husbandry and farming were able to produce more food for less effort (van Huis, 2017).

Throughout history, major events such as plagues, famines, and disease being blamed on insects contributed to their negative perceptions (Jensen and Lieberoth, 2019). Memories of these events turned the consumption of insects from being an unknown to something that should be feared and treated with disgust. The result is that insects in food are seen as a contaminant, something to be avoided in the Western world rather than celebrated as it is in other areas of the world (Sheppard, 2014; Hartmann and Siegrist, 2016). Westernised farming practices have evolved over time to allow for the mass production of food on a grand scale to accommodate our changing lifestyles. With the adoption of pesticides, insects gained a further negative perception, as something which only do harm to our food system, rather than potentially contributing to it (Jacob *et al.*, 2013; Müller *et al.*, 2016).

Despite this historical build-up of disgust and fear towards entomophagy, there have been attempts at the re-introduction of insects to the UK food chain before the current attempt. In the 1800's William Holt attempted to promote insects as a novel food for high society through the production of a book entitled "why not eat insects?" (Holt, 1885). Despite its apparent failure, the methods used within this book could have merit today. Holt discussed the potential merits of insect consumption alongside the presentation of menu's describing how to prepare insects to meet the tastes of people in that era. Additionally, the menus were presented in French, providing that high-class perception as it was commonly believed that French food was best, a perception which continues to linger today (Pinkard, 2009). A renewed interest in insects has highlighted consumer disgust, fear and neophobia as barriers to acceptance for the Western world (Looy, Dunkel and Wood, 2014; Baker, Shin and Kim, 2016; Clarkson, Mirosa and Birch, 2018; Myers and Pettigrew, 2018; Sogari, Menozzi and Mora, 2019).

1.3 The problem with Western bias

Western bias towards entomophagy has led to malnutrition in countries that consumed insects as a primary protein source (DeFoliart, 1999). It is believed that there are several linked causes of this issue stemming from the westernised phobia or disgust towards insects. Firstly, the provision of food aid is primarily made up of food that is not producible in the countries being aided which raises concerns about the psychological implications of promoting the provided food as better quality than what is available locally (DeFoliart, 1999; Harrison, Levinsohn and McMillan, 2013). The psychological impact being discussed is the potential that the provided food aid becomes aspirational over and above what is available in those countries (Harrison, Levinsohn and McMillan, 2013; Alemu *et al.*, 2016). In addition, it has been noted that tourism further promotes the Western diet, while shunning entomophagy due to unconscious bias, as disgust is often the first reaction of tourists to the thought of consuming unknown foods (DeFoliart, 1999; Yen, 2009). Ultimately, this unconscious bias leads to the dismission of traditional food systems.

Secondly, the promotion of Westernised farming systems without the consideration for other approaches has led to increased risk of toxicity in those that do continue to practice entomophagy (DeFoliart, 1997). Westernised farming utilises extensive amounts of chemicals in order to produce food. Whether it be pesticides, fertilisers, fungicides, insecticides or any other type of chemicals, people who consumed insects as part of the wild harvest have developed concerns as farming practices change in their local environment due to the perceived risk (Murefu *et al.*, 2019). There is also an aspirational effect as developing countries attempt to mimic the Western diet as wealth increases, creating desire for more meat and unhealthy diets which actively avoid insects (Goodland, 2001).

1.4 Creating a feedback loop

There are two primary purposes for the introduction of entomophagy to the Western world. The first is as an attempt to overcome the unconscious bias described above. Overcoming the bias towards entomophagy would allow for the development of better ways to treat malnutrition. The ultimate goal of which should be the development of new foods that could be easily produced within the community that is suffering from malnutrition through insect farming. This should also be accompanied by teaching the people who require help how to farm and produce these foods themselves in order to make this progress against malnutrition more permanent.

The secondary purpose appears to be the one receiving the most attention at the moment. Insects could allow for food security within the Western world to be maintained by developing suitable products that people like (Tao and Li, 2018). Insects, alongside other solutions such as in-vitro meat could be a way for the Western world to maintain its dietary practices while moving towards more

sustainable sources of food (Mandemaker, Kuttschreuter and De Vries, 2018). This potentially could be used as the steppingstone for utilising insects to help with malnutrition, but this detour takes time and allows for the systems that are causing the problems to get further established. Ultimately, it is difficult to change dietary patterns even when it is for the better (Garnett, 2013).

1.5 The modern history of entomophagy

The Food and Agricultural Organisation of the United Nations has been considering entomophagy as a pathway to food security since 2009, but their promotion began to develop major interest after the publication of their document; Edible Insects: Future Prospects for feed and food security in 2013 (van Huis *et al.*, 2013). This document put insects on a pedestal, describing how much better they are than traditional food sources in terms of efficiency, sustainability, nutritional content, and reducing harm to the environment. This created new interest in finding ways to produce and utilise insects as part of our food chain.

The response to this promotion was the rapid development of a new industry. Nongovernmental organisations such as the Bill and Melinda Gates foundation provided the funding to set up some of the first major insect farms in the Western world dedicated to human consumption (Eurasia Review, 2012). These first farms focussed primarily on the production of crickets, selling some whole, but also producing a powder that was labelled as "cricket flour" that is marketed as being able to be incorporated into a wide array of different food products.

While this industry began to grow commercially in America, Europe took a slower approach of continuing research and development. Aspects of government and research institutions have developed the potential for entomophagy further through their research and education initiatives to the point that some European countries such as Belgium and the Netherlands are beginning to normalise entomophagy (Caparros Megido *et al.*, 2014; House, 2018). In some countries, including the UK this has been hampered by an unclear legal status that allowed for the development and sale of food products that contain insects for a short time without clarification on their safety (The Scientific Committee of the Federal Agency for the Safety of the Food Chain, 2014; de-Magistris, Pascucci and Mitsopoulos, 2015; Reverberi, 2017). After closing the legal loophole that allowed for the sale of insect-based food products, the European food safety authority (EFSA) permitted established companies to continue selling their wares for a set period that ended in January 2018. However this move was ruled unlawful as the legislation at the time permitted for foods to be developed from whole animals as such those food producers can continue production until the new rules become enforced (Southey, 2020). Some countries such as Austria, Belgium, Denmark, Finland, and the Netherlands decided to make themselves an exception to this ruling and produce their own legislation that permitted the continued commercialisation of entomophagy (Grabowski et al., 2019). Since then, 13 submissions have been made to demonstrate the safety of several insect species to the EFSA via the novel food regulations. The applications for the lesser mealworm (Alphitobius diapernus), crickets (Gryllodes sigilatus & Acheta domesticus) and grasshoppers (Locusta migratoria) have reached the risk assessment phase of the novel foods approval process (Bugsolutely, 2020). Mealworms (Tenebrio *molitor*) have been approved by the EFSA but require further approval from the European Commission to finalise this approval (Lemon, Neath and O'Connor, 2021). As this approval occurred after the UK left the European Union on the 1st of January 2021, the UK FSA are enforcing a requirement for separate applications to be made to them with the expectation that this is likely to take at least a year (Lemon, Neath and O'Connor, 2021).

The rapid development of the insect industry has created some concerns. Academic literature went through a phase of speculation on the benefits and risks of insects while the field was in its infancy, however, this speculation was important as it helped narrow down those necessary areas of research but it may have been taken out of the context that the authors presented it in (Klunder *et al.*, 2012; Belluco *et al.*, 2013; Rumpold and Schlüter, 2013a). Other areas of the food industry have had centuries of research, development, and literature on which to base claims. Insects in the Western world do not have this foundation. There are minimal resources from entomophagous countries that could truly answer the questions raised in this way and research takes a considerable amount of time to definitively prove any claims. It is possible that this time of speculation has done some harm to the message being promoted as some of the claims painted a poor picture of entomophagy.

1.6 How insects are farmed

Insect farming in the West began fairly simply, modifying the practices that take place in entomophagous countries like Thailand. Crickets were placed into sealed plastic storage containers with egg cartons to provide additional space for movement (Entomo Farms, 2015, 2016). They were provided water and a commercial feed and left to grow until adulthood at which point the whole box was put into a freezer to kill the crickets inside. After this they would go onto be dried and potentially ground into a powder. This approach has multiple benefits (Clifford, Roe and Woodring, 2015). Firstly, as colonies are within their own, contained environments, disease or infections have less chance of spreading throughout the whole farm (Maciel-Vergara and Ros, 2017). Secondly, it allows for problems in growth to be noticed easily so farming times can be adjusted for the individual colony rather than risk affecting the whole farm. Thirdly, it allows for feed and water consumption to be better regulated as smaller colonies provide less competition. This method comes with some downsides too though, such as the cost of feed but it also requires a large inputof manual labour which increases the cost of production considerably (Entomo Farms, 2015). More recently some producers have begun developing methods of automation which should bring the cost of production down over time (Leary, 2017).

The production and processing methods utilised by producers are mostly kept secret which makes it difficult to accurately compare products. Microbiology for example, relies heavily on knowing the history of the product being examined. Without the history, a blind testing approach must be enforced which takes considerably longer. Knowledge of the processing involved in producing the cricket powder would allow for less broad speculation which in turn allows for testing to be specific, shortening the time that the necessary research takes. The only way to solve this issue is through greater public research into insects.

1.7 The current state of insects as human food

In modern times there appears to be a divide in how the media portrays entomophagy. Some media continues to demonise insect consumption as something negative, praying on those feelings of disgust as a form of entertainment. Television shows, such as I'm a 'Celebrity, Get Me Out of Here', use the consumption of insects as a trial, an ordeal to be overcome, forcing the negative perceptions to grow. This message is aired alongside other programmes stating the benefits of insect consumption and demonstrating innovative products. Overall, this generates confusion and when there is confusion it is simpler to default to the historical attitude of fear, disgust and apprehension (Verneau *et al.*, 2014). It is because of this duality that alternative methods of promoting entomophagy need to be explored as simply providing information is not proving as effective as people hoped (Lensvelt and Steenbekkers, 2014). According to Weigel (2016) four market research companies reported on the potential of entomophagy by 2016. Overall, the estimated size of the global market was \$106m however estimations of growth varied considerably between the four market research companies providing estimates. One predicted that growth could yield a market worth \$1.5b by 2021, the second suggested a minimum of £522m by 2023 and a third suggested the market is likely to be around \$723m by 2024. The largest areas of growth are expected in the UK, US, China and Brazil with Europe as a whole being by far the fastest growing market (Weigel, 2016). This potential for a rapidly expanding market makes insects an attractive prospect for investors as can be seen by the vast sums of money being invested in farms and production sites but without an output for those ventures, such as food products, it is unlikely that growth and investment can continue (Palmer, 2019).

The FAO have a voluntary database of stakeholders in the development of insects for feed or food. Although this database is incomplete due to its voluntary nature and not maintained, it shows a wide range of private companies, researchers and governmental organisations involved with the development of entomophagy around the world (Food and Agricultural Organisation of the United Nations, 2021). This directory lists over 150 separate entries across Europe demonstrating that growth in the short span of time that entomophagy has been popularised. The bulk of these are in the EU but the UK has 17 organisations listed, 9 research institutions, 7 private companies and 1 governmental organisation. 5 of the private companies listed for the UK are brands or sellers of insect based food products using cricket powder, although only 1 of these (Eat grub Ltd) is still producing products for sale.

While there has been considerable investment in cricket farming for the production of powders, there has been less emphasis on what this might be used for. This may be devaluing the resource from a public perspective as it may be difficult for consumers to see the value without tangible outputs. There is a circular argument to this discussion surrounding the promotion of entomophagy though. The lack of commercial availability can be solved through the creation of commercially viable products that the producers and retailers see profit from. This availability would go on to breed familiarity with insect-based foods which was highlighted as one of the primary barriers to acceptance. Greater acceptance would go on to breed further consumer desire and lead to an increase in commercial viability for the next range of insect-based foods. The key to this is making that first foray into commercialisation as good as it possibly can be, as without that quality, it risks the whole process through consumer rejection.

The bulk of products produced around the world are snacks, mainly protein bars but also crisps, cookies and other types of sweet and savoury snack foods (Engstrom, 2020). Some food staples like udon noodles, pasta and bread have been produced alongside meat replacement in the form of burgers, sausages, or a replacement for minced meat. Drinks and drink ingredients such as coffee, beer, smoothies and bitters for cocktail making have also been developed using insects as ingredients around the world. Overall, this demonstrates immense versatility in the potential of insects in food product development.

1.8 The ethics of insect consumption

At the insecta conference in 2017 the ethical implications of consuming insects were raised as an issue by Professor Potthast of the University of Tübingen, Germany. Raising questions about the appropriate rearing conditions, whether we hold a moral obligation to how we treat insects farmed for food, what the "humane" way of killing should be, whether there are species' that should not be farmed due to their characteristics, as well as further questions that linger surrounding their sensory and cognitive capabilities. There are also questions related to biosafety and biosecurity that come with rearing insect species that are not native to the country housing the farming facilities. This leaves us with the bulk of ethical discourse surrounding insect consumption being focused on the farming rather than anything that happens postfarming however, it is still important to consider this material as part of the overall context.

The primary point of this lecture lies in that the industry was in its infancy, so compared to meat production where standards have developed over many years, insect farming is running with the best practice that we know of now but is generally unregulated when it comes to these moral concerns. Freezing to kill for example is generally believed to be the best way of killing insects, as it slows their metabolism until the point it stops without inflicting pain or stress however, we have very little way of confirming this and research into other arthropods would suggest that their sensory capabilities are more complex than we had originally believed (Encyclopedia Britannica, 2018).

There are 3 issues with the farming of insects (Fischer, 2019). Firstly, because we do not know their cognitive capabilities, there is no ethical way to farm them as the

standards are based on an assumption of a lack of cognitive ability. The second is that farming insects efficiently and effectively requires harm to many more individual lives than meat farming which points towards that ethically, any harm is bad so farming plants would be better. The third aspect is the consideration of whether farmed insects have a pleasurable life while they are alive. If they spend their whole life uncomfortable or in pain then their lives are not worth living at all. More recently, there have been ethical questions raised about whether the persuasive and coercive tactics used to promote entomophagy are objectional (Waltner-Toews & Houle, 2017). The way that insects are sometimes framed as necessary to save the world pushes an obligation to consumers and could pressure them into actions that they would not otherwise take such as consuming the insect-based food. Ultimately the issue is that we do not have answers to a lot of these questions. While the researchers above highlight that with the lack of information it would be best practice to simply not begin farming insects without full understanding it is too late for that. As such we must rely on the best practices outlined at the time. The implications this has for this study is that freezing is still believed to be the best practical method of humanely killing insects, as despite some ethical objections little alternative has been proposed. Further to this when discussing food products, the aim should be to extoll the benefits that insects provide with their incorporation without being coercive to anyone that it is presented to, allowing for decisions to be entirely their own.

1.9 The future potential for entomophagy in the Western world

The future sustainability benefits that are envisaged for insects goes beyond the current state of farming and production. The current issue is that insects have to be fed on a commercial feed to comply with safety regulations (The Scientific Committee of the Federal Agency for the Safety of the Food Chain, 2014). This is feed that could be fed to other animals that are already accepted parts of our food chain. This raises the cost of production but also limits the sustainability aspect as the ideal feed for insects would be something that is not viable for rearing other animals or as food directly for ourselves (Varelas and Langton, 2017; Varelas, 2019). The limitations placed on feed and rearing, which are enforced in order to ensure safety in the short term, need to be reviewed as more information becomes available in order for the full potential of insects to be reached as further improvements to the sustainability of farmed insects are possible through the use of waste streams or inedible biomass (Dobermann, Swift and Field, 2017).

Redesigning this commercial feed stream could also allow for improvement. As insects gather some of their nutrition profile from their feed and their environment, it is entirely possible that the nutritional profile of insects could be modified by changing these aspects of production through a tailored feed and rearing environment. Ultimately this could mean insects reared to provide specific attributes to a food product such as omega 3 fatty acids (Barroso *et al.*, 2017). They could be tailor-reared to provide specific nutrients in areas of malnutrition. The modification of the insect's hedonic attributes is also possible in this way, as demonstrated by the Nordic Food Lab's experiments into how diet can affect flavour, which may make them more appealing to consumers (Lo, 2014).Their studies included feeding herbs to the insects they were rearing and finding that the herb flavour is retained.

1.10 Addressing insects as animal feed

There are several upsides to using insects as feed rather than food, such as being more acceptable to consumers and having an easier time with regulators (Verbeke *et al.*, 2015; Sogari *et al.*, 2019; Szendr, 2020). It would provide some additional food security to our current food systems and consumers can go on eating the foods that they are familiar with. Ultimately, this method would be a good way of continuing to provide existing products in a more sustainable way, but the rearing of animals for meat comes with other limits such as the availability of suitable rearing land as demand increases, which could not be overcome entirely through a change in feed. This is because 80% of all available agricultural land is devoted to some part of the chain of raising animals with roughly one third of this being used for producing feed (van der Zee, 2018).

Globally as a whole, the rearing of animals for meat is unsustainable due to population growth and increases in wealth in developing countries that yield a desire for meat (Smith, 1996;Delgado, Courbois and Rosegrant, 1998). Neither of these problems are going away and are generally predicted to get worse so utilising insects for feed production is likely to be outpaced long term.

The potential for this approach comes from the fact that large portions of arable farmland is utilised for crops which are edible by humans but instead are utilised for animal feed (Jaggard, Qi and Ober, 2010). If the consumption of insects remains abhorrent, utilising them in this manner would free up valuable farmland for the production of crops that humans will consume, ultimately increasing our food supply (van Huis *et al.*, 2013; Sogari *et al.*, 2019). There is the argument though that there is already enough food production around the world, and that this will continue to be enough for some time to come but the issue is instead, location of the food (Holt-Giménez *et al.*, 2012). One of the primary considerations for the sustainability of insects is that they can be farmed almost anywhere and will often do better in those equatorial countries that typically suffer from malnutrition, removing the issue of location (van Huis *et al.*, 2013).

The most efficient use of resources is directly farming foods which can be consumed by humans directly though. Having insects that are edible by humans, used as feed is not ideal due to feed to food conversion ratio's of those conventional foods being less than the insects being fed to them (Wegier *et al.*, 2018). Put simply, by consuming the calories and the nutrition that insects provide directly, rather than the indirect method of feeding insects to animals and then consuming tha animals, there is less waste in the food chain. Farming insects for feedhas found ground by utilising insects such as black soldier flies that are unlikely to find much use within food production (Katya *et al.*, 2017). These flies can be fed on waste or other crops which are not utilisable for food too, further increasing their sustainability due to not taking usable resources to produce them. This way, having two separate industries makes insect farming for feed viable, as although the human desire formeat is reducing in some areas of the world, it is growing in others, so finding new feed sources is one way of making the production more viable.

1.11 Why investing in insects is worthwhile

Insects offer a resource that is an increase in sustainability over other protein streams due to their significantly lower intake of water and food as well as limited reliance on specific environments to rear them (van Huis *et al.*, 2013). In general, most insects can be eaten whole so as well as the net gain through their higher feed to food conversion ratio there is an increase in edible weight over meat which has parts that cannot be consumed. Further to this, insects produce less by-products and waste that is harmful to the environment such as the methane from beef production or the ammonia from pork production. The frass from cricket rearing can be utilised as a high quality fertiliser that significantly improved the yield of corn crops furthering that sustainability (Darby *et al.*, 2018).

Nutritionally, insects vary quite considerably across the species that data is available on (Nowak *et al.*, 2016). Of the few farmed insects, generalisations of them being high in protein, containing essential fatty acids, vitamins, minerals and fibre have been made (Osimani *et al.*, 2017). Crickets have been highlighted as the species which has some of the best ratios of these aspects while being the easier to rear in large numbers than the few species like termites which contain higher protein values (Montowska *et al.*, 2019). However, due to this popularisation, concerns have been noted about the farming of a few species which may become an issue in the future as some cricket species are prone to viral outbreaks (Eilenberg *et al.*, 2015; Maciel-Vergara and Ros, 2017). The advantage that insects have is that their sustainability credentials match or exceed plant based proteins while having the benefit of a complete set of essential amino acids which plant proteins are often lacking (Aiking and de Boer, 2019). However, it is noted that insects nutritional composition changes based on their feed, environment, age at the point of harvest and their rearing conditions (Håkansson, 2018).

Aside from the value of insects sold as food or feed, a further option is being explored as the market for farming expands; what resources can be chemically or physically extracted from this new resource. Chitin, the primary component of the exoskeletons of most farmed species has an increasing number of uses both within and outside the food industry. It can be made into ingredients that provide structure to certain food types such as Surimi that is popular in Asia (Benjakul *et al.*, 2001). Typically, this resource is acquired from shellfish. Although this is typically a byproduct of the shellfish harvest for food, from a sustainability point of view, insects may help as an additional source if the uses for chitin continue to expand due to those fisheries already being stressed from overharvesting (Mohan *et al.*, 2020). The increasing popularity of chitin and chitosan for making biodegradable plastics in many industries for sustainability reasons is likely to be the catalyst in increasing value of this avenue (Srinivasa and Tharanathan, 2007).

1.12 Safety considerations

The safety aspects of edible insects are a difficult topic due to the vast number of species, rearing environments, harvesting methods, processing methods and consumption methods involved in entomophagy around the world. What has been developed, is the understanding that they are likely to pose an allergen risk, they may pose a microbiological risk, some species may pose a risk through toxins or antinutritional factors and there are risks associated with the bioaccumulation of minerals or chemicals within the insect's environment.

1.12.1 Allergens

It has been demonstrated in literature that insects can trigger an allergenic response in people who have other allergies, particularly from shellfish but also potentially dust mites (Sokol, Wünschmann and Agah, 2017). This is believed to be through both shellfish and insects containing the same allergenic proteins: arginine kinase and tropomyosin. This allergic response can be serious enough to trigger anaphylaxis, so it is a significant risk factor in the consumption of insects. To date this has been managed by labelling food products with a warning not to consume them if a person has an allergy to shellfish, but it is unclear if further warning is necessary. It is also possible to develop an allergy over time through handling and breathing within insect-containing environments such as workers on an insect farm or food processing facilities that utilise insects (Van Broekhoven *et al.*, 2016).

Literature shows that there are a range of other potential allergens of concern within insects including GADPH, myosin light chain, fructose-biphosphate aldolase, actin, α tubulin, β -tubulin & hexamerin 1B which could demonstrate similar cross reactivity to various non-edible insects, arachnids, mammals, nematodes, trematodes, plants, and fungi (de Gier and Verhoeckx, 2018). Importantly when it comes to developing food products, insect allergenicity is altered by processing. Heat treatments can increase the allergenicity of pyruvate kinase and GADPH while reducing the effect of arginine kinase (Pali-Schöll, Meinlschmidt, *et al.*, 2019). Despite these inferences, there are notably differences between species as, although some insects contain the same allergenic proteins, the Immunoglobulin E (IgE) binding process affects different areas (Pali-Schöll, Meinlschmidt, *et al.*, 2019).

1.12.2 Microbiology

Insects are typically consumed whole or processed from the whole insect into a dry powder. In conventional farm animals, the gut is removed and either discarded or thoroughly cleaned before it can be consumed. This cannot happen with insects due to their size. Although removing the feed from rearing chambers for a period of purging prior to euthanasia will have some effect on the microbiota of the gut, bacteria are likely to remain giving rise to the concerns over whether or not this would result in a safety issue (Feng, 2018). Although studies on the microbiology of insects exists, there is little consistency between rearing method and testing method of those samples making it difficult to make direct comparisons (Klunder *et al.*, 2012; Grabowski and Klein, 2017b; Megido *et al.*, 2017; Vandeweyer *et al.*, 2017; Fasolato *et al.*, 2018). Concerns have been raised to this end about studies not presenting information on how a sample was reared, processed and examined as this information is of vital importance if the results of the studies are to be understood in context. If generalisations can be made from the available data it would be that insects do pose a risk as they carry foodborne pathogens that could be processed into any food those insects are utilised in. As such, care must be taken to understand the individual sample being worked and put in place suitable methods of control for any pathogens present.

1.12.3 Antinutritional factors

Chitin has been presented as an anti-nutritional factor, binding a portion of the protein content so that there is a notable reduction in the bioavailability amongst the majority of the population who lack a functional chitinase enzyme (Rothman *et al.*, 2014). The reasoning for the lack of enzyme has not yet been made clear through research, however, the current hypothesis is that it has been a victim of evolution due to the lack of regular consumption of chitin containing foods. In addition to the effect on protein, it has been highlighted that chitin can also bind to some minerals reducing the bioavailability (Bassett, 2018).

Aside from the species containing thiaminases mentioned previously, further consideration has been given to other species that contain serine and aspartic proteases, calycin, phytic acid, oxalates, hydrocyanic acid, tannins and tropomyosin however the EFSA novel food report states that no antinutritional compounds have been found within crickets (Fernandez-Cassi et al., 2018; Patel et al., 2019).

1.12.4 Other risks

It has been suggested that when consuming whole insects, the legs and wings should be removed otherwise they can present a risk of causing a fatal intestinal blockage over time (Kouřimská and Adámková, 2016). This is due to the legs having barbs and hooks which can get stuck on their way through the digestive system. It has been pointed out that the chitin exoskeleton of which these components are made can resist the milling process, meaning this issue may persist even once the insect has been processed into a powder and made into a food product (Bassett, 2018).

1.13 Why the work is important – the problems with industrial secrecy

Research into insects for human food is important to pave the way where entomophagy can contribute significantly to the battle for sustainable food security and against malnutrition around the world. Conventional farming methodology has developed over hundreds of years to get where it is today and although some aspects of that can be broadly applied to rearing insects for food or feed, there are specific challenges that come with raising mini-livestock and producing food from that resource. The rise of the commercial entomophagy industry poses some difficulties with the ideals outlined by the FAO as important breakthroughs made by industry are likely to remain secret in order to provide a commercial advantage. Public research is a way around this issue, the information generated through research at this level can be used to benefit the many people who need it.

This thesis aims to take insects directly from the farm, all the way to the fork and considering each step of that process in order to generate a product that is suitable for the promotion of entomophagy at the end of it. The hope is that the information generated by this research can push the movement forward by demonstrating ways that each step of the process can be better and result in something that is useful for both combating malnutrition and improve on the willingness to accept entomophagy in the Western world.

Chapter 2: Impact of Species and Drying Method on the Nutritional Value of Cricket Powder

2.1 Introduction

In Western culture, consumers have a strong preference not to see that the insect is present within their food (Bisconsin-Júnior et al, 2022). The primary way this has been achieved, to date, is through the production of a dry powder which can then be incorporated into a widevariety of foods without the presence of insect material being apparent to the consumer. This approach has the advantage of aligning such insect powders with the health food industry. However, the production methods of such powders have stagnated over time compared with food production due to poor consumer perception and lack of investment in method development (Fitzpatrick and Ahrné, 2005). Recent research has suggested that the processing methods involved in the production of insect powder can have a wide array of effects on the final product, from sensory changes to nutritional and quality changes, it is important to try and understand what changes may occur in order to produce the best product possible if insects are going to stand a chance at being fully embraced in western society (Bassett, 2018; David- Birman, Raften and Lesmes, 2018).

Typically, insect powder production involves several key steps. Firstly, the insects being processed must be killed. Normally this is done by either boiling or freezing, with freezing being suggested as a more humane option due to it being a non-violent death (van Huis *et al.*, 2013). This freezing method has been found to be valuable when aiming to retain some of the nutritional value of the insects as, due to the slowed metabolism, fat is retained and there is less risk of loss compared to boiling (Adámková *et al.*, 2017). However, if there is not a subsequent process which utilises heat, an early boiling process may be a valuable trade-off to ensure food safety, as wet heat treatments are recommended by the FASFC to ensure that insectsare safe to consume (The Scientific Committee of the Federal Agency for the Safety of the Food Chain, 2014). This boiling process also alters flavour perceptions, decreasing the strength of the flavour, which could have positive implications for consumer acceptance (Kouřímská and Adámková, 2016). The effect of the killing process is likely to go beyond this, effecting the end quality of the insect powders.

Farina (2017) reported that the killing method changed the pH, saltiness, umami and overall liking of insects due to the breakdown of glycogen and formation of lactic acid. Thus, demonstrating the importance that the preparation method is kept as consistent as possible. One dilemma that arose from this study which may affect the powder quality is that the participants of the study preferred the flavour of insects that had undergone physiological stresses, such as being cooked alive rather than the less stressful freezing method of slaughter commonly used before processing which poses a moral dilemma in how best to optimise the process, as the most ethical treatment could yield an inferior product.

Secondly, the insects need to be dried. There are many options for drying processes with spray drying, drum drying, several different types of oven drying, vacuum drying and freeze-drying being the most commonly used within the food industry to produce a food-grade powder. Insects are seemingly not simple to process in this way, having a range of physiological changes that can result from the different processing methods. Spray drying, for instance, can result in the development of fishy off-flavours during the initial slurry processing that persisted into the final product (Bassett, 2018). In addition, the particle size was inconsistent, and there were notable colour and textural changes when compared with an oven-dried powder (Bassett, 2018). As noted previously, the price of an insect powder is likely to be a contentious issue.

When tested, oven drying was found to be the cheapest method in terms of energy cost as it was generally the fastest method and could process much higher quantities at once when compared to vacuum drying or freeze-drying (Kröncke *et al.*, 2019). Oven drying mealworms was found to reduce the risk of fat oxidation, but the trade-off was a loss of bioavailability of some of the minerals (Kröncke *et al.*, 2019).

Other drying methods were noted to cause quality changes to the final powder such as colour and the risk of fat oxidation (Kouřimská and Adámková, 2016; Kröncke *et al.*, 2019). The oxidisation of fats or higher moisture content can produce insect powders that are darker in colour for example (Kouřimská and Adámková, 2016). The Maillard reaction (a complex chain reaction between reducing sugars and amino acids that results in flavour compounds and brown colours) was noted as having a large effect on colour during drying processes that utilised high temperature. However, it was noted that a favourable colour was not consistent with a positive flavour. These colour changes were expected to be a result of the maillard reaction which involves a reaction between amino acids and sugars in high heat that results in the development of flavour compounds and brown pigments (Kröncke et al., 2019). Colour changes were magnified if the insects were heated in the presence of fructose, probably because it is a reducing sugar, necessary for the first part of the Maillard reaction (David-Birman, Raften and Lesmes, 2018). Milling could also have an unexpected effect on the colour of the insect powder, as it was found that powders with a smaller particle size had an increase in the L* value due to a higher amount oflight scattering (Bassett, 2018). Combining the L*, a* and b*colour values into ΔE as an overall representation of the difference between two samples can be used to compare the total colour differences between two samples by taking one value awayfrom the other. As a rough guide, a result of under 1 suggests that the difference willbe imperceptible to human eyes, below 2 will take close observation to determine a difference, 2-10 should be perceptible differences at a glance, 11-49 suggests that the colours are noticeably different but similar, while a score of 50+ demonstrates that colours are clearly different (Schuessler, 2018). In addition, spray drying resulted in a product that was grey in colour which was not favoured by consumers (Bassett, 2018).

Aside from the physical changes to the end product, this processing of the insects into a powder can potentially have both desirable and undesirable changes to the nutritional profile (Kouřimská and Adámková, 2016). Samples of the same species, bought from different vendors in the same location, or cooked in different ways, were found to have significant differences in their protein content and amino acid distribution (Köhler *et al.*, 2019). However, it is unclear if these samples originally came from the same producer, had similar diets, or were at the same growth stage when harvested. All of these factors can play a significant role in determining the nutritional profile (Kouřimská and Adámková, 2016). Other research has suggested variations in the nutritional profile as a result of processing methodology, asthe digestive fate of cricket proteins during in-vitro testing was altered in a heat-processed sample through increased gastric proteolysis, suggesting that processing using heat may improve protein bioavailability (David-Birman, Raften and Lesmes, 2018).

Under more controlled conditions, differing processing methodology was found to affect the content of Vitamin B_2 , as oven drying resulted in a lower vitamin content than when the same material was spray dried (Bassett, 2018). Oven drying, in

particular, has been shown to affect the bioavailability of some minerals, particularly zinc, reducing it to the point where only 20-40% was able to be utilised during testing (Kröncke *et al.*, 2019). This was theorised to be a reaction with the chitin. While not directly a nutrient, heat processing of cricket powder was noted to change the way antioxidant capabilities of the protein functioned as a heat treated sample had diminished electron transfer ability but an increased proton abstraction capability when compared to a non- heated sample (David-Birman, Raften and Lesmes, 2018). The ability of the protein toact as an electron donor was decreased but the potential to act as a proton donor increased to compensate. This suggests that heat processing results in conformational changes in the protein structure as the effect was more pronounced in samples that had been affected by the Maillard reaction.

The final primary step in insect powder production is typically milling. A very fine powder, below 100 microns, is usually required for most food applications, as sizes above this are detectable on the tongue. The choice of the milling process is likely to be quite important, as the chitin exoskeleton of insects can resist the milling process and fat from the insects can separate during this processing, forming deposits inside the equipment and lowering the overall nutritional value of the product (Bassett, 2018).

Once fully processed and properly stored, below 25°C, insect powder should have a shelflife of at least 18 months. When stored for longer at this temperature there were changes in the colour, particularly the lightness value or black/white scale (L*) making the powder become greyer over time which was noted in other literature to be undesirable to consumers (Kim *et al.*, 2016). However, fatty acid composition appeared to remain consistent throughout, suggesting that oxidation is not a primary risk to the quality. However, the study only took into account variations in temperature, when there are likely other factors to consider such as humidity or the presence of prooxidants and antioxidants (Kim *et al.*, 2016). The study did find that the moisture content of cricket powder does increase during storage, suggesting that this may be a factor for long term storage.

Further processing may be necessitated by the chosen killing, drying, or milling method, such as the blending to a slurry which is required by some types of oven drying and the spray drying. Some additional processes could also be included in order to increase the quality of the final product. An alkaline treatment to remove the exoskeleton of the insect resulted in a more bioavailable protein fraction and made milling easier, resulting in a more consistent powder of lower particle size (Bassett, 2018; Rumpold & Schlüter, 2013). However, the chitin content of the exoskeleton could potentially provide some relatively unique health benefits, so this is a trade with both positive and negative implications to the end product (Roos and van Huis, 2017). Removal of the legs and wings of some insects, such as crickets, has been suggested to aid in food safety as they contain barbs that can get stuck in the throat or bowel resulting in an obstruction. When tested these individual body parts had a similar nutritional profile to the rest of the insect so, although it would decrease the overall edible weight of the product, it would not negatively affect the nutritional profile (Håkansson, 2008).

Comparing reported nutritional results for insects is difficult as there are inconsistencies in the treatment of samples, their origins (including diet of the insects) and the method of analysis. In addition, some reports show clear errors in their results as figures do not align when considered as a whole sample (Rumpold and Schlüter, 2013a). Despite these issues, overall trends can be identified, such as the fact that the Orthoptera order of insects (grasshoppers, locusts, and crickets) contain, on average, the highest total protein and the lowest total fat content when compared to other orders (Rumpold and Schlüter, 2013a). These species have also been reported to be rich in essential amino acids (EAA), though there are conflicting reports on specific levels. Despite thedifference in overall protein content, Rumpold & Schlüter (2013a) reported that all ofthe tested orders of insect exceed the ideal goals for EAA set by the WHO/FAO/UNU.By contrast, Köhler et al, state that crickets and locust did not meet these requirements (Rumpold and Schlüter, 2013a; Köhler *et al.*, 2019). In the research by

Köhler et al, tryptophan was the limiting amino acid for the cricket species that they tested (Köhler *et al.*, 2019).

For some insects consumed around the world, the amino acid composition was found to be complimentary to the most common staple foods consumed in the rest of the diet. For example, some communities in South African countries which have maize products as a staple, also consume termites. These were found to provide a complimentary amino acid profile, ultimately improving the protein synthesis within the body (Bukkens, 1997). Similarly, palm weevils are consumed by communities that have tuber-based staple foods. The tubers are typically deficient in leucine and lysine, which are relatively abundant in palm weevils (Bukkens, 1997). An understanding of what EAAs are available in more insect samples would allow for a similar approach to be taken in new product development, potentially positioning insects as a supplementary food that could reduce malnutrition.

Ghosh et al found that insect fat is typically superior to animal fats in terms of the fatty acid composition (Ghosh et al., 2017). Insects have been found to contain a range of both saturated (SFA) and unsaturated fatty acids (UFA), including the essential polyunsaturated fatty acids (PUFA), linoleic acid (C18:2n6) and alphalinolenic acid (C18:3n-3). Species differences have also been shown in the amount of monounsaturated fatty acids (MUFA), which may be as a result of the presence of delta 9 desaturase enzyme in some species (including house crickets) (Yang, Siriamornpun and Li, 2006). This enzyme converts saturate fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0) to palmitoleic acid (C16:1) and oleic acid (C18:1) respectively. If the presence of delta 9 desaturase can be confirmed, it would make these species of insects attractive for product development in the Western World due to the focus on health foods, with low SFA, high MUFA and PUFA and high protein content. Other methods of comparing the fatty acids of insects such as the Atherogenic (Ai) and Thrombogenic (Ti) indices have further presented insect fats in a favourable light as these were always below the critical figure of 1 (Osimani et al., 2017).

Crickets were reported to meet the requirements of being a "source of" iron, magnesium and "high in" for zinc under the EU FSA regulations for food labelling, although this was variable within the same species depending on the source suggesting an environmental or dietary effect (Köhler *et al.*, 2019). In the same study, locust was reportedly "high in" zinc and a "source of" iron. There is, however, a suggestion that the reporting of some minerals such as calcium has been misleading due to presenting them as comparisons with meat rather than presenting the overall value (Rumpold and Schlüter, 2013a). Insects are also reported to be low in sodium, which could have a beneficial impact as a low ratio of sodium to potassium is linked with helping prevent a range of physiological disorders, including those of the renal and respiratory systems, and hypertension (Ghosh *et al.*, 2017). It is generally believed that the lower this ratio value is, the better, due to the typical excess sodium consumption of Westernised diets (Bailey *et al.*, 2015). This lack of sodium could alsomake insects suitable for the production of low sodium foods (Rumpold and Schlüter, 2013b). The ash content (representing a rough estimation of overall mineral content) of farmed crickets in Kenya was found to be between 4% and 5% regardless of the age of the cricket (Carolyne *et al.*, 2017). The crude fibre content of insects is mainly in the form of chitin, which is considered insoluble despite the presence of enzymes for chitin digestion being present in the digestive tract of animals and humans (Kouřimská and Adámková, 2016). The fibre content varies considerably with individual samples of house crickets ranging between 6% and 22% fibre, largely due to the way that the chitin exoskeleton is formed at different stages of the insects development (Rumpold and Schlüter, 2013a; Jonas-Levi and Martinez, 2017). Although methods of establishing total dietary fibre may be appealing when compared to crude fibre, it is unclear how these methods may impact on the chitin component, potentially meaning these results would not represent the total fibre content (including chitin). Despite a thorough literature search, no studies into the sugar contents of insects could be found. This could potentially be a valuable avenue to explore, as any sugars present can have physiological effects within the powder such as reducing water activity by binding water, promoting non-enzymatic browning reactions, in the case of reducing sugars, or providing sensory properties which could be useful during product development.

There impact on powder quality when considering the method used to produce insect powders has had limited study.

The current study looks at the effect of processing on the nutritional profile and quality of 4 species of crickets: Black cricket (*Gryllus bimaculatus*), Banded cricket (*Gryllodes sigillatus*), Quiet cricket (*Gryllus assimilis*), House cricket (*Acheta domesticus*) and 1 species of locust: Desert locust (*Schistocerca gregaria*). To date, a lack of consistency in methodology has made it difficult to truly assess the value that insects could have within our food system, beyond the trend that they would generally be an asset to a sustainable future. A major problem is that comparisons are often difficult to make due to a lack of information on fundamentals, such as the origin of the sample, stage of development the insect, the feed used to rear insects, the processing method used, storage conditions and how the sample was analysed (Bukkens, 1997). In addition to this, the processing of insect powders for human consumption is often regarded as commercially sensitive and is generally not disclosed. It is unclear if the organisations responsible for processing insect powder have conducted research but, if they have, very little detail has been shared publicly.

The governmental organisations responsible for food safety have suggested that they may require consistent methods of production in the future, in order to minimise the risk that adding insects into the food system poses. As such this research should provide an insight into the positive and negative aspects of different processing methods, going some way towards allowing this aspect of entomophagy to progress unrestricted.

2.2 Aims and Hypotheses

The specific aim of this section of the research is to compare and contrast samples of powdered insects of differing species, produced using differing methodologies in order to determine if there may be an optimal production method for ensuring the best nutritional profile.

A secondary objective is to consider how the production methods impact on the physical quality attributes of the powder, such as its visual appeal and properties that may impact on the consumer acceptance, willingness to purchase or willingness to consume.

The primary hypothesis is that the largest change is likely to result from heat treatments, as this is known to have an effect on some nutritional components, but it is unclear how this will affect the nutritional qualities of insects. Similarly, the heat during oven drying could also impact on quality as it may cause potentially undesirable chemical reactions such as Maillard.

2.3 Materials and methods

All analytical methods were performed according to the standard operating procedure as developed by the Division of Food, Nutrition & Dietetics, School of Biosciences, University of Nottingham unless otherwise stated.

Samples weighing 2.5kg each of five species of live insect were acquired from Monkfield nutrition (Ely, UK) and a commercial sample of cricket powder (*Gryllodes sigilatus*) was purchased from Entomo farms, Canada as a guideline control. The live insects supplied by MonkfieldNutrition were Banded Crickets (*Gryllodes sigilatus*), Black Crickets (*Gryllus bimaculatus*), Desert Locust (*Schistocerca gregaria*) and Quiet Crickets (*Gryllus assimillis*). A fifth Species, House Crickets (*Acheta domesticus*), were sourced on ourbehalf by Monkfield Nutrition from a third party due to Monkfield's stocks suffering from a viral infection.

At Monkfield Nutrition, crickets are harvested at the 3-wing stage, prior to adulthood. The crickets are primarily fed on a high quality poultry feed which includes vitamin and mineral supplements. This feed has a protein content of 28% for the first two weeks and 24% thereafter. Locusts are fed on a similar feed but with a consistent protein content of 18% and the quiet crickets are provided a variant of this feed that is higher in cholesterol. Feed for the locust's is supplemented with spring greens due to this being the only way of them acquiring moisture while the crickets have a drip feed system for water.

The house crickets were sourced from a third party, with the request that they are the same developmental stage as those sourced from Monkfield Nutrition. However, it is not possible to know any specifics of their feed. As the house crickets were the most widely raised species across farms producing for human food at the time, it was deemed worthwhile to still include them in this research despite this limitation when making comparisons.

2.3.1 Sample Preparation

As soon as they arrived all live insects were placed into a -20°C freezer overnight to kill them. As there was some debate on the topic at the time, it was chosen to not add a starvation (purging) process due to the increased mortality potentially limiting available sample size. The whole, frozen insects were then sieved using a standard kitchen sieve to remove any bedding material and other insects which were contaminating the samples before each species of insects were boiled separately in a 5-litre stock pot filled with distilled water for 2 minutes. Boiled crickets were refrozen at -20°C before being roughly chopped to allow for faster drying through their hydrophobic chitinous carapace. All samples were divided up into smaller batches (167g - 274g) and then stored frozen at -20°C until drying could take place.

The preparation of insect samples and further analysis of the prepared samples outlined throughout the remainder of this thesis did not include any biological replication, as such any mention of replicates were purely technical repeats.

2.3.2 Drying

2.3.2.1 Oven

Oven drying was carried out in a laboratory drying oven (Genlab Ltd, Cheshire, UK) set at 105°C until the sample tray for each species achieved a consistent weight. Samples were removed from each drying method, typically once per day and weighed to calculate the lengthof drying time.

2.3.2.2 Vacuum Oven

A vacuum oven (Fistreem International Ltd, Leicestershire, UK) set at 105°C with a pressure of 1 bar was used to dry a set of samples until a consistent weight was achieved.

2.3.2.3 Freeze Drying

One sample of each species was moved into -40°C storage to facilitate freeze-drying, before being placed inside a freeze dryer (Edwards SuperModulyo, West Sussex, UK) set at -40°C with 0.01mbar vacuum. Samples were weighed (twice per day) until they achieved a consistent weight indicating that no more moisture would be lost utilising this method.

2.3.2.4 Drum Drying

Samples for drum drying were blended to a slurry using additional distilled water equal to the weight of the sample in a bench-top blender (Kenwood). Liquid samples were loaded into the loading well of a pilot- scale drum dryer (R. Simon (Dryers) Ltd, Nottingham, UK) running at 4 bars of steam pressure with a drum temperature of 120°C. Each sample was scraped off the drum at the end of one full rotation through the whole machine. Rotation speed was adjusted throughout to ensure a visibly dry sample.

2.3.3 Milling

The bulk of the samples for further analysis were processed on a cutting mill (Fritsch pulverisette 19, Northamptonshire, UK). Small samples of silent crickets were processed on a hammer mill (Perten Laboratory Mill 3100, Hägersten, Sweden), Ball

Mill (Fritsch, Northamptonshire, UK) and Coffee Grinder (Braun Aromatic KSM 2,

Germany) to act as a comparison of possible milling techniques.

2.3.4 Sample Naming

Post drying, the following naming abbreviations (Table 1) were utilised throughout the remainder of this study. Entomo where it appears on any of the results refers to the commercial sample of cricket powder sourced from Entomo Farms (Canada).

	Drying	Oven	Vacuum	Freeze	Drum
	Method	Dried	Oven Dried	Dried (FD)	Dried
		(OD)	(VD)		(DD)
Insect Species					
Banded Crickets		BaOD	BaVD	BaFD	BaDD
(Ba)					
Black Crickets (BI)		BIOD	BIVD	BIFD	BIDD
Quiet Crickets (Q)		QOD	QVD	QFD	QDD
House Crickets (H)		HOD	HVD	HFD	HDD
Desert Locust (L)		LOD	LVD	LFD	LDD

Table 1: Explanation of Naming abbreviations.

2.3.5 Nutritional Study

2.3.5.1 Energy

Duplicate 1-gram samples were weighed and pressed into metal crucibles designed for use with the Parr 6300 Bomb Calorimeter (Illinois, USA). Control samples of benzoic acid were used to test the machines operating capacity before and after sample analysis. Samples were ignited under oxygen and the energy recorded in MJ/KG of the sample. Results were subsequently converted into KJ and Kcal per 100g.

2.3.5.1 Water Content

The water content of each sample was analysed on an Ohaus MB25 moisture balance (Naenikon, Switzerland). Triplicate three-gram samples were spread onto the disposable foil inserts and slowly heated to 120°C. Before and after each species, pure water was used as a control sample to confirm equipment functionality.

2.3.5.2 Protein

50 milligrams of each sample was weighed into foil crucibles in triplicate and analysed using the Thermo Scientific Flash EA1112 Nitrogen Analyser (Massachusetts, USA). Aspartate standards were analysed prior to samples and 50mg sucrose blanks standard protein conversion figure of 6.25 was used for the calculation of total protein. The recent research suggesting that the conversion figure should be lower is acknowledged but currently there are no definite conversion figures for these species of insects (Lakemond, 2017). As such, the nitrogen figures will be reported as appendix A in order to allow for accurate conversions when the factor for these species has been adequately researched.

2.3.5.3 Amino Acids

The samples for the amino acid study were designed to provide a snapshot of the samples with the intent to extend the study if any unusual data was produced. Thus, the primary comparisons are between samples that had been dried at a high and low temperature as it was hypothesised that if anything, this would be the cause of differences between drying methods. The wider inclusion of all the house cricket samples, provides further confirmation of those differences should they occur.

Samples containing approximately 10 mg nitrogen were oxidised in 100 ml screw capped (with Teflon liners) Duran bottles for 16-18 hours at 4°C in with 5ml of chilled and freshly made oxidation solution which was 10% of hydrogen peroxide (30%) incubated (1hour at 20-30 °C) in 87% formic acid with 0.55% (weight/volume) phenol added as oxygen scavenger.

After oxidation, the samples were added 0.84g of sodium metabisulphite to decompose any excess oxidation reagent. The samples were then hydrolysed with 50 mL of 6 N HCl containing 0.1% phenol (w/v): The contents were thoroughly wetted and mixed on a vortex mixer until all the sample was finely distributed in the acid. After mixing, the solutions were hydrolysed at 110 °C for 24 h in an oven. The sample solutions pH was adjusted with sodium hydroxide to 2.20, and made up to volume of 200ml with tri-sodium citrate buffer in a volumetric flasks, with internal standard norleucine added in. Then 15 ml of the solutions were centrifuged at approximately 3000 rpm for 2 minutes, and about 1-2 ml supernatant were passed through 0.22 mm filter, ready for HPLC analysis.

The results of the amino acids were initially expressed as grams of amino acid per kg of sample which was then converted to mg of amino acids per gram of protein so that the results can then compared to the requirements outlined bythe FAO and also compared to other protein-rich foods. Table 2 below provides the figures used for comparison (Anaeto *et al.*, 2010; Yi *et al.*, 2013).

Amino acids	mg/g	protein	mg / g protein		WHO/FAO/UNU
(mg / g protein)	Beef	Pork	Casein	Soya Bean	mg/g protein
Total Essential aa	408	401	371	307	263
Histidine	29	32	32	25	15
Isoleucine	51	49	54	47	30
Leucine	84	75	95	85	59
Lysine	84	78	85	63	45
Methionine	23	25			16
Phenylalanine	40	41			
Threonine	40	51	42	38	23
Valine	57	50	63	49	39
Total Conditionally Essential aa	223	201			
Arginine	66	64			
Glycine	71	61			
Proline	54	46			
Tyrosine	32	30			
Total non-essential aa	284	287			
Alanine	64	63			
Aspartic	88	89			
Cysteine	14	13			
Glutamic	144	145			
Serine	38	40			

Table 2: Figures for comparison of amino acid quality.

Total Aromatic = Phenylalanine + Tyrosine, Total Sulphur = Methionine + Cystine, Total Savoury = Aspartic + Glutamic, Total Sweet = Glycine + Alanine (Zielińska *et al.*, 2015; Köhler *et al.*, 2019).

The limiting amino acid was calculated as the lowest ratio of what is present in the protein to what is required for protein synthesis using the estimates for amino acid requirements in adults presented in Recommended Dietary Allowances: 10th Edition (National Research Council (US) Subcommittee on the Tenth Edition of the Recommended Dietary Allowances, 1989).

2.3.5.4 Fat

Soxhlet extraction was used in order to determine the total fat content of the processed insect samples. Duplicateone-gram samples were placed inside filter paper and loaded into glass vials filled with 155ml of petroleum ether. The ether was boiled off using the Gerhardt Soxtherm SOX-6 place system with a Gerhardt Variostat controller (Königswinter, Germany) leaving the fat behind in the glass vial. The total Fat % was calculated by:

F<u>at + Crucible – Crucib</u>le Sample x100

2.3.5.5 Fatty Acids

The fat remaining from Soxhlet extraction was put through a methylation procedure followed by chromatographic analysis. The methylation procedure involved dilution of each sample in 0.7ml 10M potassium hydroxide and 5.3ml 100% methanol. Samples were then incubated in a water bath at 55°C for 90min, with a 5-second

vortex mix every 20 minutes. Samples were placed into an ice bath for 10 minutes to cool. Afterthis 0.58ml 12M sulphuric acid was added and the samples were mixed and incubated as before. Samples were cooled again in the ice bath followed by the addition of 3ml 100% hexane, then vortex mixed for 30 seconds. Samples were then spun in a centrifuge for 5 minutes at 2000rpm before the top, hexane layer was transferred to a solventresistant LP4 tube and stored at -30°C until analysis.

Samples were analysed using a Perkin Elmer Clarus gas chromatograph (Massachusetts, United States) with a 'CP-sil 88 for fame' 90m column with an internal diameter of 250um. An initial temperature of 45°C was ramped firstly for 13°C/min to 175°C and held for 20 minutes, secondly, 8°C/min to 200°C, hold for 4 minutes, thirdly 10°C/min to 215°C and held for 25 minutes. Hydrogen was the carrier gas set at 37.5 PSIG with a split ratio of 100:1. Results were compared with a Fatty Acid Methyl Ester (FAME) C4-C24 10mg/ml control sample in order to determine which fatty acids were present in samples.

Atherogenic index was calculated by this equation: $(\Sigma SFA)/(\Sigma n-6 + \Sigma n-3 + \Sigma MUFA)$ and the Thrombogenic index was calculated by this equation: $(\Sigma SFA)/(0.5 \times \Sigma n-6 + 3 \times \Sigma n-3 + (\Sigma n-3 / \Sigma n-6))$.

2.3.5.6 Ash

One-gram samples were heated to 500°C in a Carbolite muffle furnace (Hope Valley, UK) operating on a 4 hours heat, 4 hours cool cycle. Samples were left to cool to room temperature overnight before calculating the values for the remaining ash using the following equation to determine the overall percentage of inorganic material present in each sample.

A<u>sh + Crucible – Crucib</u>le x100 Sample

Nitrogen free extract

The nitrogen free extract is calculated by subtracting the gram value of the protein, fat, water, fiber and ash from the total sample weight with the remaining figure being representative of the total digestible carbohydrates.

2.3.5.7 Crude Fibre

The crude fibre was analysed using the Gerhardt manual fibrebag system (Königswinter, Germany). Fibre bags were dried for 4 hours in a 105°C drying oven before being weighed. One-gram samples were placed inside dried fibre bags and heated in 360ml of 0.128M sulfuric acid held just below boiling point (approx. 90°C) for 30 minutes, before being washed 3 times in hot distilled water. Samples were placed back into the system and immersed in 360 ml of 0.313M sodium hydroxide held just below boiling point for a further 30 minutes. After this stage samples were removed,

washed with distilled water and placed in a drying oven at 105°C for 4 hours. Samples were left to cool in a desiccation chamber for at least 15 minutes before being weighed. The bags were then placed inside weighed crucibles suitable for the muffle furnace and ashed at 600°C for 4 hours. Once cool, crucibles were re-weighed. The final value was recorded as the loss in weight during ashing using the following equation:

% Crude Fibre = ((Bag+ Residue Wt. - Bag wt.) – (Beaker + Ash wt) - Beaker) x 100. Sample wt.

2.3.5.8 Sugars

Analysis of sugars began with hydrolysis in trifluoroacetic acid (TFA) followed by HPLC analysis on the Dionex Chromatograph with Carbopac column and PAD detection system (Thermo Scientific, Massachusetts, USA). Triplicate 10mg samples were suspended in 1ml of 2M TFA and autoclaved at 120°C for an hour. The resulting hydrolysed sample was centrifuged to allow removal of the supernatant which was diluted in NaOH to achieve a 1:100 ratio and analysed using the Dionex comparing it to standards for Arabinose, Xylose, Galactose and Glucose at known concentrations. Results shown are from an initial pilot study to determine the value of completing this analysis.

2.3.5.9 Minerals

Analysis of minerals was achieved through nitric acid digestion under pressure in an Anton Parr Microwave Pro (Graz, Austria) followed by Inductively coupled plasma mass spectrometry (ICPMS) to provide figures for B, Na, Mg, P, S, K, Ca, Ti, Li, Be, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Cs, Ba, Tl, Pb & U. 0.2g of each sample was weighed into the digestion vessels in triplicate followed by 6 ml of concentrated nitric acid (HNO₃). The digestion vessels were placed into the microwave set to ramp up to 140°C over 10 minutes and held at that temperature for 20 minutes before cooling to 55°C over a further 15 minutes. Once complete, the vessels were vented, and contents diluted with 4 ml Milli-Q water before being transferred to a universal sample bottle in preparation for ICPMS. The digestion vessel was washed two more times, each with 5 ml of Milli-Q water, to ensure all of the sample had been accounted for and the correct dilution had been achieved.

Multi-element analysis of dilute solutions was undertaken by ICP-MS (Thermo-Fisher Scientific iCAP-Q; Thermo Fisher Scientific, Bremen, Germany). The instrument was run employing three operational modes, including (i) a collision-cell (Q cell) using He with kinetic energy discrimination (He-cell) to remove polyatomic interferences, (ii) standard mode (STD) in which the collision cell was evacuated and (iii) hydrogen mode (H2-cell) in which H2 gas was used as the cell gas. Samples were introduced from an autosampler (Cetac ASX-520) incorporating an ASXpress™ rapid uptake module through a PEEK nebulizer (Burgener Mira Mist). Internal standards were introduced to the sample stream on a separate line via the ASXpress unit and included Ge (10 µg L-1), Rh (10 µg L-1) and Ir (5 µg L-1) in 2% trace analysis grade (Fisher Scientific, UK) HNO₃. External multi-element calibration standards (Claritas-PPT grade CLMS-2 from SPEX Certiprep Inc., Metuchen, NJ, USA) included Ag, Al, As, Ba, Be, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Rb, S, Se, Sr, Tl, U, V and Zn, in the range 0 – 100 μ g L⁻¹ (0, 20, 40, 100 μ g L⁻¹). A bespoke external multielement calibration solution (PlasmaCAL, SCP Science, France) was used to create Ca, Mg, Na and K standards in the range 0-30 mg L⁻¹. Phosphorus, boron and sulphur calibration utilized in-house standard solutions (KH₂PO₄, K₂SO₄ and H₃BO₃). In-sample switching was used to measure B and P in STD mode, Se in H2-cell mode and all other elements in He-cell mode. Sample processing was undertaken using Qtegra™ software (Thermo-Fisher Scientific) utilizing external cross-calibration between pulsecounting and analogue detector modes when required. Minerals which do not provide a function within the human body were omitted and the remaining group (B, Na, Mg, P, S, K Ca, Cr, Mn, Fe, Co, Cu, Zn, Se & Mo) termed "Nutritionally relevant minerals" for the purposes of discussing the data. The full table is available in the appendices.

2.3.5.10 Iodine

Iodine analysis followed a similar procedure to minerals but utilised tetramethylammonium hydroxide (TMAH) digestion prior to being analysed using ICPMS. 0.2g of each sample was weighed into the microwave digestion vessels followed by 5ml of 5% TMAH solution. The microwave was set to ramp up to 110°C over 5 minutes and hold for a further 20 minutes before being set to cool to 50°C over a further 10 minutes in order to complete the digestion. Samples were vented, decanted and then the containers are washed twice using 10ml applications of Milli-Q water to ensure all sample had been removed from the digestion vessels and provide appropriate dilution for the next step. Samples were transferred to centrifuge tubes and spun at 3000rpm for 30 minutes. The supernatant was then filtered through Millipore filters using a syringe before being stored ready for ICPMS analysis.To analyse the iodine content the ICPMS was run in STD mode using Re as the internal standard with samples diluted to 1% TMAH.

2.3.6 Quality Study

2.3.6.1 Particle size and Shape

Particle size and shape was assessed qualitatively using an EVOS FI microscope (Advanced Microscopy Group, Washington, USA).

2.3.6.2 Colour

A Hunter Lab Colour Quest XE spectrophotometer (Murnau, Germany) was used in the analysis of the powder's colour. Prior to use, the instrument was calibrated using the black and white calibration panels. Samples were scanned three times inside clear plastic pouches, moving the scanning point each time to ensure an average would be representative of the entire due to the variation within the powders.

 ΔE is calculated by: $\Delta E = \sqrt{L^2 + a^2} + b^2$

Tuble 5. Delta E categorisations (Seneassier, 2020)						
Delta E	Perception					
<= 1.0	Not perceptible by human eyes.					
1 - 2	Perceptible through close observation.					
2 - 10	Perceptible at a glance.					
11 - 49	Colours are more similar than opposite					
100	Colours are exact opposite					

Example colour images were produced by entering the average LAB values to a paint producing website (E-Paint, 2019). As such, these are only meant as a visual representation as the accuracy of this method is uncertain.

2.3.7 Statistics

Each measurement was conducted in duplicate or triplicate, unless otherwise stated. It is important to note that these replicates were all taken from the same batch of insects and, as such, represent technical, as opposed to biological, replicates. As a result, where possible, measurements were individually checked for statistical differences by 1-way ANOVA between 'insect species' and 'drying method' using Microsoft Excel. It was not possible to look at the interaction of these factors used due to lack of biological replication.

2.4 Results

2.4.1 Powder processing

Figures 1-3 show that the majority of the drying was completed within 24 hours, but it took a significant amount of time to ensure that no further moisture could be removed from each sample. Samples fluctuated by less than a gram each time weighed beyond the 96/98-hour mark. Oven drying continued for 173 hours while vacuum oven drying continued this variation for 194 hours. On average the moisture content lost during freeze-drying is higher while the oven-based methods are both similar. The vacuum oven dried black, banded, locust and quiet samples took longer to reach the same point.

Due to the methodology involved in the drum drying, it is impossible to detail the moisture loss results due to water added, and because a considerable amount of product is lost through being stuck to the drum or dropping to the floor. Despite this drawback, the drying time was less than an hour per batch.

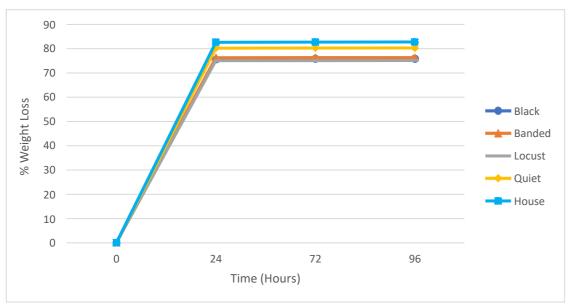


Figure 1: Time taken to complete oven drying (n=1)

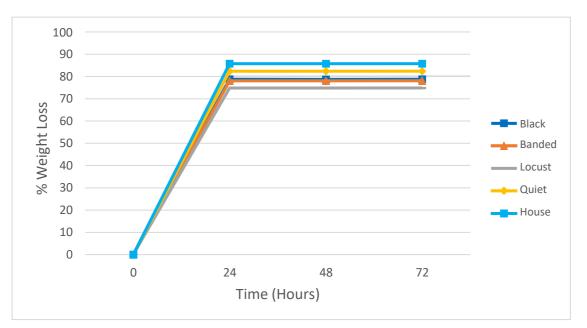


Figure 2: Time taken to complete freeze-drying (n=1)

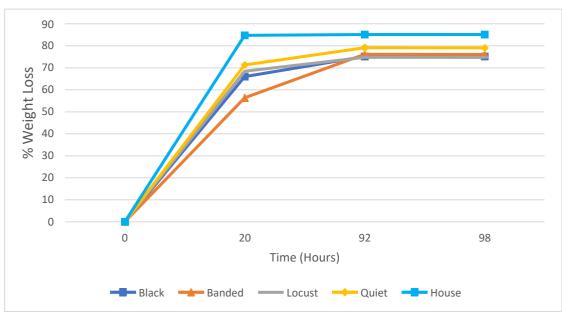




Figure 4 shows the total loss of weight of each species dried by each method with the exception of drum drying for the reasons outlined above. Statistical analysis indicated significant effects of both drying method as oven drying removed the least moisture while freeze drying removed the most on average (P = 0.02). There were also significant differences between the species of insect, with the house crickets loosing on average 10% more moisture than the locust (P <0.001). No significant differences were seen in residual moisture compared across the species' (P = 0.3) but the comparison between drying method was statistically significant (P <0.001) (Figure 5). Drum drying left behind the most residual moisture on average and vacuum oven left behind the least.

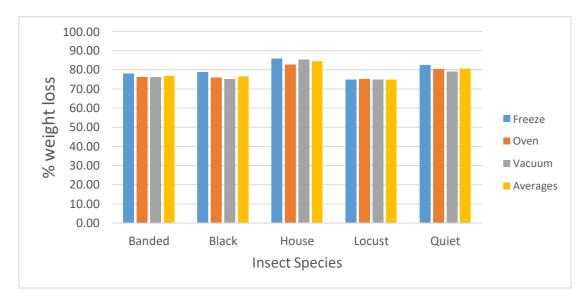


Figure 4: Total weight loss during drying (n=1)

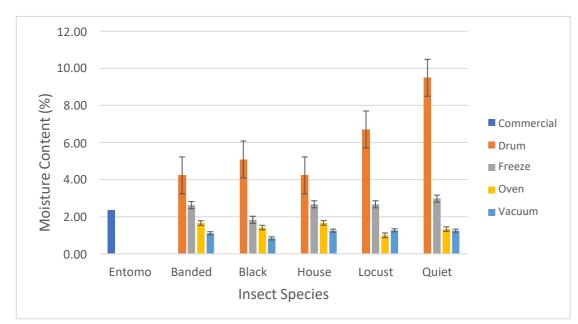


Figure 5: remaining moisture content of each sample post drying (n=3)

2.4.2 Nutrition

The total energy content of dried insect powders as measured by bomb calorimetry is shown in Figure 6. While significant differences were seen between insect species (P <0.001), no significant effect of drying method was demonstrated by these results (P = 0.30). House crickets stand out as having less overall energy provision than the other samples tested whilethe black cricket samples provided the highest amount of energy on average.

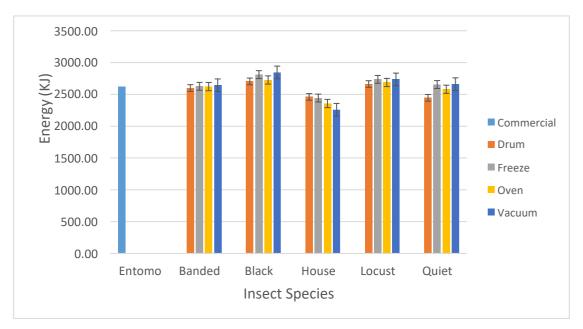


Figure 6: Energy in Kilojoules per 100g of sample (n=2)

Protein content of the sample was estimated from Nitrogen content. As can be seen in Figure 7 significantly different values were found between species (P < 0.001) with House showing the highest, and Black the lowest protein content of the Cricket species. No consistent impact of drying method on protein content was seen to be statistically significant (P = 0.067). The average results are similar suggesting that it is individual outliers that are driving this potential significance. The outliers are different for each drying method limiting what, if anything can be concluded from this.

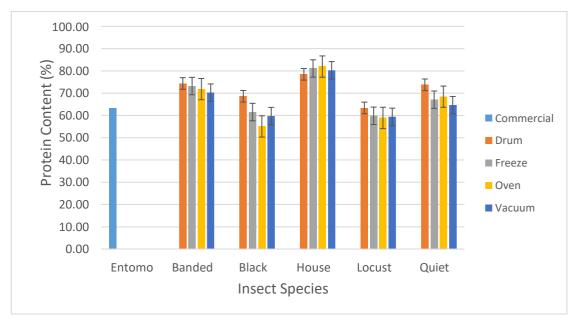


Figure 7: Dry weight protein content of each sample (n=3)

All of the samples exceed the percentage of energy from protein required to label the powders as high in protein with house, banded and quiet crickets containing double this value (Figure 8).

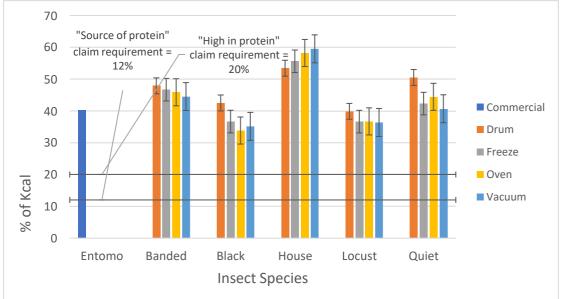




Table 4, below shows that the values for the essential amino acids are close to meeting the ideal figures set by the FAO for each individual amino acid but with the absence of tryptophan from these results, it cannot be confirmed that these samples would meet the 40% total EAA figure. For all samples, the limiting amino acid is histidine. The most prevalent essential amino acid is leucine across all samples.

When using individual ANOVA tests to compare the overall amino acid results of freeze-dried powders to oven dried across all species, there are significant differences in the house, banded and quiet crickets ((P < 0.001 for all 3) but not the black (P = 0.1) or the locust (P = 0.5). These differences occur from the results of oven dried samples being lower in almost every amino acid on each sample. The differences between species when not factoring in the drying method are not statistically significant (Overall: P = 0.97, freeze dried: P = 0.22, oven dried: P = 0.57). The values for the sulphur containing amino acids and the aromatic amino acids more than meet the WHO guidelines on all samples.

Amino acids							Samples						
(mg / g protein)	Entomo	HOD	HFD	HDD	HVD	BAOD	BAFD	BLOD	BLFD	LOD	LFD	QOD	QFD
Total Essential aa	297.95 ± 15.35	287.51 ± 5.36	289.02 ± 6.68	306.99 ± 17.30	292.16 ± 8.26	285.84 ± 3.74	310.77 ± 2.88	291.53 ± 3.20	307.57 ± 6.48	285.52 ± 6.04	290.48 ± 9.06	284.75 ± 1.33	308.54 ± 2.89
Histidine	19.20 ± 0.92	17.96 ± 0.34	19.67 ± 0.68	20.21 ± 0.96	18.61 ± 0.75	16.37 ± 0.28	21.35 ± 0.12	16.43 ± 0.18	20.67 ± 0.47	19.35 ± 0.31	21.29 ± 0.07	16.39 ± 0.15	19.89 ± 0.24
Isoleucine	38.65 ± 1.63	34.52 ± 0.23	34.62 ± 1.07	37.99 ± 1.20	35.40 ± 1.64	34.78 ± 0.70	35.66 ± 0.25	36.83 ± 0.67	35.80 ± 1.19	36.49 ± 1.41	36.41 ± 0.30	34.04 ± 0.10	36.23 ± 0.69
Leucine	63.12 ± 3.33	61.06 ± 1.07	59.82 ± 0.79	63.29 ± 3.15	61.84 ± 1.28	60.57 ± 0.49	62.36 ± 0.68	63.85 ± 0.15	62.75 ± 0.85	58.44 ± 0.87	57.78 ± 0.63	61.18 ± 0.06	63.56 ± 0.35
Lysine	44.19 ± 2.46	44.57 ± 1.09	48.76 ± 0.83	51.41 ± 2.66	45.82 ± 1.42	41.36 ± 0.82	52.77 ± 0.74	36.89 ± 0.65	51.03 ± 0.63	40.10 ± 0.07	45.65 ± 0.43	42.32 ± 0.03	51.62 ± 0.22
Methionine	15.55 ± 0.90	15.67 ± 0.49	14.38 ± 0.42	15.53 ± 3.10	14.48 ± 0.40	16.52 ± 0.54	18.52 ± 0.08	14.65 ± 0.20	17.06 ± 0.07	11.27 ± 0.08	12.03 ± 3.29	15.98 ± 0.32	17.44 ± 0.01
Phenylalanine	31.08 ± 1.62	29.34 ± 0.56	28.97 ± 0.60	31.57 ± 1.64	31.00 ± 0.25	31.02 ± 0.36	32.45 ± 0.26	33.02 ± 0.82	32.42 ± 1.01	29.90 ± 0.75	28.27 ± 4.06	31.04 ± 0.42	32.50 ± 0.13
Threonine	33.47 ± 1.82	33.02 ± 1.06	31.96 ± 0.05	33.30 ± 2.01	32.50 ± 0.22	33.82 ± 0.19	34.95 ± 0.34	34.18 ± 0.42	34.57 ± 0.28	32.58 ± 0.36	32.11 ± 0.20	34.07 ± 0.01	34.60 ± 0.31
Valine	52.70 ± 2.68	51.38 ± 0.51	50.84 ± 2.24	53.70 ± 2.59	52.50 ± 2.29	51.41 ± 0.36	52.73 ± 0.41	55.68 ± 0.11	53.26 ± 2.00	57.39 ± 2.21	56.94 ± 0.07	49.72 ± 0.23	52.71 ± 0.93
Total Sulphur (Met + Cys)	34.37 ± 1.53	31.07 ± 1.89	30.11 ± 0.62	31.5 ± 3.93	28.78 ± 0.5	31.77 ± 1.15	36.32 ± 0.49	29.69 ± 1.19	35.77 ± 0.65	25.06 ± 0.53	27.21 ± 3.9	32.95 ± 0.45	36.27 ± 0.33
Total Aromatic (Phe + Tyr)	72.56 ± 2.21	73.47 ± 0.78	75.98 ± 0.81	81.93 ± 4.48	80.79 ± 0.31	73.69 ± 0.49	79.44 ± 0.85	79.00 ± 2.24	81.92 ± 2.41	77.22 ± 1.84	74.39 ± 7.40	75.26 ± 0.68	79.81 ± 1.28
Total Protein %:	63.13	81.96	81.10	78.49	80.30	71.85	73.22	55.09	61.53	58.90	59.85	68.45	67.08
AA %	50.58	62.78	62.74	64.27	63.39	54.51	59.10	42.99	49.78	46.35	46.62	52.13	54.79
% Protein Recovery	80.11	76.59	77.35	81.88	78.94	75.86	80.71	78.05	80.90	78.69	77.89	76.15	81.67
% unknown	19.89	23.41	22.65	18.12	21.06	24.14	19.29	21.95	19.10	21.31	22.11	23.85	18.33
% EAA	36.92%	37.54%	37.37%	37.49%	37.01%	37.68%	38.51%	37.35%	38.02%	36.28%	37.29%	37.39%	38.17%

Table 4: Overview of the essential amino acid composition of all insect samples (n=2)

Amino acids							Samples						
(mg / g protein)	Entomo	HOD	HFD	HDD	HVD	BAOD	BAFD	BLOD	BLFD	LOD	LFD	QOD	QFD
Total Conditionally Essential aa	199.86 ± 6.97	191.56 ± 4.78	200.09 ± 2.43	213.52 ± 7.47	207.85 ± 4.16	185.92 ± 12.16	198.80 ± 11.57	188.20 ± 7.19	201.51 ± 5.92	202.97 ± 3.34	194.57 ± 12.82	187.22 ± 8.78	204.80 ± 6.61
Arginine	55.36 ± 3.44	54.63 ± 1.37	55.03 ± 0.90	56.44 ± 1.86	53.46 ± 1.44	53.20 ± 0.95	61.17 ± 0.46	52.30 ± 0.58	59.03 ± 0.96	48.17 ± 0.15	50.14 ± 2.07	54.03 ± 0.75	59.11 ± 0.60
Glycine	47.17 ± 1.31	40.52 ± 0.61	45.97 ± 1.17	47.33 ± 2.15	46.79 ± 0.73	37.87 ± 0.28	39.99 ± 0.46	43.15 ± 0.53	43.20 ± 1.27	46.91 ± 0.53	48.71 ± 0.15	38.58 ± 0.15	40.95 ± 0.43
Proline	55.85 ± 1.63	52.28 ± 2.59	52.08 ± 0.15	59.39 ± 0.61	57.81 ± 1.92	52.18 ± 10.81	50.65 ± 10.05	46.76 ± 4.67	49.78 ± 2.29	60.58 ± 1.58	49.61 ± 7.25	50.39 ± 7.63	57.42 ± 4.43
Tyrosine	41.48 ± 0.59	44.13 ± 0.22	47.01 ± 0.21	50.36 ± 2.84	49.79 ± 0.06	42.67 ± 0.13	46.99 ± 0.59	45.98 ± 1.42	49.50 ± 1.40	47.32 ± 1.09	46.12 ± 3.34	44.22 ± 0.26	47.31 ± 1.15
Total non-essential aa	290.30 ± 13.54	271.43 ± 6.25	268.67 ± 4.99	282.35 ± 16.06	275.12 ± 5.49	271.62 ± 2.70	279.70 ± 3.81	285.70 ± 3.61	281.24 ± 5.10	284.62 ± 0.63	278.73 ± 41.49	272.61 ± 0.73	276.10 ± 1.99
Alanine	74.93 ± 1.98	66.48 ± 0.81	74.64 ± 1.42	80.11 ± 3.75	77.35 ± 1.41	61.82 ± 0.35	63.56 ± 0.74	66.90 ± 2.43	65.58 ± 2.23	84.11 ± 0.20	82.19 ± 0.84	61.49 ± 0.44	61.89 ± 0.03
Aspartic	74.52 ± 3.98	71.29 ± 1.50	66.05 ± 1.73	68.92 ± 4.85	68.18 ± 1.01	74.79 ± 0.63	75.86 ± 1.08	80.28 ± 0.87	76.34 ± 0.81	66.45 ± 0.32	64.73 ± 39.52	74.95 ± 0.12	75.68 ± 0.24
Glutamic	103.06 ± 5.42	100.16 ± 2.72	93.78 ± 1.41	97.73 ± 5.03	96.14 ± 2.47	102.30 ± 1.59	104.69 ± 1.27	105.80 ± 0.13	103.27 ± 1.74	98.28 ± 0.03	95.79 ± 0.84	102.32 ± 0.15	103.87 ± 0.80
Cysteine	18.82 ± 0.63	15.40 ± 1.4	15.73 ± 0.2	15.97 ± 0.83	14.30 ± 0.1	15.25 ± 0.61	17.80 ± 0.41	15.04 ± 0.99	18.71 ± 0.58	13.79 ± 0.45	15.18 ± 0.61	16.97 ± 0.13	18.83 ± 0.32
Serine	37.78 ± 2.17	33.50 ± 1.22	34.19 ± 0.44	35.60 ± 2.43	33.45 ± 0.61	32.71 ± 0.14	35.59 ± 0.72	32.73 ± 0.18	36.06 ± 0.33	35.77 ± 0.07	36.02 ± 0.30	33.85 ± 0.03	34.65 ± 0.93
Total Sweet (Gly + Ala)	102.53 ± 4.75	95.15 ± 1.98	100.99 ± 2.07	103.77 ± 4.01	100.25 ± 2.18	91.07 ± 1.22	101.15 ± 0.93	95.45 ± 1.11	102.23 ± 2.23	95.08 ± 0.68	98.85 ± 2.22	92.61 ± 0.89	100.07 ± 1.03
Total Savoury (Asp + Glu)	177.59 ± 9.39	171.44 ± 4.22	159.83 ± 3.13	166.65 ± 9.89	164.32 ± 3.47	177.09 ± 2.21	180.55 ± 2.35	186.07 ± 1.00	179.60 ± 2.55	164.74 ± 0.36	160.52 ± 40.35	177.27 ± 0.26	179.56 ± 1.03
% NAA	63.08%	62.46%	62.63%	62.51%	62.99%	62.32%	61.49%	62.65%	61.98%	63.72%	62.71%	62.61%	61.83%

Table 5: Overview of the non- essential amino acid composition of all insect samples (n=2)

The total fat appears unaffected by the drying method chosen (P = 0.2 for variability between methods) but the insect species vary greatly (P < 0.001) with House crickets generally having the lowest amount of fat and Black Crickets the highest.

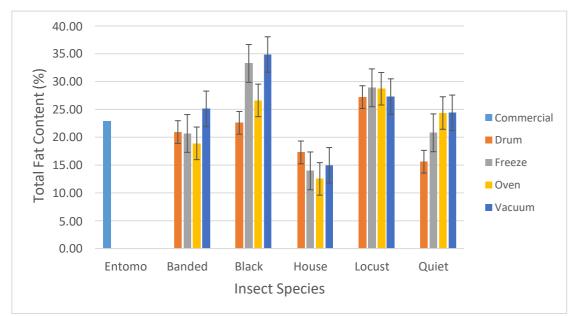


Figure 9: Total fat content of each sample (n=2)

Crickets have slightly more saturated fatty acids while the locust samples have more monounsaturated fatty acids. Saturated fatty acids generally made up 30-40% of total fatty acid composition, though this was higher in oven-dried samples, resulting in a statistically significant difference between drying methods (P < 0.001), due to the higher saturated fatty acid content of the oven dried samples when compared to freeze dried. There were relatively small, but significant differences between species (P = 0.02) which could either be driven by the higher stearic acid content of the banded samples or the differences in the locust samples. By contrast, the MUFA content appears to be unaffected by the choice of drying method (P = 0.18) but is statistically different between species (P < 0.001) and ranged between 20-30% between cricket species but was much higher (almost 50%) in locust. The polyunsaturated fatty acid content appears to vary by insect species (P < 0.001) and is affected by the drying method (P < 0.001) as the oven dried samples have significantly decreased polyunsaturated fatty acids when compared to any of the other drying methods. Table 6 shows that despite small variations, all of the insects are primarily made up of palmitic acid, oleic acid, linoleic acid, and stearic acid with small amounts of a few other fatty acids.

	614.0	646.0			-			620.0	C10.2.2
Fatty Acid	C14:0	C16:0	C16:1	C18:0	C18:1n9c	C18:2n6c	C18:2n6+	C20:0	C18:3n3
	Myristic Acid	Palmitic Acid	Palmitoleic Acid	Stearic Acid	Oleic Acid	Linoleic Acid		Arachidic Acid	Alpha-Linolenic Acid
BADD	0.69 ± 0.01	27.73 ± 0.13	0.65 ± 0.00	13.12 ± 0.03	22.93 ± 0.17	24.40 ± 0.16	0.38 ± 0.04	0.30 ± 0.07	1.28 ± 0.38
BAFD	0.66 ± 0.00	27.78 ± 0.04	0.67 ± 0.00	12.67 ± 0.07	23.16 ± 0.03	24.54 ± 0.07	0.33 ± 0.00	0.35 ± 0.00	1.40 ± 0.00
BAOD	0.87 ± 0.01	37.01 ± 0.00	0.69 ± 0.00	17.97 ± 0.00	22.77 ± 0.09	9.01 ± 0.04	0.14 ± 0.02	0.88 ± 0.00	0.48 ± 0.00
BAVD	0.65 ± 0.01	26.32 ± 0.09	0.68 ± 0.00	11.24 ± 0.19	23.18 ± 0.46	25.18 ± 0.07	0.41 ± 0.01	0.33 ± 0.02	1.38 ± 0.03
BLDD	0.62 ± 0.02	27.18±0.17	0.64 ± 0.02	13.10 ± 0.19	22.45 ± 0.49	24.53 ± 0.94	0.51 ± 0.19	0.42 ± 0.01	1.30 ± 0.08
BLFD	0.48 ± 0.00	24.94 ± 0.33	0.70 ± 0.01	8.92±0.16	32.33 ± 0.4	26.25 ± 0.31	0.75 ± 0.00	0.53 ± 0.01	1.11 ± 0.02
BLOD	0.75 ± 0.01	35.80±0.38	0.77±0.00	13.05 ± 0.32	32.15 ± 0.41	7.61±0.03	n.d.	0.72 ± 0.38	0.23 ± 0.00
BLVD	0.49 ± 0.00	23.82 ± 0.42	0.71 ± 0.00	8.06±0.65	31.43 ± 0.70	28.05 ± 1.79	0.64 ± 0.00	0.45 ± 0.13	1.22 ± 0.17
Entomo	0.59 ± 0.14	23.08±0.42	0.67±0.02	9.91±1.47	27.17 ± 5.01	31.22 ± 1.65	n.d.	n.d.	1.83 ± 0.83
HDD	1.45 ± 0.16	24.90 ± 0.68	0.91 ± 0.05	10.36 ± 0.37	24.16 ± 0.56	29.51 ± 1.12	0.49 ± 0.09	0.39 ± 0.01	1.84 ± 0.10
HFD	1.02 ± 0.04	23.49 ± 0.39	0.90 ± 0.01	8.90±0.37	26.52 ± 0.65	30.63 ± 2.52	0.35 ± 0.01	0.24 ± 0.02	2.08 ± 0.21
HOD	1.15 ± 0.02	27.91 ± 0.18	0.91 ± 0.01	11.84 ± 0.09	25.59 ± 0.26	19.14 ± 0.10	0.18 ± 0.00	0.79 ± 0.00	0.84 ± 0.09
HVD	0.88 ± 0.00	22.48±0.29	0.83 ± 0.00	9.05 ± 0.22	24.15 ± 0.48	31.32 ± 0.37	0.44 ± 0.10	0.46 ± 0.02	1.76 ± 0.01
LDD	2.05 ± 0.01	26.69±0.05	1.52 ± 0.01	6.55±0.07	47.89±0.01	7.48 ± 0.00	0.03 ± 0.00	0.21 ± 0.00	7.03±0.03
LFD	2.03 ± 0.00	26.28±0.06	1.57 ± 0.00	5.98±0.13	45.86±0.05	7.61±0.15	0.04 ± 0.00	0.19 ± 0.01	6.97±0.00
LOD	2.24 ± 0.01	29.16±0.31	1.57 ± 0.03	6.89 ± 0.36	46.18 ± 0.22	5.15 ± 0.55	0.03 ± 0.00	0.27 ± 0.00	3.83±0.43
LVD	2.08 ± 0.13	27.65±1.81	1.53 ± 0.02	6.69 ± 0.58	46.06±0.17	6.40 ± 1.75	0.05 ± 0.00	0.25 ± 0.03	5.21±0.03
QDD	0.75 ± 0.06	26.34±0.9	0.78±0.02	13.08±0.27	21.27 ± 0.97	22.31 ± 2.10	0.67±0.02	0.44 ± 0.00	1.54 ± 0.25
QFD	0.63 ± 0.01	24.36±0.28	0.95 ± 0.02	9.07±0.01	25.35±0.18	30.10 ± 0.01	0.60 ± 0.01	0.38 ± 0.01	2.18 ± 0.01
QOD	0.84 ± 0.00	34.02 ± 0.08	1.10 ± 0.00	13.42 ± 0.09	27.60 ± 0.10	13.16 ± 0.12	0.51 ± 0.00	0.78 ± 0.01	0.56 ± 0.00
QVD	0.71 ± 0.01	24.86±0.04	1.00 ± 0.00	8.80±0.20	26.84 ± 0.01	28.91 ± 0.19	0.63 ± 0.00	0.41 ± 0.03	2.37±0.03

 Table 6: Fatty acid composition for each sample (n=2)

	Tuble 7. combined july usu results and by type and ratio values.								
	SFA	MUFA	PUFA	% Unknown	PUFA/SFA	n-6/n-3	Ai	Ti	
BADD	41.84 ± 0.24	23.58 ± 0.17	26.06 ± 0.58	9.20	0.62	19.36	0.63	1.49	
BAFD	41.46 ± 0.11	23.83 ± 0.03	26.27 ± 0.07	9.12	0.63	17.76	0.62	1.45	
BAOD	56.73 ± 0.01	23.46 ± 0.09	9.63 ± 0.06	11.68	0.17	19.06	1.24	3.19	
BAV	38.54 ± 0.31	23.18 ± 0.46	26.97 ± 0.11	11.37	0.70	18.54	0.58	1.33	
BLD	41.32±0.39	23.09 ± 0.51	26.34 ± 1.21	10.19	0.64	19.26	0.62	1.47	
BLFD	34.87 ± 0.5	33.03±0.41	28.11 ± 0.33	4.74	0.81	24.32	0.45	1.04	
BLOD	50.32 ± 1.09	32.92 ± 0.41	7.84 ± 0.03	8.92	0.16	33.09	0.96	2.40	
BLVD	32.82 ± 1.20	32.14 ± 0.70	29.91 ± 1.96	6.22	0.91	23.52	0.42	0.96	
Entomo	33.58±2.03	27.84±5.03	33.05 ± 2.48	4.94	0.98	17.06	0.43	0.96	
HDD	37.1±1.22	25.07 ± 0.61	31.84±1.31	6.88	0.86	16.30	0.56	1.12	
HFD	33.65±0.82	27.42 ± 0.66	33.06±2.74	6.46	0.98	14.89	0.47	0.95	
HOD	41.69±0.29	26.50 ± 0.27	20.16 ± 0.19	11.84	0.48	23.00	0.71	1.64	
HVD	32.87±0.53	24.98 ± 0.48	33.52 ± 0.48	9.54	1.02	18.05	0.46	0.98	
LDD	35.5±0.13	49.41±0.02	14.54 ± 0.03	0.79	0.41	1.07	0.61	0.70	
LFD	34.48 ± 0.20	47.43±0.05	14.62 ± 0.05	3.70	0.42	1.10	0.62	0.70	
LOD	38.56 ± 0.68	47.75±0.25	9.01 ± 0.98	4.98	0.23	1.35	0.72	1.00	
LVD	36.67 ± 2.55	47.59±0.19	11.66 ± 1.78	4.38	0.32	1.24	0.67	0.84	
QDD	40.61±1.23	22.05 ± 0.99	24.52 ± 2.37	13.26	0.60	14.92	0.65	1.49	
QFD	34.44 ± 0.31	26.30 ± 0.20	32.88±0.12	6.76	0.95	14.08	0.47	0.98	
QOD	49.06±0.18	28.70 ± 0.10	14.23 ± 0.02	8.01	0.29	24.41	0.88	2.14	
QVD	34.78±0.28	27.84 ± 0.01	31.91±0.22	5.88	0.92	12.46	0.48	0.97	

Table 7: Combined fatty acid results and by type and ratio values.

The ash content of the samples was not significantly affected by species (P = 0.19) or drying method (P = 0.44).

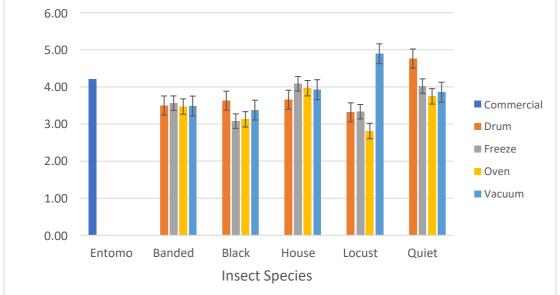


Figure 10: Ash remaining after combustion in a muffle furnace (n=3)

The crude fibre content was unaffected by the drying method (P = 0.7) but there was some variation between species as the house crickets had considerably more fibre than the other samples (P = 0.03).

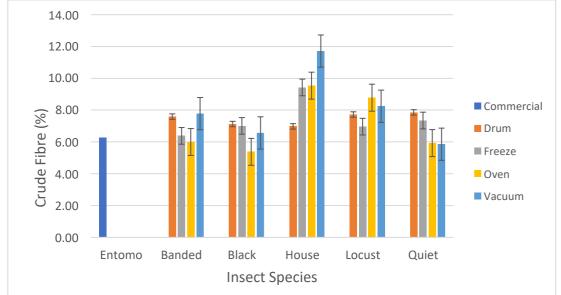


Figure 11: Percentage of crude fibre within each sample (n=2)

The nitrogen free extract was primarily affected by the species of insect (P = 0.04) as opposed to the drying method (P = 0.25). The house crickets had very little on average (0.8%) while the nitrogen free extract accounts for 5.90% of the black crickets, 5.71% of the quiet crickets and 7.70% of the locust on average.

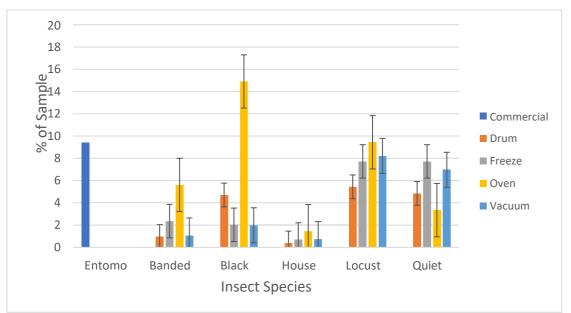


Figure 12: Nitrogen free extract calculated by difference

Analysis of the nutritionally relevant minerals (as defined by the mineral serving a function in the body) revealed quantities of Iodine, Sodium, Magnesium, Phosphorous, Potassium, Calcium, Iron, Selenium, Copper and Zinc that were more than 10% of the RNI in 100g of powder. An overview of the full results from the ICPMS study can be found in Appendix B.

There appears to be no significant difference established through the drying method (P = 0.5) but there is a notable difference between insect species (P < 0.001) as the locust contains the lowest average nutritionally relevant mineral content while the highest amount was found in the quiet cricket samples. There was variation between the cricket species too as there is just over 500mg difference between the average total mineral contents of the banded and quiet crickets.

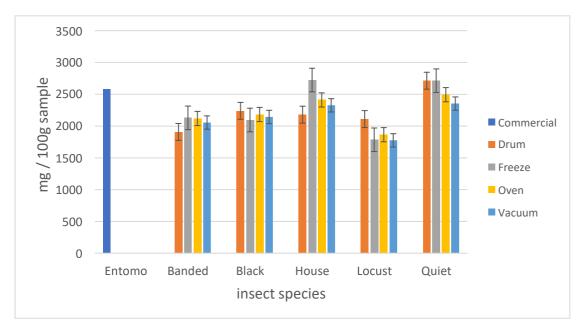


Figure 13: Sum of the nutritionally relevant minerals in each sample (n=3)

The black and banded crickets contained significantly more iodine than other species (P <0.001). There was a significant difference between the drying methods too (P = 0.002) as freeze drying resulted in a much lower value than the other methods (Fig 14). However as detailed in figures 15-24, with the exception of iodine, all minerals presented significant differences only when compared by species (P = 0.01 or less for each mineral when compared by species with the exception of iron which P = 0.08) Quiet crickets presented the highest values of sodium, magnesium, phosphorous, potassium, calcium and manganese. Black crickets contained the highest average results for iodine and iron but the lowest content of calcium and copper. Banded crickets presented the highest results for selenium and copper but the lowest content of iron, potassium and magnesium. The highest result for zinc came from the house crickets. Locust presented the lowest values for sodium, phosphorous, selenium, zinc and manganese.

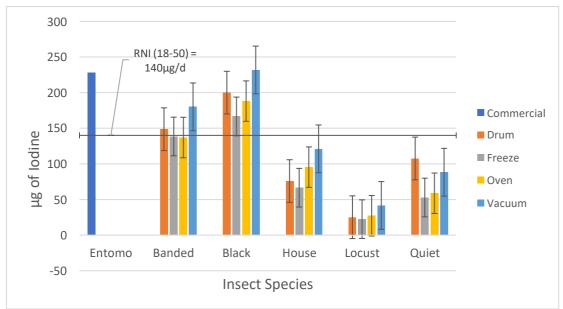


Figure 14: µg of iodine in 100g of each sample (n=3)

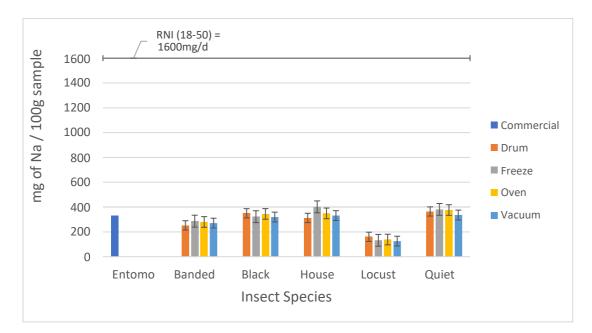


Figure 15: mg of sodium per 100g of sample (n=3)

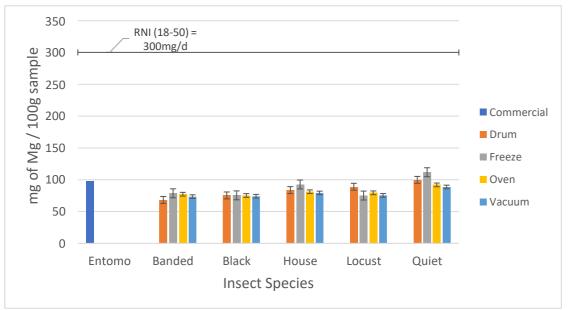
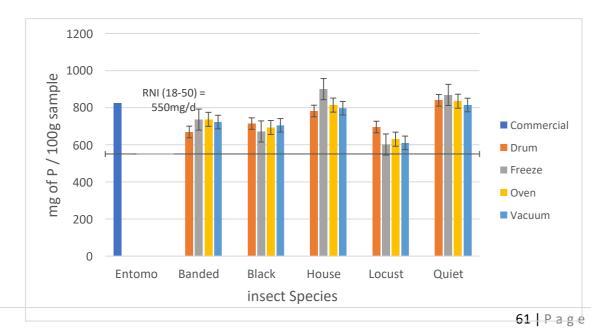


Figure 16: mg of Magnesium per 100g of sample (n=3)





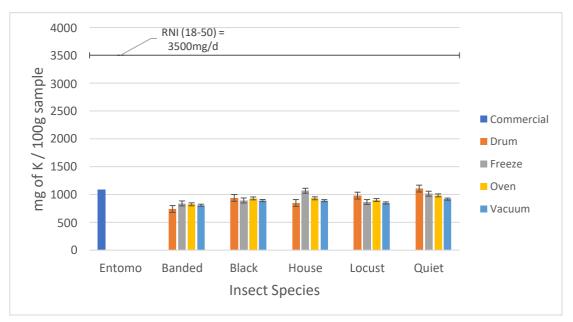


Figure 18: mg of Potassium per 100g sample (n=3)

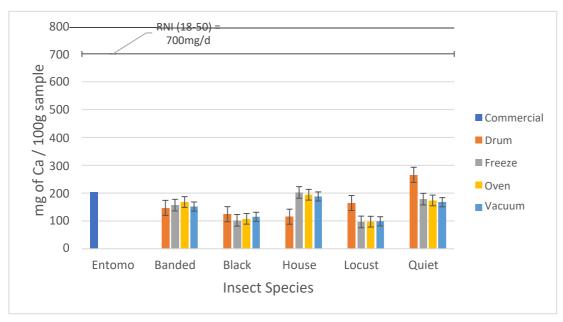


Figure 19: mg of Calcium per 100g of sample (n=3)



Figure 20: mg of Iron per 100g sample (n=3)

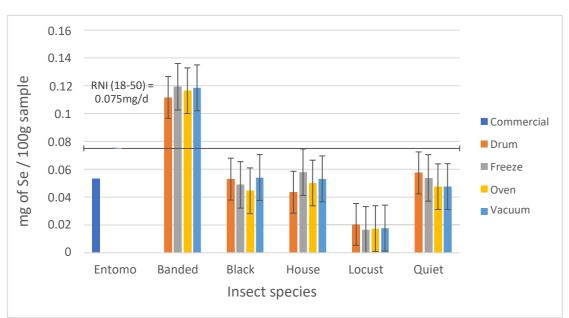


Figure 21: mg of Selenium per 100g sample (n=3)

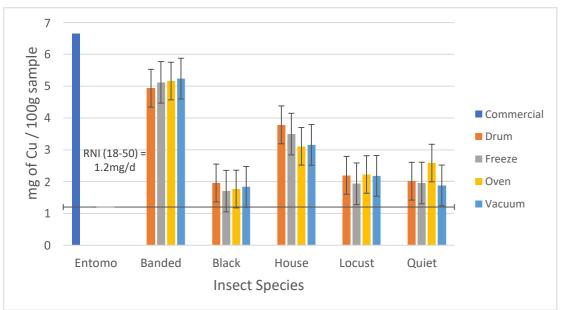
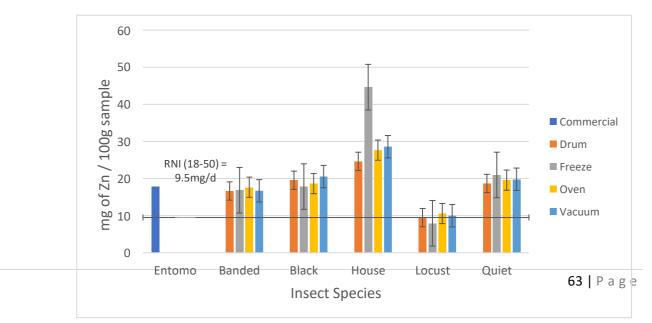


Figure 22: mg of Copper per 100g sample (n=3)



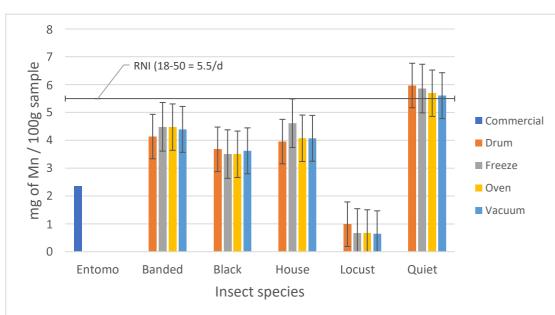


Figure 23: mg of Zinc per 100g sample (n=3)



The primary sugar in all of the samples was glucose followed by two unknown sugars. Statistically, the difference between the species had low significance (P = 0.07) that appears to be driven primarily by the locust having more than twice the total sugars of the cricket samples.

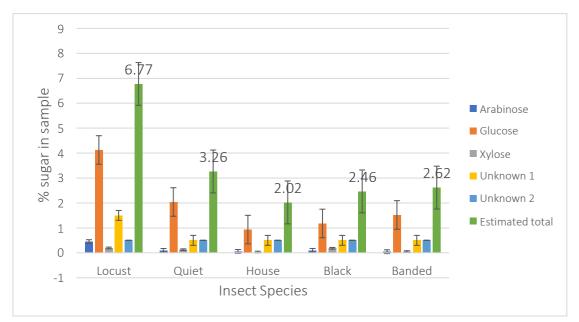


Figure 25: Sugar content of the vacuum-dried insect powder samples (n=2)

2.4.3 Physical Properties of Insect Powders *2.4.3.1 Particle size and Shape*

Under the microscope, ball milling appeared to perform best as most of the visible particulates were below 100µm with a few pieces above that as highlighted in figure 26. Hammer milling (figure 27) and the coffee grinder (figure 29) produced inconsistent results, some particles below the 100µm goal, but most above that. The cutting mill appeared to perform the worst with particles above 1000µm. Particles of the fine commercial sample were generally below 100µm with a few larger. Parts of the coarse cricket powder were above 400µm in length while the bulk of it appeared to be above 100µm.

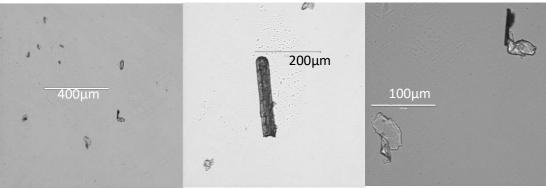


Figure 26: EVOS microscopy images of Ball Milled cricket powder

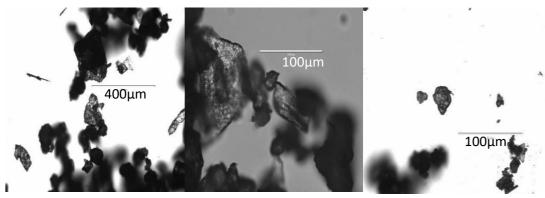


Figure 27: EVOS microscopy images of Hammer Milled cricket powder

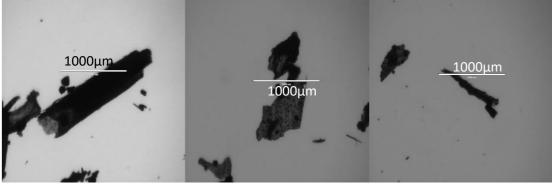


Figure 28: EVOS microscopy images of Cutting Milled cricket powder

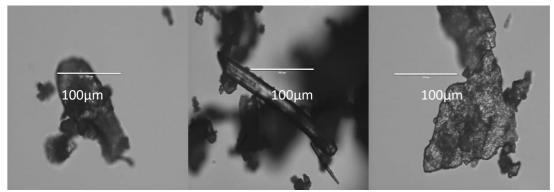


Figure 29: EVOS microscopy images of cricket powder ground using a coffee grinder

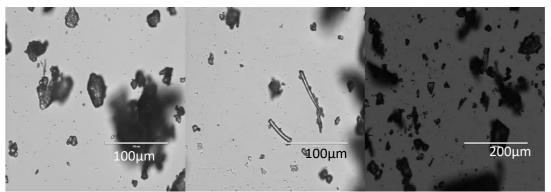


Figure 30: Commercial example - fine ground cricket powder from Entomo farms, Canada

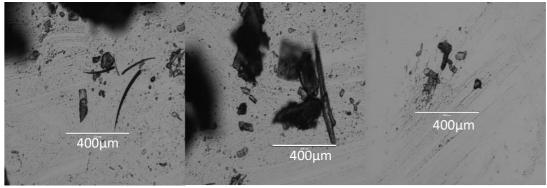


Figure 31: Commercial example - coarsely ground cricket powder from Entomo farms, Canada

As the image below shows, there was an issue milling the Oven-Dried Black crickets. All other samples were able to be milled to a reasonable standard like the Drum Dried Black shown above. The oven-dried black crickets clogged the mill resulting in a poorly milled product. Despite attempts to re-mill this sample, the issues persisted.



Figure 32: Failure to adequately mill BLOD using the cutting mill - Left: Oven-Dried, unable to be completely milled using the cutting mill. Right: Drum Dried Powder for comparison

2.4.3.2 Powder colour

Greyscale (L*) appears to be affected by the drying method (P = 0.019) with no statistical difference between insect species (P = 0.65). The colour values (a* and b*) were solely affected by the insect species (a*: P = 0.001 for species variation and P = 0.1 for method variation), (b*: P = 0.004 for species variation and P = 0.51 for variation caused by drying method). However, when combined to ΔE , only the drying method was statistically significant (P = 0.02). This was down to freeze drying producing samples which were grey in colour and oven drying producing more brown pigments.

	Average L*	Average a*	Average b*	ΔΕ	Colour
BADD	42.61	4.84	5.41	43.22	
BAFD	55.97	3.29	8.31	56.68	
BAOD	42.10	5.17	8.54	43.27	
BAVD	43.04	4.04	7.46	43.87	
BLDD	35.61	3.02	2.69	56.12	
BLFD	42.45	2.56	3.98	42.37	
BLOD	31.82	1.70	1.74	43.11	
BLVD	36.50	3.02	4.14	36.86	
HDD	48.45	3.73	6.92	49.08	
HFD	53.62	2.44	7.92	54.26	
HOD	44.31	4.34	8.09	45.25	
HVD	47.24	3.29	8.85	48.17	
LDD	48.53	8.56	13.81	51.18	
LFD	50.80	5.36	12.94	52.70	
LOD	36.10	5.43	6.39	37.06	
LVD	37.64	5.43	8.11	38.88	
QDD	44.07	3.60	5.53	44.56	
QFD	55.04	2.64	9.21	55.87	
QOD	42.01	4.96	8.55	43.15	
QVD	44.41	3.81	7.65	45.22	

Table 8: Results of Colour analysis of the dried insect powders (n=3)

2.5 Discussion

The data presented in this chapter indicates significant difference in the nutritional composition of different insect species, even when their diet and rearing conditions are similar. There is also evidence that the drying methods impact on some specific nutrients. Notable differences were found in the overall protein content, the amino acid profile, the total fat, fatty acids profile, and the crude fibre as outlined below. House crickets had the highest protein content while also having the lowest available energy and total fat suggesting these as the best species for the purposes that insects are typically attributed to. Black crickets had the highest protein content. However, there are also some important notes on how this research was conducted and some errors in the results.

Although the nitrogen conversion for the insect used within this study are expected to be less than the x6.25 conversion factor used here, the values presented by Janssen et al (2017) are for three species of insect which have a holometabolous metamorphosis preventing a similar comparison due to how the chitin is formed for those species. How much the formation of chitin effects the protein content can vary as much as 30% depending on the stage of the insects life and its metabolism (Jonas-Levi and Martinez, 2017). Utilising the x6.25 figure for this study but presenting the nitrogen results (Appendix A) allows for the comparison to other research which has utilised this figure to determine protein content while leaving it able to be interpreted accurately in the future.

The results for the nitrogen free extract suggest that generally the drying method has little effect on the macronutrients. The large spike in the BLOD sample on Figure 12 suggests compounded minor errors in other areas of the study, likely caused by the incomplete milling process. This means that some aspects of this sample are likely to be under-reported. Removing this obvious error from the results does not impact the statistical significance of the results though, the methods remain insignificant, while the species remains a significant factor. Although it is also important to note that the methodology used in this study does not rule out the potential for interaction, where the drying method affects one species more than another resulting in a higher or lower nutritional value.

The ash content was fairly similar across all species and methods of drying. With the

overall average of 3.6% ash, this is a reasonable indicator that these insect species' could be a valuable source of micronutrients.

Finally, identifying sugars in insect samples poses some challenges. The methodology utilises strong hydrolysis reactions at high temperatures which breaks down some of the longer chain carbohydrate polymers within the sample. This leaves it unclear where the sugars presented have originated. It is expected that some of the results are a by-product of the breakdown of chitin during the hydrolysis, rather than this being the amount within the sample. Despite this, if a method for sugar analysis could be developed, the results demonstrate that there may be value in studying this areafurther, to identify the unknown sugars because N-acetyl glucosamine, glucosamine and other micronutrients that could be present have gained recent value as health supplements. One potential is that the compounds registering by this technique are breakdown products of chitin such as glucosamine, which have recently been gaining popularity as components in health food or supplements.

2.5.1 Species differences

In general, the largest differences between the samples were found when comparing the species. Comparing the locust samples to the crickets often yielded the biggest differences but there were clear differences between the cricket samples for the majority of the analysis carried out in this study.

2.5.1.1 Protein and Amino Acids

Considering the nutrition results, House crickets contained the most protein, followed closely by the banded samples with the locust generally containing the least. In all of the cricket samples, the protein provides most of the energy, however, for the locust, it is fairly evenly distributed between the fat and the protein (average of 39% from fat vs 37% from the protein). House crickets are on average over 10% better than the closest sample for Kcal from protein (56% in the house compared to 46% in the banded). Regardless, all samples meet the criteria to count as "high in" protein for product labelling purposes.

In line with previous research it appears that the amino acid patterns vary between all insect species, even within the same order (Bukkens, 1997). The limiting amino acid in this study is always methionine, with one exception; BLOD which has slightly lower histidine. All samples meet the requirements set by the WHO/FAO/UNU for histidine, isoleucine, phenylalanine, threonine and valine. All crickets meet the requirements for leucine, but the locusts do not. Lysine appears to be particularly affected by the oven drying as only the oven dried sample of each species are below the WHO/FAO/UNU guidelines. Both banded cricket samples along with the freeze dried black and quiet meet the requirements for methionine. Generally, they are slightly below these requirements. All samples meet the requirements for total sulphur and total aromatic amino acids. None of the samples meet the 40% total essential amino acid goal either, however, as tryptophan is missing from these results, it could go some way towards making up this shortfall. As the results for protein recover ranges between 75% and 81%, it seems likely that the full results should demonstrate that the insects would meet the 40%. When compared, there is only a single point of data where the insects exceed beef for amino acid content. This is when freeze dried locust has a 0.39 mg higher valine content per gram of protein. A comparison to pork offers similar results, except all but QOD has a higher valine content. None of the samples have amino acid values above what is presented for casein while the soya bean values are only exceeded on valine. As such, the protein quality of these samples is overall lower than that of meat and lower in the amino acids that the plant-based proteins provide but insect protein contains all essential amino acids where plant proteins do not.

2.5.1.2 Fats and Fatty Acids

Total fat varies a lot between the different species, with house crickets having the lowest total fat content. This could make them a better choice for the production of health foods as lower total fat could be a desirable trait in this type of product.

The ratio of n-6/n-3 is considerably higher than the ratio's suggested to be beneficial to health for all cricket samples edging into figures that are associated with adverse effects (Simopoulos, 2002). It is possible that some of the unknown fatty acids may contribute to this figure for the cricket as there is a notable amount of long chain fatty acids which are not identified by the method used in this study. The locust though, due to a decreased amount of linoleic acid and considerably more ALA than crickets, have ratio's close to 1:1 which could be a major upside to their consumption. The low atherogenic index across the board suggests that the ratio of fatty acids in insects would favour reducing the adhesion of fatty acids to artery walls. The thrombogenic index, on the other hand, indicates that all of the cricket species are mildly favouring clotting which is less than ideal but the ratio is fairly low even forthose samples above 1:1. The primary differentiator is that due to the oxidation of PUFA during oven drying, the ratio is higher for OD samples.

2.5.1.3 Crude fibre

The crude fibre content for all samples is fairly high. These results come with a few caveats though, as it is unclear whether the methodologies used for fibre analysis would affect the chitin content. This concern is because the method for converting chitin to chitosan involves boiling in a strong alkali in the same way that is required for the determination of crude fibre. Overall house crickets come out as the highest fibre content at an average of 9.41% with locust the next closest at 7.93%. Compared to literature, the results are considerably higher than the locust and banded cricket results but as the study uses an enzymatic method it is unclear how comparable the result is (Zielińska *et al.*, 2015). The fibre results for house crickets reared in the USA are almost double the findings of this study but the study utilises acid detergent fibre as its methodology (Finke, 2002; Rumpold and Schlüter, 2013a). The result for black crickets presented by Ghosh et al are comparable to the results found here but they do not list their methodology at all (Ghosh *et al.*, 2017). Despite the lack of directly comparable literature, the results here add further value to house crickets being the species which could be best for health food products in the western world.

2.5.1.4 Minerals

The nutritionally relevant minerals content of the tested insect species seems particularly high as several minerals such as phosphorous and copper are above the daily reference nutrient intake. One hundred grams of all samples would be enough to label them as "high in" phosphorous, iron, zinc, potassium, copper, manganese, chromium and molybdenum while also being a "source of" magnesium. In addition, all of the cricket samples could be counted as "high in" iodine and selenium while also being a "source of" calcium. Quiet and house crickets have the highest average content of nutritionally relevant minerals driven by the higher phosphorous and zinc values. The iron content may be affected by the drying method as there is consistently lower results for the oven dried and vacuum dried samples however it is unclear why without further study.

The lower content of sodium compared to other types of protein powders, especially in the locust samples, means that it may be possible to formulate foods that are "low in sodium" (product has to be below 120mg of sodium per 100g) using insects as a protein source (United States Department of Agriculture, 2008).

2.5.2 Drying method differences 2.5.2.1 Protein and Amino Acids

The potential protein variation by drying method is just below the level of definitive significance (P = 0.067) however there was nothing consistent about the results that would allow for insight into whether the drying method can affect overall protein content. This certainly needs further study as it indicates overall protein content may be being affected. There are known reactions which could cause this, such as the Maillard reaction which have been documented in the literature, however further analysis of the sugars could be used to confirm this as there would be a reduction in the present reducing sugars too (David-Birman, Raften and Lesmes, 2018). It is interesting though that drum drying is the method which appears to maintain the highest protein levels for most samples as it can reachtemperatures above that of the oven drying suggesting that the time spent drying may be a larger factor or that moisture left behind in the drum drying process limits the rate of degradation.

The potential significance of this protein difference caused by drying method is reinforced by differences in amino acid composition. The low temperature freeze drying method yielded a slightly higher result for almost all amino acids. This could be explained through the protein conformational changes; Maillard reaction in the oven dried samples, or the heating having an effect on the amount of protein bound to Chitin (David-Birman, Raften and Lesmes, 2018; Kröncke *et al.*, 2019).

2.5.2.2 Fats and Fatty Acids

There appears to be an increase in saturated fatty acids and a decrease in polyunsaturated fatty acids by over 20% in some cases during oven drying. This does not occur during vacuum oven drying, and as these take place at the same temperature, this suggests that the PUFA may be being oxidised rather than lost in another manner. This leaves the samples with a fatty acid profile which has a reduced nutritional benefit, due to the loss of essential fatty acids like linoleic and alpha-linolenic acid. Drum drying has some effect but not as dramatic as the oven drying, likely due to the shorter period of heating. Maintaining these could be a potential selling point worth the choice of drying process. Locust is vastly different compared to the crickets. It is made up predominantly of oleic acid (C18:1n9c) whereas the crickets contain similar amounts of palmitic acid (C16:0), Oleic acid and, when not affected by oxidation; Linoleic acid (C18:2n6c). It is unclear whether on balance, the locust's fatty acid profile that is predominantly MUFA would be better overall than the crickets profile that has the trade-off between SFA and PUFA.

2.5.2.3 Crude Fibre

Although differences between drying methods was not concluded to be statistically significant, if proven to be accurate through biological replication, the small differences may be commercially significant when the insect powder is sold. This is because all samples except for BLOD, QOD and QVD qualify for the labelling requirements of "high in" fibre (6g/100g).

2.5.3 Powder Preparation

Literature suggests that the boiling used within the initial preparation process may inactivate enzymes and other biological processes which could change the quality of the end product (Hyun-Jung, 2015). Enzyme activity, creating components such as lactic acid was noted to be a positive influence during consumer sensory testing so this initial processing may be worth further focus (Farina, 2017). In addition to this, it could also leach water-soluble micronutrients and fats during this procedure lowering the overall nutritional value (Adámková *et al.*, 2017).

Drum drying is by far the fastest method of producing a dry powder that was tested, but it produces a considerable amount of product loss through either sticking to the drum or falling off before being completely dry. An upside to production using this method is that it creates a visibly uniform powder reducing the need for further milling, however, it is entirely possible that product quality could be further increased if a suitable milling step was added afterwards. It may be the case that drum drying is the most commercially optimal if loss during production can be avoided as it gets the moisture down to a safe level, leaving behind an increase in end-product weight. As these products are often sold on a weight basis, it could add value to optimise the drying process to the maximum permissible moisture based onwater activity. An aW of below 0.6 is typically enough to prevent the growth of microorganisms but it should be recommended to go below this value as it has been noted that the powders will draw in atmospheric moisture during storage (Kim *et al.*,2016). The study by Bassett (2018) suggested that there were noticeable development of aromas during the blending of insects to a slurry for drying however, this was not noticeable by the researcher when producing the slurry for drum drying.

Vacuum drying appears to have taken the longest amount of time to reach the initial plateau of dryness, but it is unclear as to why. Theoretically drying under vacuum should speed the process up but it does not seem to have been the case with these samples. While vacuum drying removed the most moisture on average, minor variations in the total moisture removal is unlikely to be significant to the food industry. The trade-off of total moisture removed versus the cost of using vacuum oven or freeze-drying methods is likely to make it unappealing even if the lower moisture content is desirable. When compared for the production of dried mealworms, the cost of freeze-drying and vacuum oven drying were found to be more than four times as expensive as oven drying due to the amount of power required to sustain the equipment and the amount of product that can be processed at the same time (Kröncke *et al.*, 2019). Overall, the house crickets lost the most moisture during drying but when comparing the vacuum drying figures, it appears to dry the fastest indicating that there may be an interaction between the drying method and species of insect which could benefit from more research.

Comparison of milling demonstrated that the choice of milling method is likely to have a large impact on the quality of the powder product. A desirable particle size is below 100 microns as this is the detectable limit of the average tongue. This would allow for the creation of texturally smooth products such as drinks where particulates would be viewed negatively to the consumer. Out of the methods tested, only ball milling seemed to achieve this consistently. As this is a laboratory standard method rather than something regularly used within the food industry, using this method in large scale food production is likely to be cost-prohibitive. There may be further optimisations to be made with the cutting or hammer mill that could improve what is demonstrated here or particle sizes could be reduced through including a sieving process to remove large particles. The issues with the milling of the oven-dried black crickets are as yet unexplained and require further investigation see if this is a regular occurrence or just a one-off fault. The sample had a greasy feel indicating that it could be linked to the changes in the fatty acid profile as the oxidation of fats would change them from being liquid at room temperature to solid.

2.5.4 Quality Study

Unfortunately, none of the milling methods trialled resulted in consistent particle shape. This is one of the key parameters for ease of incorporation into products like baked goods, as sharp shapes such as those from antennae could result in damage to the structure of a food product, such as during bread proving for example (Gan *et al.*, 1989). The ball milling resulted in the lowest and most consistent particle size. However, this is a laboratory/pharmaceutical method that is considerably more expensive to implement than the others trialled. Hammer milling has potential. The microscopy shows some particles that are below the ideal 100 microns but quite a wide variation. It is possible this methodology could be optimised by someone with more experience to produce a high-quality powder.

The colour of the resulting powder differed significantly with drying method. The L* value was reduced in the freeze-dried samples resulting in a greyer appearance. The reddish- brown appearance in the samples which used a heated drying method (particularly oven drying) showed further potential to the theory that the oven-dried samples may have been affected by the Maillard reaction as this generates colour by protein reduction. This reaction also generates flavour and aroma though, which may have a larger influence on consumer perception than the colour. The food industry generally favours white protein powders as they would have limited impact on the colour of the foods they are being added into. This could mean that the greyer freeze-dried samples may be preferred for mass production despite there being suggestions that the colour was not favoured by consumers. Overall, the powders are pretty similar when compared, usually falling into the high end of the "perceptible differences at aglance" category.

2.6 Conclusions

In conclusion, this study confirms that insects represent a potential important source for a range of nutrients. They are high in protein that is rich in essential amino acids, contain some valuable essential fatty acids and would be a good source of a diverse group of minerals.

As hypothesised, the drying methods that relied on heat did yield some differences between the samples. This was particularly notable in the fatty acids but potentially more significantly to the product, the amino acids changed resulting in a protein quality difference. This protein quality difference was likely induced, at least in part by the Maillard reaction as the colour changes would also be consistent with those changes.

In general, house cricket seems to be the best species here for food product development as it had the lowest total fat, highest fibre and highest protein content overall, giving it the best macro-nutritional profile. In terms of micronutrients all of the crickets had the same presentable values although the quiet cricket samples had more nutritionally relevant minerals than the other species' when considering a serving weight of 100g. Basing nutritional results on the consumption of 100g of powder may be unrealistic though, and while they would still be good, this value over-estimates the nutritional value due to the way "high in" and "source of" are described. This value is likely to come down as insect powder consumption becomes more mainstream and the typical serving weight becomes apparent.

The species of insect appears to matter far more than the method used to produce insect powder although the drying method does impact on some key nutrients. In particular, the reduction of essential PUFA during oven drying should be a concern as this is the method which is the most economical in terms of large-scale production. Other methods maintain these fatty acids but at a cost increase which could be prohibitive for use in food products. The potential for the overall protein content to be affected by the choice of drying method needs further exploration as this is the primary selling point for insect powders and results here are inconclusive. Similar further testing should also be considered for iron content as a lot of the advertising surrounding insects focuses on this value.

As for the drying methods, commercially, drum drying followed by an optimised hammer mill would yield the best / most cost-effective product from the methods

detailed during this study. The factors which are affected by drying method such as the oxidation of PUFA, are minimally affected by drum drying and the potential for less protein degradation during production should not be overlooked as insect powder is primarily sold on its protein value. The remaining moisture provides additional product weight without risking the immediate safety and all of this is achieved in a much lower production time. However, further studies should consider whether the formation of the slurry causes any negative sensory properties. There are methods not trialled in this study such as microwave drying that would be potentially more expensive due to smaller throughput and higher initial cost of equipment. Spray drying, if optimised should yield a powder with a very low particle size, minimising the need for further milling, however literature reports it producing off flavours during the processing (Bassett, 2018).

Chapter 3: The safety considerations for utilising insect powder in food development

3.1 Introduction

There have been a host of concerns raised about introducing insects into the food system such as the risk of allergens, microbiological pathogens, anti-nutritional factors, metal toxicity and the risk posed by insect particles within the human body (Zhuang, Zou and Shu, 2009; Kouřimská and Adámková, 2016; Grabowski and Klein, 2017b; de Gier and Verhoeckx, 2018; Fasolato *et al.*, 2018; Fernandez-Cassi *et al.*, 2018; Pali-Schöll *et al.*, 2019).

In the western world, the primary production of insects for human consumption has focused on crickets, drying and grinding them into a protein-rich powder which can be incorporated into a wide array of food products. Typically, in the production of commercial cricket powder, crickets are reared in small, temperature-controlled storage containers for 6-8 weeks, and fed on commercial animal feed, in order to comply with legal guidance, which suggested that moving away from a certified feed poses a risk to human health (The Scientific Committee of the Federal Agency for the Safety of the Food Chain, 2014). The production process of cricket powder varies between producers, but one example might be that once at the adult stage of development, the crickets are frozen as an ethical kill step before being boiled at 100°C for 5 minutes in order to reduce the microbial load as suggested by the FASFC (The Scientific Committee of the Federal Agency for the Safety of the Food Chain, 2014). The boiled crickets are then oven-dried at 90°C for 110 minutes and going through a further milling process if necessary, to produce the grade of powder required. Finally, the powder is packaged in sealed containers with a desiccant and/or oxygen absorbent packet in order to extend the shelf life.

In this example, the freezing, boiling and the drying stages could all potentially have a detrimental effect to any present bacteria, while the milling and processing stages have the potential to introduce additional micro-organisms by contamination, typical of milled food ingredients. The boiling particularly, should effectively reduce the quantity of any surface bacteria that the crickets were carrying, however, a study carried out later has shown that reducing any internal bacteria is likely to prove more difficult (Grabowski and Klein, 2017b).

The methodology of farming seems to have a great effect on the safety of the insect powders. Minerals in the environment that the insects are raised in, directly correlate to the minerals found during nutritional studies and the environmental microbiota are going to influence what is found on the insect during safety studies (Belluco *et al.*, 2013). The composition and microbiota of the insect feed will also correlate to what is found within the insect's gut. This presents a problem as there are a growing number of insect farms focussing on different insect species and utilising different methods to produce their products.

Typically, food safety is ensured through blanket legislation that covers an entire animal type or food product category (The Instutute of Food Science and Technology, 1997). With there potentially being so many different types of edible insects that could be used in food production, this approach may not be viable but there needs to be a focus on this area of research in order to ensure that all insects entering the food chain will be safe. This variability poses some problems as legislators need definitive results so that they can ensure food safety.

In the literature, these risks have been heavily speculated upon, with far less studies providing definitive answers (Klunder *et al.*, 2012; Belluco *et al.*, 2013; Rumpold and Schlüter, 2013a). This speculation itself is a risk, as insects have already entered the food chain without definitive research to ensure that they are safe to consume. Speculation creates delays as researchers have to chase dead ends rather than confirming actual risk. Therefore, studies that can provide some definitive insight into the safety of insects should prove valuable.

3.1.1 Microbiology

Several studies into the microbial safety of insects and their products have taken place yielding inconsistent results (Garofalo *et al.*, 2017; Megido *et al.*, 2017; Vandeweyer *et al.*, 2017). One such study indicated that the microbiological potential for whole insects could include a wide range of pathogenic bacteria from 13 different species, but the primary pathogens that pose a risk in crickets were *Klebsiella*, *Yersinia* & *Citrobacter* (Schlüter *et al.*, 2017).

It is unclear where exactly the bacteria come from within the insect. Surface bacteria are likely to be environmental and could be controlled through changes to the environment, while gut microbiota is likely to be influenced by the insect species and the feed it consumes (Fernandez-Cass et al., 2018). When producing insect powders, both areas are going to be mixed during the milling process. If the heat treatments during production happen while the insect is whole, care must be taken to account for the size and shape to ensure that the gut is also affected.

There are few studies which analyse commercial cricket powder directly. In one of these studies, DNA for *Listeria*, *Staphylococcus*, *Clostridium* and *Bacillus* were present within the powder but in low quantities. The study did conclude that due to using a genomic methodology, some of these bacteria may have been initially present as part of the insect's gut microbiota but are unlikely to have survived the processing into powder (Garofalo *et al.*, 2017). Further to this suggestion of low pathogenic risk, a different study suggested that the Enterobacteriaceae and spore-forming bacteria that are isolated from cricket powder are typically not pathogenic and that a heating process followed by refrigeration would be enough to guarantee safety for at least 2 weeks in a food product (Belluco *et al.*, 2013).

Despite the production methods involved in cricket powder providing some reduction in bacterial counts, Grabowski & Klein (2017b) found that drying methods are not enough to reduce intestinal bacterial counts within insects and that dried and powdered insects had higher bacterial counts than deep-fried or cooked insects partly because of this. In this study, the powdered insects contained a wide array of bacteria and moulds which could be hazardous to human health including *B. cereus*, coliforms, Serratia liquefaciens, Listeria ivanovii, Mucor spp, Aspergillus spp, Penicillium spp and Cryptococcus neoformans (Grabowski and Klein, 2017b). Further studies by Osimani et al., also found that cricket powder yielded higher microbial counts than other types of cooked insect food products (Osimani et al., 2017). This research linked the higher counts to the additional processing involved in producing cricket powder – particularly the milling. The counts presented posed some figures, such as the 5 log of *Bacillus cereus* pathogen found in cricket powder, which would be particularly concerning when considering making the powder into food products for human consumption, as this is above the limits suggested by the IFST for dried foods $(10^3 - 10^4)$ (The Institute of Food Science and Technology, 1997).

This increased risk posed by the milling process suggests the insect powders require careful handling in order not to garner further pathogenic risk from the environment. Bacteria, moulds and other microflora can be introduced through contamination from human involvement in food production, residues remaining on processing harbour bacteria from these sources of contamination under regular usage conditions within a food production facility.

Overall, the findings so far are of some concern, as there are large differences across each source of cricket powder or insects tested. Although this is likely to be linked to the environment in which the insects are raised, it makes for some difficulty when considering the regulations required to secure food safety. It has been estimated that each insect species brought into the food chain could bring with it 10 new bacterial species which will also need to be conclusively studied for pathogenicity and risk to food quality (Larsen *et al.*, 2017). As several countries within Europe have announced that they will accept food products containing insects, they may be lacking the conclusive literature surrounding the necessary control mechanisms to create a safe food product (Reverberi, 2017).

In an attempt to bring in some control prior to defining insects as a novel food product, the Scientific Committee of the Federal Agency for the Safety of the Food Chain (FASFC) in Belgium published a document suggesting that, until more studies were carried out on the microbiota of insects for human food, a full botulinum heat treatment process (12D cook) would be necessary to ensure safety due to the presence of spores (The Scientific Committee of the Federal Agency for the Safety of the Food Chain, 2014). Despite the suggested 12D cook, the FASFC's suggested standards for microbiota within insects followed the criteria for minced meats and shellfish providing thresholds for *Salmonella* at absence in 10g, *E. coli* at 500 CFU/g, *L. monocytogenes* at 100 CFU/g and a total aerobic count for the raw insects at a maximum of 5 x 10⁶ CFU/g. This report also highlighted the risk posed by mycotoxins and suggested the inclusion of a pH control within a product formulation, freeze-drying to produce products such as cricket powder and wet cooking methods for microbial control.

However, when it comes to commercial cricket powder, standards similar to other dry, high protein powders may be more appropriate than meat, particularly as the standards set in place by the FASFC may miss some organisms that are more prevalent in dried foods. The control methods necessary during food production of cricket powder will be highly influenced by those used in the production of the ingredient itself so getting this documentation right is important to securing food safety when using these novel ingredients for mass production. An example of the criteria for dry food products that require further cooking, can be gained from the Institute of Food Science and Technology's (IFST) microbiological criteria. They set the thresholds for key organisms such as *S. aureus*, *B. cereus* and *C. perfringens* at 10^2 CFU/g at point of food production, rising to a strict maximum of 10^3 CFU/g or 10^4 CFU/g for *B. cereus* and *S. aureus* at point of consumption. In addition to this, it is also suggested that indicator organisms such as *E. coli*, yeasts and moulds should be monitored for this type of product. Ready-to-eat foods and those that have been heavily heat-processed should also include a test to confirm the absence of *Salmonella* in 25g of product (The Institute of Food Science and Technology, 1997).

The influence of processing has been studied within some other insect species covering aspects of nutrition and quality but the effect of processing on microbiology remains an area with minimal literature. Inferences could be made through looking at the impact of these techniques on other food products, but it has been demonstrated in other areas of literature that insects do not always behave the same as other food analogues. One example taken from the nutrition is that the chitin exoskeleton can change its behaviour during heating, binding more minerals when heated (Bassett, 2018). This may also have an effect on microbial viability as the minerals are required for some microbial pathogenesis too so if they become bound to the chitin, it could make the cricket powder less hospitable to microorganisms (Terwilliger, Maresso and Baylor, 2016). Due to the minimal number of species that have been compared when discussing processing methodology, it remains valuable to see if different species within the same order behave differently (Klunder *et al.*, 2012).

An understanding of the microbiota present within the cricket powder will allow for determination of adequate controls when the powder is utilised within a food product. This way, it should be possible to ensure food safety even without defined controls. These are often specific to the food product, as using the wrong control mechanism would influence consumer perception of the food product. However, a change in pH, low water activity, removal of oxygen or the inclusion of natural antimicrobial ingredients could all benefit the safety of a food product that contains insects.

3.1.2 Heavy metals and minerals

It has been proposed that insects may accumulate minerals and heavy metals from their feed and environment over the course of their life (Fernandez-Cassi *et al.*, 2018). For example, there have been cases of lead poisoning from imported crickets in the USA (Handley *et al.*, 2007). In addition to this case, it was found that zinc and copper can accumulate along the food chain, potentially posing a risk if high amounts are consumed (Zhuang, Zou, & Shu, 2009). Although the risk was discussed, it was deemed that it should not be considered as a risk factor when the insects are utilised as feed as the heavy metals accumulated in parts of the animals, not typically consumed. (Belluco *et al.*, 2013). Aside from these studies, it seems that little consideration has been given to the risk of mineral toxicity as the primary studies which consider mineral content focus solely on their contribution to nutrition (Rumpold and Schlüter, 2013a; Zielińska *et al.*, 2015; Patel, Suleria and Rauf, 2019).

3.2 Aims & Hypothesis

The aim of the pilot study was to determine if the commercial sample would potentially be contaminated by the environment under typical use conditions in the food industry. In addition to this, the study was designed to gain an overall understanding of the microflora present in a sample directly from a commercial cricket powder producer through the use of selective and non-selective media. This also completed the necessary risk assessment of the commercial powder required for product development (chapter 5) using the university food production facilities.

The enrichment study followed this up by considering how the small number of bacteria present, may grow under abuse conditions and to understand what the specific species of bacteria are in order to understand what controls will need to be in place during product development.

The third part of the microbiology study is to consider how the production process used within chapter 2 affects the microbial safety of powdered insects when compared to a commercial sample, in order to be able to develop safe products.

Finally, data relating to mineral content (from chapter 2) will be considered in terms of the risk of toxicity, and the particle size will be reviewed due to the potential safety risk it can pose.

The hypothesis is that due to the processing involved in the production of the cricket powder, the initial levels of microflora in the powders will be low. However, any surviving microflora of samples produced without the extended heat treatments of the oven-based samples may yield potentially harmful bacteria.

As for minerals, the hypothesis was that the expected amount of powder consumed as part of a normal diet would not yield toxicity on its own, however, it may be cause for concern when considering diets as a whole.

3.3 Materials and methods

3.3.1 Water activity

An Aqualab 4TC running at 20°C was utilised in determining the water activity of the dried samples produced during chapter 2. To ensure functionality of the machine, pure water was run before and after a set of samples as a control with a reading of 0.95 or lower being cause forrecalibration.

3.3.2 Microscopy

Particle size and shape was assessed qualitatively using an EVOS FI microscope (Advanced Microscopy Group).

3.3.3 Minerals from a safety perspective

Results from mineral analysis (See chapter 2.3 for full methodology) were compared to safe upper limits (SUL) and tolerable upper limits (TUL) (note: TUL used for selenium only due to the absence of available SUL) set to prevent toxicity (Expert Group on Vitamins and Minerals, 2003).

3.3.4 Microbiology pilot study

3.3.4.1 Sample preparation

Samples used within this study were commercially-sourced powdered crickets (*Gryllodes sigillatus*) intended for human consumption produced at a production facility in Canada. The exact method of production is commercially sensitive; however, it is described as being roasted before being ground into powder (Entomo Farms, 2016).

The sample for this study was opened and used within a simulated factory environment based at the Sutton Bonington campus of The University of Nottingham, UK, in order to permit possible atmospheric contamination which would be consistent with regular use during a commercial product development cycle.

Duplicate samples (5 g) were transferred into a filter lined stomacher bag (Seward BA6041/STR) along with 45 ml of sterile buffered peptone water (Oxoid CM0509). Samples were homogenised using a stomacher (Seward Stomacher 400 Circulator, West Sussex, UK) for two minutes at 275 RPM. These samples were treated as the 10^{-1} dilution. One stomacher bag was set in a water bath at 80°C for 10 minutes. Each sample was further diluted by 10-fold serial dilution; the unheated to 10^{-6} and the heated to 10^{-4} using the sterile buffered peptone water.

3.3.4.2 Microbial analysis

Samples (0.1 ml) were spread plated in duplicate on selective and non-selective media. Plate count agar (PCA) (Sigma 70152) was used to identify aerobic mesophilic bacteria. PCA was also utilised to identify anaerobic mesophilic bacteria by incubating in a sealed container with an AnaeroGen sachet (Oxoid AN0035) and an anaerobic indicator patch (Oxoid BR055). Enteric bacteria were identified using MacConkey Agar (MAC) (Sigma M7408). Moulds were counted on rose Bengal chloramphenicol (RBCA) (Sigma 17211) and DeMann, Rogosa and Sharpe agar (MRS) (Sigma 30912) layer plates were used for identifying any mesophilic-psychrotrophic lactic acid bacteria at a 10⁻¹ dilution. Plates were incubated according to manufacturer's instructions (PCA & MAC at 35°C for 2 days, MRS at 30°C for 2 days followed by 22°C for 1 day, RBCA at 22°C for 5 days) before being inspected visually for growth followed by microscopy of any unique colonies. All plates were returned to their respective incubators for the remainder of a week, to permit the recovery of damaged/sporulated bacteria or the growth of any slow-growing organisms present within the sample. Counts were recorded at both stages. As is standard for microbiological assessment only counts between 30 and 300 are considered confirmed results as outside of that range the probability of serious errors increase.

3.3.5 Bacterial identification in the commercial sample

3.3.5.1 Enrichment study

Two grams of cricket powder from the three bags of Canadian cricket powder (two previously unopened, one used within a simulated factory environment) was added to sterile vials containing 18ml brain heart infusion broth (BHI) (Oxoid CM1135) in duplicate to provide an initial 10⁻¹ dilution. After vortex mixing for 1 minute, 0.1 ml of each sample was spread plated onto PCA in triplicate. The remaining vials of 10⁻¹ were incubated alongside the plates as an enrichment procedure. This process was carried out at both 25°C and 35°C. The 10⁻¹ plates were analysed for growth after 2 days of incubation. Following an inspection, plates were returned to incubation at both temperatures for a further 5 days to ensure that any slower growing bacteria could be visualised.

After 1 day of enrichment, visual inspection and microscopy (Nikon Optiphot – 2, Nikon Instruments Europe B.V., Amsterdam, Netherlands) took place for any vials of the inoculated BHI demonstrating growth. Following this, each vial was streaked out for single colonies onto duplicate PCA plates. The plates were incubated at the temperature at which the enrichment took place for two days, before being inspected for growth. Enrichment broths were returned to incubators for a further 6 days before being plated onto PCA using the same method and then discarded. After 2 days of incubation, the plates from the 7-day enrichment vials were visually inspected for growth with additional microscopy taking place on any colonies that appeared to have unique colony morphology.

3.3.5.2 Sample selection

Nine colonies, with differing colony morphology, were isolated and streaked for single colonies onto PCA and incubated at the same temperature as their source plate. A single colony from each of these plates was inoculated in 1 ml of sterile water and frozen at -80°C until the DNA extraction could begin.

3.3.5.3 DNA extraction

The following extraction protocol was adapted from previous unpublished work by Charlotte Jane Gray-Hammerton who originally constructed the procedures from three published articles (Sabat *et al.*, 2000; Chakravorty *et al.*, 2007; Tremblay *et al.*, 2015). The samples were defrosted and put through heat lysis at 95°C for 10 minutes in a heating block (Grant QBT2, Grant Instruments, Cambridgeshire, UK). The vials containing lysed DNA were centrifuged at 13,000 rpm for 1 minute in a Mikro 185 Hettich (Tuttlingen, Germany) and the supernatant was separated into a sterile Eppendorf tube. The extracted DNA concentration and purity was confirmed at the 260nm wavelength by using a Nanodrop[™] ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE). The extracted DNA was re-frozen to be later used as the template for the PCR reaction.

3.3.5.4 PCR amplification

The following protocol was also adapted from the same unpublished research conducted by Charlotte Jane Gray-Hammerton. All single-use equipment used in the PCR reaction was placed under UV light at 650nm to prevent any environmental DNA contamination during the process with all further work being carried out in a contained PCR cabinet (Astec Omni PCR workstation, Hampshire, UK). During this time, primers for the V1-4 region of 16S rRNA (MWG Eurofins) (16S V1-4: 8 Forward: AGAGTTTGATCCTGGCTCAG & 16S V1-4: 80S Reverse: GACTACCAGGGTATCTAATC) were sequentially diluted to 100µM, then to 10µM using Ultrapure water and the DNA samples were retrieved from -80°C storage and set on ice to slowly defrost. A master mix consisting of 300µl of Dreamtaq 2x buffer containing DreamTaq DNA Polymerase, 2X DreamTaq buffer, dNTPs, and 4 mM MgCl₂ (Thermo Scientific, Wilmington, DE), 24 µl of both the forward and reverse primers and 204 µl of MilliQ water was created and then aliquoted out at 23 µl per PCR tube. Two control samples were created by adding 2 µl of MilliQ water to the master mix and 2 µl of sample DNA was added to the remainder of the PCR tubes to form the samples.

PCR amplification was carried out in a programmable heating thermocycler (BioRad C1000TM Thermal Cycler, Hertfordshire, UK).

The complete PCR tubes were placed into a Bio-Rad C-1000 thermal cycler under the following conditions: initial denaturation for 5 minutes at 95°C, denaturation at 94°C for 1 minute, annealing at 52°C for 1 minute and elongation at 72°C for 2 minutes. The denaturation, annealing and elongation process was repeated for 30 cycles which were followed up by a final elongation procedure which lasted for 10 minutes at 72°C.

Confirmation that no contamination had occurred was carried out by testing the control samples using the Nanodrop[™] spectrophotometer to demonstrate DNA absence. The resulting PCR products underwent a clean-up procedure using a Wizard SV Gel and PCR Clean-Up System as per manufacturer's instructions (Promega, Southampton, UK).

The cleaned PCR product was checked by electrophoresis on a 1% TAE agarose gel (Sigma Agarose A9539) containing ethidium bromide (0.5mg per ml) in 1x TAE running buffer (40mM Tris base, 20mM acetic acid, 1mM EDTA) at 75 V for about 45 minutes. The amplified DNA was compared to a 100 base pair ladder (Promega G210A) visualised under a UV transilluminator (GelDoc XR, Bio-Rad Laboratories, Hertfordshire, UK) and images were recorded using The Quantity One 4.6.5 Basic software, (Bio-Rad Laboratories, Watford, UK).

Finally, the PCR products were diluted to $5ng/\mu I$ DNA using DNA free water to meet the analysis requirements.

3.3.5.5 Sequencing

The prepared samples were shipped to MWG Eurofins who carried out the sequencing. The resulting sequences were accessed using SnapGene viewer (GSL Biotech LLC, Chicago, USA) before being input into NCBI Blast (NCBI, Bethesda, USA)

for the final identification.

3.3.5.6 Confirmation of Bacillus cereus

As *Bacillus cereus* notably shares a similar rDNA sequence with some other *Bacillus*, confirmation of Bacillus cereus was carried out using the microscopy methodology to look for toxin parasporal crystals outlined by Zhou et al (2011).

3.3.6 The effect of processing

3.3.6.1 Sample preparation

Sample preparation follows the methodology outlined in Chapter 2.3.1 – 2.3.4, with the addition of a batch of each species being left frozen after the sieving stage for later analysis as the raw sample (RAW) and a second sample being frozen immediately after the boiling (PB). The remaining samples were taken after each different drying method was complete.

Post drying the following naming abbreviations (Table 9) were utilised throughout the remainder of this study. Entomo where it appears on any of the results refers to the commercial sample of coarse cricket powder sourced from Entomo Farms (Cananda). The raw samples were taken after the freezing step, the post boil samples are taken after the frozen samples were boiled as outlined above.

Drying Method	Post	Oven	Vacuum	Freeze	Drum
	Boiling	Dried	Oven Dried	Dried	Dried
	(PB)	(OD)	(VD)	(FD)	(DD)
Insect Species					
Banded Crickets	ВАРВ	BaOD	BaVD	BaFD	BaDD
(Ba)					
Black Crickets (Bl)	BLPB	BIOD	BIVD	BIFD	BIDD
Quiet Crickets (Q)	QPB	QOD	QVD	QFD	QDD
House Crickets (H)	НРВ	HOD	HVD	HFD	HDD
Desert Locust (L)	LPB	LOD	LVD	LFD	LDD

Table 9: Explanation of Naming abbreviations

3.3.6.2 Microbiological analysis

Below is a table of abbreviations for the different media used in the microbiology study and their function.

Abbreviation	Full Name	Purpose
MRD	maximum recovery diluent	Dilution of samples
PCA	Plate Count Agar	Non-selective agar allowing for the enumeration of all species
MAC	MacConkey Agar	Enumeration of gram-negative bacteria with selective identification via lactose metabolism
RBC	Rose-Bengal, Chloramphenicol Agar	Selective enumeration of yeasts and moulds
MRS	de Man, Rogosa and Sharpe Agar	Selective agar for the enumeration of fermentative bacteria
RV	Rappaport-Vassiliadis	Broth for the selective enrichment of <i>Salmonella spp.</i>
XLD	Xylose Lysine Deoxycholate agar	Agar for the selective growth of Salmonella spp.

Table 10: Microbiology Abbreviations

Agar plates were produced following the manufacturer's instructions inside a laminar flow cabinet that had been cleaned with antibacterial wipes and transferred to a cleaned sealed container and refrigerated to ensure minimal risk of contamination prior to work beginning.

Samples for microbiological assessment were taken from the insects post freezing (Raw), post boiling (PB) and after drying from each method. Two-gram samples were diluted in 18 ml maximum recovery diluent (MRD) followed by serial dilution down to 10⁻⁵. Samples on PCA were plated using Miles Misra down to 10⁻⁵, while the samples on MAC, RBC & MRS were spread plated at 10⁻¹ in triplicate. PCA plates were incubated at 2 different temperatures (25°C and 35°C) for two days both aerobically and anaerobically using sealed containers with Anaerogen patches and anaerobic indicator paper. MAC plates were incubated at 35°C for 2 days, MRS was incubated in a low oxygen environment using an anaerogen pack at 30°C for 2 days followed by a further day at 22°C and finally RBC was incubated at 25°C for 5 days before being analysed forgrowth.

Presence of *Salmonella* was analysed in the raw insect samples using standard methodology for determining absence in 25g of sample. Twenty-five grams of each sample was added into 225 ml of buffered peptone water and incubated at 35°C overnight. 0.1 ml of each sample was added into Rappaport-Vassiliadis (RV) enrichment broth (Oxoid CM0669) and incubated at 42°C for 48 hours. This was then streaked onto Xylose Lysine Deoxycholate agar (XLD) (Oxoid CM0469) in triplicate and incubated at 35°C for a further 24 hours before being checked for the red colonies with black centres indicative of a presumptive *Salmonella* result.

3.4 Results

3.4.1 Microbiology pilot study

Overall, the sample contains very low numbers of bacteria demonstrating minimal risk in the commercial sample. Each case of growth was only on 1 of the 2 replicate plates.

Pilot study				
Media Type	Growth from 10 ⁻¹ Raw Sample?	Growth from 10 ⁻¹ Heated Sample		
Plate Count Agar	2 colonies	1 colony		
MacConkey Agar	No	No		
de Man, Rogosa and Sharpe Agar	1 colony	No		
Rose-Bengal, Chloramphenicol Agar	1 mould	1 mould		
Anaerobic Plate Count	No	No		

Table 11: Results from the pilot plating study analysis of the Entomo Farms cricket powder sample (Coarse)(n=2 plates)

3.4.1.1 Microscopy

The microscopy image demonstrates the presence of spore forming rods.

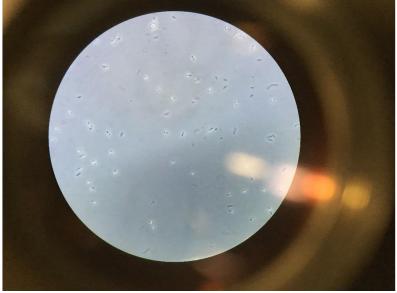


Figure 33: Microscopy showing spore forming rods

3.4.2 Bacterial identification in the commercial sample

3.4.2.1 Enrichment study

The results confirm the results of the pilot study and demonstrate that the bacteria

presence will grow rapidly and provide a risk if given the right conditions.

Enrichment Study				
Test	Growth on PCA?			
All Sources – Enrichment	Pellicle formation visible from 1 day (Figure 34)			
Day 0 – 25°C	No visible growth			
Day 0 – 37°C	Present but below countable levels			
1-day Enrichment – 25°C	Visible haze over all plates, no defined colonies			
1-day Enrichment – 37°C	Confluent growth			
1-week Enrichment – 25°C	Confluent growth			
1-week Enrichment – 37°C	Confluent growth			
Day 0 plates 25°C – 1-week incubation	Additional bright yellow colony apparent after 1 week			
Day 0 plates 37°C – 1-week incubation	No additional colonies			
1-day enrichment – 1-week incubation at 25°C	Variation in colony morphology is apparent			
1-day enrichment – 1-week incubation at 37°C	Variation in colony morphology is apparent			

 Table 12: Results of the enrichment study carried out on 3 samples of Entomo Farms

 cricket powder (n=2 plates per sample)

This type of pellicle layer is often present in top-fermenting bacteria such as those responsible for vinegar production.



Figure 34: Pellicle layer in the enrichment vial

3.4.2.2 Sequencing

The results show the presence of two human pathogens (*Bacillus cereus & Bacillus lichenformis*), one insect pathogen (*Brevibacillus Laterosporus*) and two relatively unknown bacterial species (*Rummeliibacillus stabekisii & Lysinibacillus pakistanesis*). *Bacillus cereus* was confirmed by microscopy but no image was captured.

Table 13: Organisms chosen for identification and the results of sequencing. A = powder opened for use in the food production facility, B&C = packets opened for this study.1The percentage of matches in the positioning of nucleotides to the reference.

Sample #	Source	BLAST result	Ident %1	Query % ²
1	A – Plated Day 0 – 1-week incubation at 35°C	Rummeliibacillus stabekisii	100%	98%
2	A – Plated Day 0 – 1 week incubation at 25°C	Lysinibacillus pakistanesis	96%	99%
3	A – Plated Day 0 – 1 week incubation at 25°C	Micrococcus luteus	99%	99%
4	B – 1 week enrichment – 2-day incubation at 35°C	Bacillus cereus	99%	99%
5	B – 1-day enrichment – 2-day incubation at 25°C	Bacillus lichenformis	100%	99%
7	C – Plated Day 0 – 2-day incubation at 25°C	Brevibacillus laterosporus	98%	98%
8	C – 1-day enrichment – 2-day incubation at 35°C	Rummeliibacillus stabeksii	100%	98%
9	C – 1-day enrichment – 2-day incubation at 35°C	Bacillus cereus	99%	99%

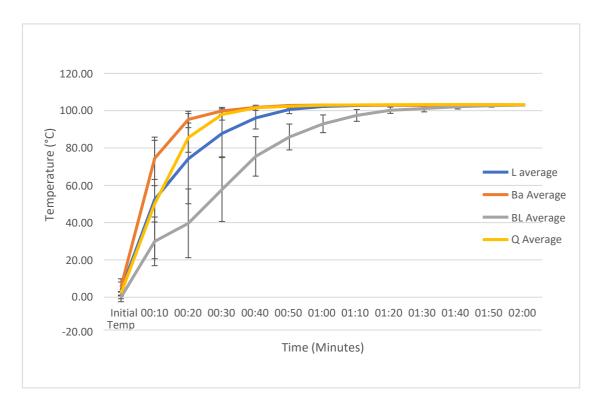
3.4.3 The effect of processing

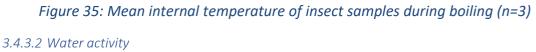
3.4.3.1 Effectiveness of the blanching cycle

treatment. (n=3)					
	Locust	Banded	Black	Quiet	
Average Mass (g)	1.12 ± 0.17	0.33 ± 0.02	1.24 ± 0.11	0.59 ± 0.19	
Average size (mm)	8 ± 0.82	4.67 ± 0.47	10.00 ± 0.82	6.67 ± 0.94	

Table 14: Average weight and size of the samples involved in testing the heat treatment. (n=3)

Black crickets took the longest period to reach 100°C at 1 minute 30 seconds whereas both the quiet and banded crickets both reached 100°C after 40 seconds.





All drying methods reach an aW value below that which can support growth of microorganisms. Drum drying retains more moisture than other methods. Statistically, the species is not a factor in the water activity of the insect samples(P = 0.82) while the drying method is significant (P < 0.001).

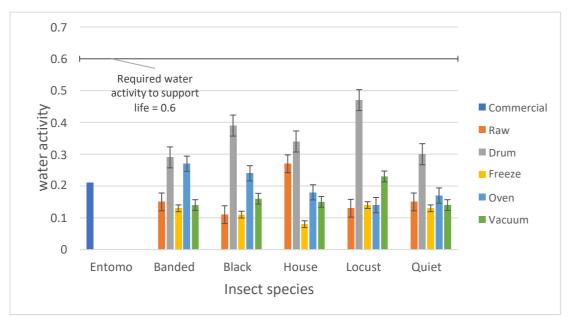


Figure 36: The water activity of the dried and powdered insect samples (n=3 technical replicates)

3.4.3.3 The effect of processing on the microbiota

The methodology used in this study has limitations on the counts. As a maximum the results for the plates are 9 Log CFU/g with the exception of RBC and MRS that has a maximum of 5 Log CFU/g. Values in the graphs above these limitations should be read as though this is the minimum the result could be as the actual value is unknown. There is also a minimum of 2 Log CFU/g for all samples. No value displayed on the table should be understood to be <2 log CFU as there may be microorganisms present but at levels that are not detected by this methodology with the exception of errors noted in the text. The 3 results marked with an X (Figs 39, 40, 41) were uncountable due to merging colonies.

The effect of the boiling procedure appears to be inconsistent across the different species. Boiling created a minimum of a 5-log decrease in bacterial counts in the banded crickets (Fig 37A) and the absence of Gram-negative bacteria on MacConkey agar which is maintained during drying and milling. Results for anaerobic PCA at 37°C are absent due to a failure of the containerseal. The boiling process results in a 4-log decrease in counts in the black cricket samples (Fig 37B) without complete removal of the Gram-negative bacteria. The boiling process does not appear to be effective in reducing counts in the locust samples (Fig 37C) however hot drying processes do reduce the counts. The boiling process reduces counts by at least 3-log CFU/g in the quiet samples (Fig 37D) but this is not maintained when the samples are vacuum dried or freeze dried. Overall counts within the house cricket sample were reduced by at least 4-log CFU/g by the boiling process. However, it was unsuccessful in completely removing the Gram-negative bacteria. Drying methods which employed heat, were able to removethose bacteria.

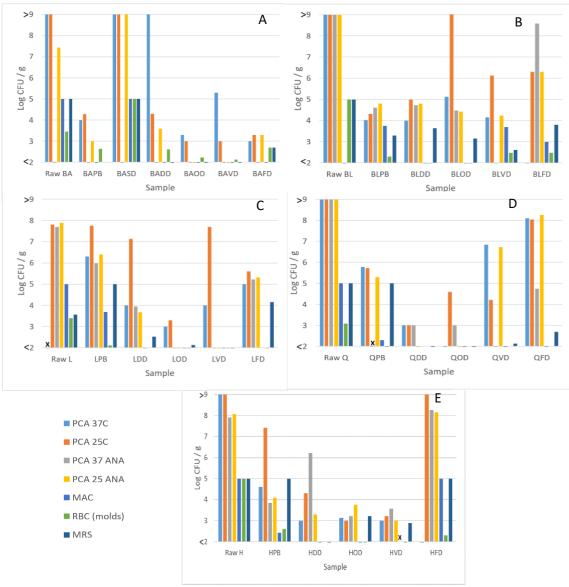


Figure 37: Plate count results for the samples (A = Banded crickets, B = Black Crickets, C = Locust, D = Quiet Crickets, E = House Crickets) across different media (n=3 plates)

3.4.4 Minerals from a safety perspective

None of the minerals reach the SUL/TUL in 100g of sample and there appears to be no consistency in mineral accumulation across the species that would make one species present more risk than another (Fig 38). The commercial sample contains the highest aluminium and copper risk, providing 57% and 66% of the daily safe upper limits respectively. Quiet crickets present the highest risk of manganese toxicity due to them providing 48% of the SUL in 100g of sample on average. Black crickets contain the highest risk of iron toxicity although this is only 19% of the SUL. The risk of zinc toxicity is highest in the house cricket samples at 67% of the SUL (53% if the HFD outlier is removed from the sample). The banded crickets have accumulated the highest risk of selenium toxicity at 25% of the TUL. Locust samples have the least risk on average as they present the lowest values for aluminium, manganese, zinc and selenium.

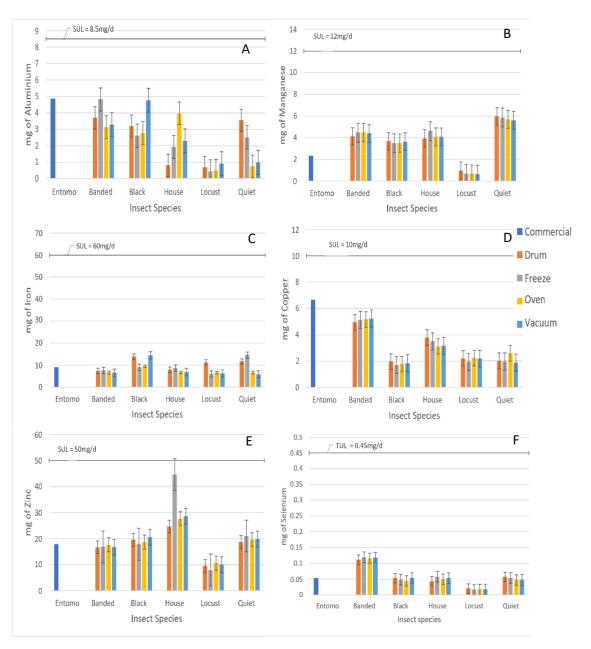


Figure 38: Analysis of the mineral results from a safety perspective (A = Aluminium, B = Manganese, C = Iron, D = Copper, E = Zinc, F = Selenium) across different species / drying method.

3.4.5 Microscopy of particle size and shape

The large particulates left behind by the cutting mill (Figure 48D) may be a risk of causing blockages in the digestive tract. Other methods appear (fig 48 A-C) to be successful at reducing the risk posed by the hooks and barbs within the sample.

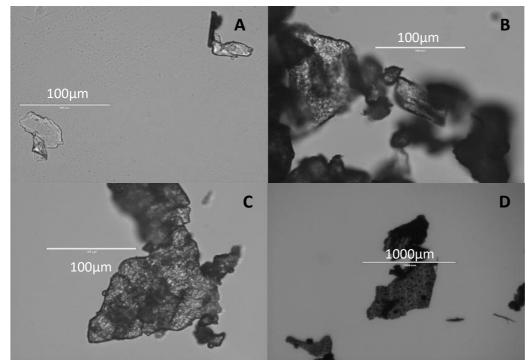


Figure 39: Comparison of particle size and shape post milling on Oven Dried Banded Crickets (A = Ball Milling, B = Hammer Milling, C = Coffee Grinder, D = Cutting Milling)

The large sharp particles in the coarse version of the commercial sample (Figure 49A) may pose a risk, however, the fine sample (Figure 49B) show that these can be successfully reduced.

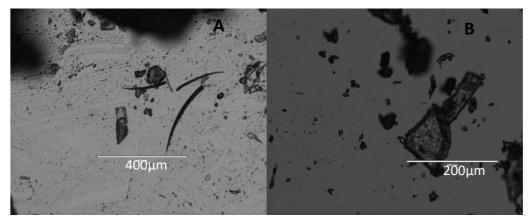


Figure 40: Comparison of particle size and shape between commercial samples (A = Coarse grade, B = Fine grade)

3.5 Discussion

This microbiology study had 3 primary aims. The first was to conduct a broad spectrum analysis of the commercial cricket powder sample and understand if it is likely to pick up microbiological contaminants during food production trials. The second aim was to understand how the organisms discovered during that exploratory study would grow under optimal growth conditions. This is relevant if there are failures in the safety procedures of a food production facility or if contaminated product is utilised for combatting malnutrition in equatorial climates. A sample of the colonies produced during this study were sent off for identification so that the safety measures that would need to be implemented during food production could be discussed. Finally, the powders produced during chapter 2 were analysed in a similar way to the exploratory pilot study to determine if the drying methods used, impacted microbiological safety. Further to this, the mineral and microscopy data produced for those samples in chapter 2 were analysed further to determine if those results may impact on the safety of those powders.

The levels in the raw commercial sample are below the minimum detection level of this study, suggesting a very minor bacterial presence, which in its current state, should not be any concern for food use according to the IFST standards for food microbiology while also complying with the requirements laid out by the FASFC.

Microscopy during the pilot study revealed the presence of a spore-forming rod shaped bacterium which was expected to be *Bacillus* due to the heat treatments involved in the production of cricket powder. This was confirmed by the sequencing and identification during the enrichment study. Alongside this, the low count for moulds suggests that, contrary to the suggestion by the FASFC, there should also be minimal potential risk posed by mycotoxins within this sample. Although the growth on MRS was a single colony, due to the selectivity of the plate, it does suggest that there may be fermentative or a lactic acid-producing bacterium present which is consistent with the results of Belluco et al (2013). However, its absence during the enrichment study suggests that it is quickly outcompeted by the *Bacillus*. Despite this, a fermentative bacterium would create cause for concern with food producers due to its potential to be a detriment to product quality unless careful controls are put in place. The complete absence of growth on MacConkey agar or under anaerobic conditions indicates a general level of safety for the cricket powder contrary to the reports by Grabowski & Klein (2017), as this limits the potential

presence of the Gram-negative pathogenic bacteria.

The results of the enrichment study (when the sample was provided with optimal growth conditions) confirmed that the presence of *Bacillus* was not generated through contamination. However, the sample which had previously beenexposed to the atmosphere produced notably larger colonies during the day 0 plating. This suggests that the organisms present may have had an easier recovery period due to having available air or be different entirely.

Plating the 1-day enrichment yielded confluent growth at 37°C from all sources, however, the growth at 25°C was considerably less. Extending the incubation period to one week for the day 0 samples at 25°C yielded a colony with considerably different morphology from the opened sample. This suggests potential contamination, but also that the contaminant requires very specific conditions to grow that had not been entirely met through this study. Under microscopy, these bacteria were packets of cocci.

The week-long enrichment under optimal conditions, once plated, allowed for confluent growth at 25°C suggesting that there may be a risk of bacterial growth if any food is made with this powder that has a permissible aW level, or if food products without further microbial controls were sold in countries where higher temperatures are common. As one of the primary goals for entomophagy is aiding with malnutrition in developing countries, this is a concern as many of those areas are of a warm climate. Colonies with visually different morphology became more apparent after a week-long enrichment, however, under microscopy, the bacteria were all consistent with spore forming rods suggesting the potential for variations in the types of bacteria present at the species or sub-species level.

The study on the effectivity of processing method provides interesting results. In the cricket samples, the boiling process reduces the samples to below the safe levels described by the FASFC and IFST (The Instutute of Food Science and Technology, 1997; The Scientific Committee of the Federal Agency for the Safety of the Food Chain, 2014). However, locust seems considerably less affected by this process (Fig 37C) suggesting that the bacteria contained within this sample may be different as the heating curve suggests it receives a similar level of heat treatment. Figure 37C does show that the bacteria contained within the locust sample appear to be reduced to a safe level after a drying process that utilises high heat (OD, VD) though.

This requires further study with biological replication, as this could mean that the suggestions of the FASFC of a wet heat treatment being the best option may not be completely accurate, instead it may actually be dependent on the species of insect. The fact that moulds remain after 2 heat treatments in some samples, requires further study as this could be attributed to heat resistant moulds which can sometimes produce toxic secondary metabolites (Tournas, 1994).

With the exception of the banded crickets, Gram negative bacteria remained present after the boiling process (Fig 37). The high heat drying processes appeared successful in a complete inactivation though. Freeze drying maintained or caused an increase in the bacterial presence. This suggests that in order to ensure safety, either a longer heat treatment or a hot drying process is absolutely necessary unless other techniques of bacterial control are put in place. Overall, this study suggests that the best way of ensuring safety may be hurdle technology, which involves employing a set of less aggressive microbial control techniques that work in tandem to result in a safe level while providing minimal risk of affecting the nutritional profile.

3.5.1 Details of the specific organisms

It is important to note that using the 16s RNA identification method, it is impossible to distinguish between *Bacillus cereus* and *Bacillus thuringiensis*. It is only through the microscopy revealing the parasporal crystals that the presence of *Bacillus cereus* was confirmed. This differentiation is likely to be of major importance to cricket powder producers as *B. thuringiensis* is a commonly used insecticide so its presence would also be an issue, but for different reasons. Molecular analysis revealed a mixture of different Bacillus species. Bacillus cereus and Bacillus lichenformis both pose a pathogenic risk when consumed, so although their presence was minimal, when developing food products careful controls will need to be in place to prevent their growth to levels above 10^5 CFU/g, as that would pose a risk to human health (Jones, 2012). The Bacillus cereus creates three different kinds of toxin responsible for outbreaks of food poisoning. The emetic toxins that can remain viable after high degrees of processing (126°C for 90 minutes) and causes nausea, vomiting and malaise (Doyle, 1989; Granum and Lund, 1997). Notably, there are recorded cases of outbreaks of food poisoning with this toxin from dried food products such as spices. The other toxins that are produced are heat labile and should be removed via processing,

however, if the *Bacillus cereus* present is permitted to grow either in the food or in the gut there would also be enterotoxins produced that primarily cause diarrhoeal syndrome (Doyle, 1989; Granum and Lund, 1997).

Micrococcus luteus is classified as an opportunistic pathogen, particularly in patients with a compromised immune system (Peces *et al.*, 1997). As this bacterium was only found in the opened sample, it is likely that aerobic contamination of the sample is responsible, however, this organism has been noted to remain in a dry sample for extended periods of time (Greenblatt *et al.*, 2004). It is unlikely this will pose any major food-borne risk due to it being unable to survive the gastric transit, however, it is an organism to consider during the hygienic design of factories which are utilising insect powders to make food products, particularly if the ingredient is to be exposed to the air for any significant period. This may occur during large scale food production as cricket powder is considerably cheaper if bought in huge bulk due to import taxes. The production of food products will generally only need a small quantity (<10% of the product matrix) in order to fulfil the requirements for "high in protein" labels so there is the potential that it may sit in factory dry stores for extended periods of time.

Aside from the known pathogens, not much is known about *Lysinibacillus pakistanesis* and *Rummeliibacillus stabekisii* beyond their characterisation (Vaishampayan *et al.*, 2009; Ahmed *et al.*, 2014). *Lysinibacillus pakistanesis* has been suggested to have uses breaking down carcinogenic material from the leather industry and inhibit the growth of some pathogens, notably *E. coli* (Brazlauskaite and Mason, 2017). *Rummeliibacillus stabekisii* has been tested as a potential probiotic, aiding in the growth and disease resistance of Nile tilapia but that appears to be the limit of the information available on both of these organisms (Tan, Chen and Hu, 2019). This is in line with the concerns expressed by Larsen et al (2017) when they suggested that the introduction of insects to the food chain could introduce at least 10 new bacteria per species however more could have been found through a more rigorous sampling method. However, despite the limitations of the sampling methodology, this still demonstrates the risk of the unknown that entomophagy poses. Hence further research is required.

Finally, the presence of *Brevibacillus laterosporus* found in a commercial cricket powder sample may be of concern for the insect farmers. It is notably an insecticide for the orders of Coleoptera which includes crickets, Lepidoptera and Diptera (Ruiu, 2013). It's current uses are in the prevention of flies indairy farming however, due to experimentation demonstrating that it is not a cause of European foulbrood in honey bees it may become a more popular choice of natural insecticide in the future due to the protection of bees becoming a larger focus in insecticide choice (Ruiu, 2013). The fact that they have this organism present within their colonies poses a risk to colony survival and may reduce the farms overall productivity. It is likely present due to it being the control mechanism for pests in some part of the insets diet. This organism has also been identified as having a broad-spectrum antimicrobial activity which has been tested successfully against an array of Gram-positive pathogens. This bacterium has also been sold as a probiotic in capsule form for humans under the brand name Flora-Balance due to the anti-microbial activity (Hong, Le and Cutting, 2005). Therefore, its presence within the endfood product could be a benefit but it is difficult to say whether that would outweigh the risk to insect growth and death due to consumption of the insecticide. From the food producer's point of view, this may be a monitor species to indicate the quality of the product as a high count of this organism may indicate disease, but the symptoms of that disease and how it would affect the end product would need to be identified.

3.5.2 Suggested control mechanisms when producing food products

Overall, the primary concern when considering the production of food is the spore presence of the two pathogens. Therefore, more focus needs to be put on confirming the absence of spore-forming bacterial pathogens in the processed commodity at the point of use. Care should be taken during food production in order to minimise the potential for these spores to grow and pose a risk to human health. Fortunately, there are many well-defined control mechanisms available, including low pH, low water activity, sterilisation to standard botulinum requirements or kept under refrigeration conditions prior to consumption with a carefully regulated shelf life.

For *Bacillus lichenformis*, which poses a risk of gastro-enteritis with a further risk of septicaemia, a pH of 4.2 would inhibit growth, as would an aW of under 0.9. A heat treatment of 100°C gives a decimal reduction time (D value; defined as the number of minutes exposure to a defined temperature to reduce viable bacteria by 90%) of 13.5 minutes with a z value (the number of degrees the temperature has to be increased to achieve a tenfold) of 6°C (Campden & Chorleywood Food Research Association Group, 2006). Ohmic heating (heating by passing electrical current through food) has also been noted as an effective control mechanism (Pereira *et al.*,

2007). *Bacillus cereus* can form a heat, pH and proteolysis-stable toxin if permitted to grow significantly, however, the organism is generally less resistant to pH and heat treatments than *B. lichenformis*. A 100°C heat treatment in neutral buffer gives a D value of around 8 minutes with a z value of 10.5°C and a pH below 4.3 would also act as an effective

control (Campden & Chorleywood Food Research Association Group, 2006; Jones, 2012). Refrigeration is also an effective control mechanism for both pathogens as their optimal growth temperatures are typically above 30°C; however, careful temperature monitoring must be in place as there are strains of *B. lichenformis* and B. cereus that are known to grow at 6°C but not at 4°C giving this method of control quite a narrow range of effectivity (Nissen et al., 2001). However, certainty in refrigeration as a method of delivering safety requires complete definitive identification that is not present with this study due to the sampling procedures as there are some *Bacillus* species which do grow at 4°C such as *Bacillus* weihenstephanensis. In addition, low water activity, developed by the drying of the insects would also inhibit bacterial growth. An aw of around 0.91 is considered the minimum to be supportive of growth for most *Bacillus* species (Jones, 2012). Further recommendations for controlling the proliferation of bacillus involves continual cooling of cooked foods with a goal to be under 55°C within 4 hours (Jones, 2012). Care must be taken to consider the thermal conductivity of the food products and also the macro-composition of the food which is being heat treated due to conditions such as available oxygen, fat content, available nutrient content and water activity all having an effect on the effectivity of the processing (Jones, 2012).

Although not a focus of this study, one unanticipated issue with the production of insect-based foods is that they may need tailor-made production facilities due to the allergen risk not being common for the type of products being produced. Very few dry powders conventionally used in the food industry come with a potential shellfish allergen warning, meaning that they would haveto radically modify their existing production processes to accommodate insect-based foods safely. This requires a high level of investment which adds further importance to generating commercial viability. In the example of the noodles, the allergen which is cross-reactive with shellfish allergies is unlikely to be found within a factory that produces noodles regularly. This makes their production require additional cleaning andsafety steps which would be costly to maintain in already existing factories.

3.5.3 Food quality concerns

When it comes to quality control, the type of product must be carefully considered if these organisms are not to be removed prior to food production. As an example, *Bacillus lichenformis* notably forms rope in bread suggesting that the extracellular amylase activity may also have detrimental effects on other product types before it gets to the point of being a safety risk (Pepe *et al.*, 2003). This also applies to the fermentative and pellicle forming bacteria (Fig 34) that are present, as both of these are likely to be undesirable properties in many types of food product.

3.5.4 Powder production

When it comes to the initial preparation, the boiling process appears to be successful as all samples reached an internal temperature of 100C within the 2-minute time (Poulsen, 2007). The length of treatment required appears to directly correlate with the insects' mass which means that this needs to be carefully calibrated as the size of the insects varies even when care has been taken to produce insects of the same stage of their life cycle. Although a 10-minute blanching process is what is suggested, based on this trial, that time could be reduced as it requires much less time to inactivate the majority of concerning bacterial species (The Scientific Committee of the Federal Agency for the Safety of the Food Chain, 2014). The present spore forming microorganisms are likely to sporulate to survive this process and concerns have been raised that removing all other bacteria leaves these without competition within a food product (Fernandez-Cassi *et al.*, 2018).

As demonstrated by the time/temperature graph (Fig 35), it may be possible to further optimise this heating time in order to provide the level of safety required while reducing the risk of damaging or leaching nutritional components. However, as this process is suggested by regulatory authorities, it is unlikely that it can be removed altogether unless a suitable heat treatment is included elsewhere in the process (TheScientific Committee of the Federal Agency for the Safety of the Food Chain, 2014)

All of the drying processes produce powders with a low enough water activity to prevent the growth of microorganisms. This does not mean that organisms will be absent however as the spores would remain unless other methods of inactivating them are carried out during the powder production. These spores would then be able to grow again once the conditions are suitable for them to do so. As the lack of water and the heat treatment is what would cause the sporulation, it would be the availability of water and a suitable environmental temperature that would permit the growth of the organisms present. This suggests the powders would remain safe if kept in the cool, dry and dark conditions that is typical for dried food products. However, it is when the powder is used as an ingredient for a food product with aW that permits the growth of microorganisms that risk is developed.

3.5.5 Mineral toxicity

Within regular food products, the insect powders would likely only make up a small component minimising the risk of consuming too much of any given mineral. The primary risk would be presented by consumers who utilise a range of dietary supplementation, incorporating mineral supplements into their diet in addition to the incorporation of insect protein powders as this lifestyle could permit the ingestion of dangerous mineral levels. Although all samples show results that are below the SUL/TUL in 100g of powder, based on these results, there is a potential risk posed by minerals as there is no defined portion size for cricket powder. It is plausible that combined with a regular diet or used in high supplementary doses (such as those used by athletes or body builders) that the minerals could provide risk of toxicity. Outside of this potential avenue of risk, it is unlikely that the consumption of these insect powders could provide a mineral toxicity risk, however, this is only one source and one feed, others may differ and the risk could be greater if more higher minerals were provided to the crickets unknowingly by the farm.

Analytical techniques used within the food industry will only describe total amount as standard procedure so this could present an issue for those creating products. It is unlikely that a product matrix could be made that reaches the suggested SUL's but it is possible that these results may be misjudged by those who do not know the risks as it does not take into account the bioavailability of these minerals. The bioavailability has been demonstrated to be negatively affected by some of the anti-nutritional factors within the samples such as the chitin and the processing method used (Kröncke *et al.*, 2019).

It is unclear if the rubidium content is a concern or not. The level set was for use within pharmaceutical components with a continuous use (USEPA, 2016).

3.5.6 Particle size and shape

Through microscopy it can be demonstrated that these barbs and other sharp areas of the insect anatomy are separated during the milling process although they can be viewed as whole pieces. The type of milling may have an influence on this as it can be seen when the commercial samples are compared, that it is possible to reduce the presence of the sharp components. If it is larger particles that increase this risk, the cutting mill produced the largest particle size out of the milling methods tested.

Unfortunately, although the risk of blockage is described in literature, it is unclear how it is caused. It is possible to speculate that the barbed areas on the back of legs catch the membrane of the digestive tract and build up over time through repeated consumption based on the original report (Zeitschrift *et al.*, 2016). However, it is not clear if these barbs need to be a part of the whole leg for this to occur or if the barbs alone can cause the issue.

3.6 Conclusions

In general, this study should act as further evidence of the microbial safety of massproduced insect powder for human consumption. The evidence presented so far across the literature, suggests that the microbiota is highly influenced by the growing conditions, production methods and the post-production storage conditions used in the creation of the cricket powder products.

The microbiology results from the commercial sample of cricket powder which had been opened for use within the simulated factory environment, when compared to the samples which were opened under sterile conditions, suggest that the risk posed by environmental contamination once a cricket powder package is opened during food production is minimal. A typical factory, where dry ingredients are held and processed under strict control conditions, should not pose any additional risk. Currently, the commercial cricket powder investigated meets the requirements set out by the IFST for a dried food product and the FASFC guidelines. The results also indicate that careful management needs to be in place to ensure that the pathogens present do not pose a risk to human health. As Bacillus cereus appears to be a consistent organism throughout the tested insect powder products in literature, it may be prudent to suggest that control mechanisms utilised during the production of insect-based food products consider this a target organism when devising food safety protocols for the end products.

The presence of an insecticidal bacterium within the commercial cricket sample suggests that the insect farming industry needs to carefully regulate its feed, as the inclusion of this bacterium poses a risk to the productivity of the farming. The effect of processing on the microbiota demonstrates that the boiling process suggested by the FASFC may not be a uniform solution for all species of insect. The locust sample appears to be affected more by a dry heating method than the boiling, meaning that there needs to be further consideration in this area before true legislation can be developed and enforced.

The water activity in both the commercial samples and generated through the drying procedures all reach levels below what would be able to support microbial life. However, this still permits the existence of spores which will remain dormant until they enter an environment conducive of growth. This could potentially occur within a product matrix so care must be taken to take these into account when developing insect-based food products. Although the minerals presented here are high, none of them reach the safe upper limits. Within the context of this project, insects are not likely to be consumed in 100g batches, as they are intended to be incorporated within a food product to provide additional nutritional benefits. As such excessive intake would only occur in the extremes of circumstance, such as consuming insects in addition to a high level of dietary supplementation.

Without understanding exactly how the intestinal blockages occur, as well as how much needs to be consumed and how often, it is not possible to determine if the milling process is successful or not in making the powder safe. Therefore, it would be prudent to take care during processing to reduce the risk as much as possible, by employing appropriate milling processes to limit the risk as much as possible.

As the market for insect-based food products grow, further research is required to gauge the wider implications of introducing insect-based ingredients to our commercial food system. Based on the results of this project, and the literature reviewed, it would seem that there is potential to introduce these ingredients in a safe manner, but the regulations surrounding them need greater consideration and more definition on how these ingredients should be produced and used.

Chapter 4: The psychology behind the promotion of entomophagy

4.1 Introduction

4.1.1 Why expectations are important

Subconsciously our expectations of a food product, of which we have limited prior knowledge, are what dictates whether we would consider trying it for the first time (Baker, Shin and Kim, 2016; Clarkson, Mirosa and Birch, 2018; Sogari, Menozzi and Mora, 2018). We take cues from our past experiences, details on the packaging, and our interactions with other people, to make a decision about whether its contents will be good or not, before we can confirm this for ourselves (Looy, Dunkel and Wood, 2014; Clarkson, Mirosa and Birch, 2018). This makes expectations of insectbased food products exceptionally important because, in the Western world, there are very few people who have direct knowledge of what to expect from such products. With little potential for past positive experiences to generate these expectations, it is easy to default towards the negative and follow the reactions people have towards the living insect, which tend to be those of disgust and fear, due to their association with uncleanliness and disease (Looy, Dunkel and Wood, 2014).

Further to this, when a consumer is persuaded to try a product which they are unsure about for the first time, the confirmation of expectations, or the disproving of them, can have a strong psychological impact on willingness to consume similar products in the future (Tan *et al.*, 2016). If they assume a product will be bad, and this is confirmed, it is highly unlikely that they will ever try anything similar again under normal circumstances due to that negative experience. If the product is good, that can allow for a lowering of their neophobia and a willingness to try more things of a similar nature (Sogari, Menozzi and Mora, 2019).

4.1.2 Review of the Entomophagy Expectations Literature

As previously stated, it is typically prior experience that consumers base their perceptions on (Tan *et al.*, 2015). However, when prior experience is absent, they fall back on expectation of sensory properties to make a judgement about food (Tan *et al.*, 2015). In the case of insects this could lead to inaccurate sensory predictions resulting in rejection of the foods. This makes expectations particularly important to insect-based foods as, it is still fairly rare for Western consumers to be familiar with the sensory properties of insects or insect-based products. The study goes on to discuss how these expectations are predominantly culturally derived and vary from

country to country dramatically based on what the participants had learnt about food while growing up (Tan *et al.*, 2015). This study was also able to clearly demonstrate that these expectations can exist without any prior experience of insects or insectbased products at all.

The influence that expectation has on food enjoyment and acceptance is a fairly well documented area of sensory science. Hedonic expectancy is where the brain makes predictions as to taste, texture, aroma, and potential satiety of a food. Expectations have the potential to influence overall liking and enjoyment of a food in dramatic ways (Spence and Piqueras-Fiszman, 2014). A basic example is that a red coloured yogurt would provide the expectation of a berry flavour and the consumer considering trying that product would make their assumption of liking based on that berry flavour being present.

Generally, matching or exceeding expectations can result in higher approval ratings and influence overall opinion of a food product (Tuorila *et al.*, 1998). However, a negative experience that meets a negative expectation has been shown to hold a much longer influence over a consumer, resulting in future rejection, ignoring any changes. As such, care must be taken when introducing people to novel foods that are expected to taste bad, because in this instance it is likely to be a detriment to insect-based food products as a whole, rather than just the product type or category that was tried (Spence and Piqueras-Fiszman, 2014).

A study in Belgian consumers demonstrated clear preferences for appropriate meal occasions, whether the insects should be sweet or savoury, how products should be formulated and the paired flavours (Caparros Megido *et al.*, 2014). This demonstrates potential expectations of consumers, despite a general lack of prior knowledge of insects, which may be useful when aiming to promote entomophagy. The expectations for appropriate flavour pairings were further documented in another Belgian study which found that their participants had a clear expectation for savoury Asian flavour pairings, as insects had been promoted in Belgium as a meat replacement solution (Tan, van den Berg and Stieger, 2016). This study only offered a comparison between Asian & Western flavour profiles though, so there may be other culinary expectations worth exploring. Although most literature points towards familiar flavour pairings resulting in positive expectations, a recent study posed the idea that utilising insects in familiar foods may result in contamination disgust (Tan *et al.*, 2016). This could lead to an overall lowered willingness to eat and accept insects

as food. As such it may prove hugely beneficial to understand which route is preferred for generating products for the UK consumer base, in order promote the acceptance of insects as a sustainable protein source.

When it comes to creating insect-based products it is not just sensory expectations that have to be met. Participants in one study demonstrated clear expectations regarding ethical and sustainability implications of products made from insects, which may limit potential ingredients to those with similar credentials within these products. Participants in this study demonstrated reduced interest in consuming and purchasing an insect-based product when these ethical and sustainability motivations were not matched by other parts of the product (Tan, van den Berg and Stieger, 2016). Further studies describe consumers as having an expectation that novel foods would be potentially harmful or dangerous, which reduced willingness to eat or accept them as part of their diet (Martins and Pliner, 2006). As the foods consumed in a country follow cultural perceptions, it is plausible that consumers may link eating insects to a reduction in cultural, economic or social standing (Yen, 2009). This may also be a factor in acceptance of insect-based foods in the future.

As insects have been promoted primarily for their benefits to sustainability, it is reasonable to assume that any consumer exposed to them, through various media channels, may have built up expectations towards their usage within foods (Tan, van den Berg and Stieger, 2016). On the other hand, the popular reality TV series, 'I'm a Celebrity... Get me out of Here', may have created negative expectations as insect consumption is posed in a negative light, often viewed as a challenge to overcome, rather than being a positive experience.

Expected aversive textural properties was found to be the largest factor in rejection in a study comparing a wide range of attributes linked to disgust. This was hypothesised to have been because the participants linked some of the properties to decay and spoilage (Martins and Pliner, 2006). Products such as meat substitutes are expected to have a negative sensory profile, limiting uptake by some of their intended target market (Caparros Megido *et al.*, 2016). Contrary to insects though, this has been driven primarily by prior poor experiences. Until recently, in the Western world, there is very limited perceived threat when it comes to the protein shortage, so consumers feel very little pressure to make more conscious ethical decisions regarding food (Vermeir and Verbeke, 2006). Instead, the focus is almost purely based on sensory pleasure which is better delivered by regular meat products. Participants of some studies who were familiar with insect-based products through previous trials, gave increased in sensory liking, willingness to eat and acceptance scores, demonstrating the importance of expectations in such studies (Caparros Megido *et al.*, 2016). In this study participants were asked to identify insect-based burgers amongst several other preparations. There was a definite correlation between low rating and identifying the burger as insect containing. However only 44% of the participants successfully identified the correct burgers, suggesting that participants were linking the potential of insects to a low sensory liking, despite having no basis for that thought process. This poses the hypothesis that expectation of the negative may be the first key barrier to overcome when proposing insects as a future sustainable protein source.

4.1.3 Survey design considerations

Utilising an online survey to explore the expectations surrounding entomophagy overcomes a range of issues that other methodology would encounter (Porter, 2004). Firstly, the method is convenient for the participant as they can complete it in their own time, in the comfort of their own home. The survey platform offers a wide array of different formatting options such as skip logic that would be difficult to present using other methods like an in-person questionnaire. Further to this, not having the researcher present when the survey is being completed has been demonstrated to limit some forms of participant bias. In particular, there is a likelihood that participants will answer more positively when the researcher is present. The convenience provided by the format allows for a more in-depth exploration than would be permitted by other methodology as a participant is more likely to be willing to answer more questions as well as them having the time to think about more difficult questions.

Questionnaires have been utilised in consumer behaviour studies for a long time due to the ability to present the questions and subjects of the questions in a wide range of different formats such as in person; paper-based questionnaires, postal surveys or more recently online survey platforms such as Survey Monkey. Methods which require participants to be questioned in person suffer on a number of fronts; firstly, that participants are less likely to be honest about subjects which they may find sensitive and also it will be more difficult, costly and time consuming to recruit an adequate representative populace. As the subject of entomophagy is seemingly considered a taboo in the western world, the influence of the interviewer being present when asking questions should not be underestimated when aiming to gather conclusive data (Krumpal, 2013).

Postal votes would also be difficult to gather a defined populace for the survey without utilising costly postal databases of people who register and are paid to complete surveys. It was therefore decided to conduct an online survey, which would benefit from being able to use convenience samples within the university accessed through email groups set up by the university administration to target particular groups of staff and students. This method allows for the survey to be distributed both to students who may be expected to have knowledge of the field, such as those within food related degrees, and those from other disciplines who may be able to provide a more un-influenced opinion.

It is recognised that there is a risk of using a university-based convenience sample in that education levels and age will be biased towards those with A-levels or a form of higher education and those people who are between the ages of 18 and 22. Previous studies have suggested education to be a non-factor (Lacey, 2016). However, age may be a factor due to the perception that the millennial generation in particular is more accepting of novel foods, particularly those that come with potential benefits to health and are also more willing to try new things (Ares & Gambaro, 2007). Having this knowledge prior to the surveys production ensures that no comparisons are made to a wider UK population that would be inaccurate while also allowing any valid comparisons to yield useful results that can influence future studies. This is a key aspect when discussing particular demographics that hold particular beliefs, expectations or views on the subjects being discussed within the study.

High quality research needs to yield data that is both reliable and valid. Reliability is demonstrated through the ability for it to be consistent between one study and the next. While validity incorporates several aspects to ensure that the data itself serves the purpose that it appears to. This is the primary reason for a keen search of previous methodologies as through peer review these studies have been identified as using valid and reliable methods. Validity comes in three main forms when approaching a survey (Mora, 2011). Content validity is the creation of questions that can accurately yield all of the information pertaining to the concept being studied. Internal validity questions whether the methods will define the concept that is the aim of the study. External validity focuses on whether the study will yield information that is attributable to a wider populace. If all of these aspects are met, the study should yield

valid data that is suitable for high quality research. The justification of individual questions is described in Appendix C.

4.2 Aims and Hypotheses

The primary goal of this research is to define a potential route forward for using food products for the promotion of entomophagy in the UK. Developing an understanding of consumer expectations of insect-based food products is valuable to this goal as it will allow for a prediction of how participants in future studies may act. Another implication of consumer expectation is that it can be used to influence the product design part of the research programme. As the overall goal of the research is to facilitate acceptance of insects as a food, using any positive expectations to steer product design could result in products that are better suited in promoting some aspects of acceptance, especially initial willingness to eat as this aspect is primarily based off what is visible at the time and any predisposed expectations that the consumers or participants may already have prior to tasting.

The overall research question for the study is "What are the potential impacts of consumer expectation on the acceptance of insect-based foods?" This will be divided into several areas predominantly focusing on the explicit and implicit expectations but also incorporating aspects of interpersonal expectations, in order to study overall expectations towards potential flavours and cuisines linked with insects. The aim of the work is ultimately to gather an understanding of people's views towards insectbased food in the UK so that those expectations can be utilised in attempting to make a suitable product for the promotion of entomophagy.

The questionnaire is designed to test the following hypotheses:

The emotional expectations of insect consumption will lean towards fear and disgust, particularly for people who have never consumed them before.

Participants will believe that the cuisines that are most appropriate may be the ones they are least familiar with, due to inherent biases as they attempt to distance themselves from the food that they find disgusting.

Dry, fine powders will be the most acceptable way to incorporate insects to the food system.

Insects will generally be expected to be a poor-quality food product that is inappropriate to incorporate into the western diet.

4.3 Materials and methodology

4.3.1 Focus groups

Two focus groups were arranged in order to determine the best way of asking questions related to sensory or emotional expectations as part of the survey. The focus groups were asked about the layout and wording of these questions as well as determining whether questions and chosen responses were fully understood by all present as a reasonable proportion of the campus is made up of people whose first language is not English.

The emotions focus group was focused on the modification of the GFEE list of emotions used in previous work on insects (Gmuer et al., 2016). This modification was felt necessary due to the original list being a translation from German and the belief that some important emotions were missing from the list while others on the list would not be directly understood. Participants were asked to highlight emotions from the list that they do not understand and any that they believed were the same. Following this they were asked to write a definition for each emotion that they do understand and provide any additional emotions that they felt were missing. Additional emotions were assessed within the group to ensure that they were unique and that everyone present would understand them if they were to be included in the survey. Using the group's numbers, the list was organised into confidence tiers based on how many of the participants voted in favour of a particular emotion remaining on the list. This tiering system in combination with the participants views on emotions that were lacking from the list would be used in order to form the list of emotions used within the survey. In order to follow the same methodology, the participants were finally asked to categorise the emotions that they have provided between positive, negative and neutral emotions. The final list was reviewed by the supervisory team who suggested to keep some of the middling (unsure) tier emotions due to them being distinct from everything else on the list.

The survey focus group was presented with sensory descriptors used in studies from literature and questioned whether all of the descriptors are understood, unique and whether any are missing. This was repeated for the primary senses; sound, taste, aroma, visual and texture.

4.3.2 Survey

The survey (see appendix C for Question layout, purpose and justification) was developed using Surveymonkey[®], an online survey platform, as this software permitted the use of skip logic that was not available in alternative formats. This was to be used in order to separate those participants who are capable of consuming insects and those who are not due to dietary restrictions or allergies as questions directly asking participants about consumption scenarios would be irrelevant for those that could not consume insects. Where possible and suitable to the question, the order of the answers were randomised for each participant by the platform. The images used within the survey were either examples taken from previous work in the field of entomophagy by the researcher or stock photo examples of a typical product.

The survey was sent out to the population of the Sutton Bonington Campus of The University of Nottingham mailing list made up of 3258 unique email addresses on the 28th of January 2019 and was then closed on the 8th of April 2019. During that time, 2 reminders were sent out: one on the 18th of February and the second on the 4th of April. The population of the mailing list consisted of staff and students from the school of biosciences and the school of veterinary medicine made up of 1835 undergraduate students, 768 postgraduate students and 655 staff members. The email invitation and the reminders can be viewed as part of the appendices.

The data from the Survey was analysed and visual representations were made by the SurveyMonkey platform.

4.3.2.1 Ideal Sample Size

Based on a student and staff population of 3,258 accessed by university mailing list – assuming all associated emails are delivered to unique recipients, the sample size required for a 95% confidence level with a 5% margin of error would be 344 (Penwarden, 2014). The survey was fully completed by 312 people providing a confidence level of 95% with a margin of error of 5.3%.

4.3.2.2 Ethics

Ethics for the survey was applied for within the university by application to the social sciences ethics committee who are familiar with the approval process and can define what necessary ethical considerations are required for the survey to be opened to public participation. In general, the format will follow what is defined as good practice by the university, including an introductory information page describing the research aims as well as contact details of the author and how a participant can remove consent. Consent to participate will be provided by check box on that introduction page. They will also be asked to type a series of numbers and letters into a text box on that introduction page so that their data may later be identified and removed if they desire. This way the data presented is anonymous and only the participant themselves knows which code would belong to them.

Aside from the general ethical considerations afforded to other surveys which are worked through by the inclusion of the introduction and ending page of the survey, there may be minimal risk posed to participants through the discussion of insects. Insects in general are a common phobia so any inclusion of graphic imagery may require some form of warning.

4.3.2.3 Data Analysis

The resulting data from the survey was analysed using the survey software.

4.4 Results

4.4.1 Focus group

The focus groups consisted of 10 participants from the staff and student body of the Sutton Bonington campus of The University of Notingham. There were 4 female and 6 male participants, 8 were students aged between 18 and 25 with the remaining 2 being staff members who were older. Ultimately, they determined that there was no viable way of running those questions within a survey format. Although this has been done within literature, it was impossible to form a consistent and complete list of sensory attributes that all present participants understood and had the same definition for. As such, including these questions in the survey would have likely provided inaccurate results through misunderstanding which would defeat the purpose of the survey. It was determined that an alternative format, such as sensory testing, where examples of a descriptor could be provided, would be the best way of providing insight into those areas.

The focus group on emotions determined that several of the GFEE list provided no benefit to the work as they were either unclear, misunderstood or were alternate ways of saying the same thing. In addition, certain words such as "aroused" were removed as the participants felt that they would never use them when describing food.

Based on the responses of the focus group participants, the emotions listed on the GFEE list were categorised into confidence tiers based on how many participants responded (Fig 50) that they both understood the word and could provide a suitable description.

		Participan	ts views on the GF-EE list for	use in a survey format		
Definite Keep	Кеер	Probably Keep	Unsure	Probably Remove	Remove	Definitely Remove
Dreadful	Bad	Energetic	Cheery	Charged	Aroused	Quiet
Excited	Bored	Grossed out	Exhilarated	Exuberant	Chipper	Solemn
Нарру	Content	Invigorated	Impassioned	Thankful	Dazed	
Refreshed	Disappointed	Irritated	Inferior	Torpid	Lusty	
Relaxed	Dissatisfied	Motivated	Unique	Vital	Outstanding	
Surprised	Good	Terrific			Overwrought	
Uneasy	Greedy	Well			Tired	
	Sickened					
	Strange					
	Strengthened					

Figure 41: Confidence tiering of emotions from the GFEE list by focus group participants.

The emotional descriptors below are what the focus group determined were not suitable for the study from the GFEE list. When questioned as to why, it was consensus that emotions surrounding sexual arousal such as lusty or aroused were not something they would typically use to describe food without external context. Similarly, this applied to tired, solemn and dazed. Emotions such as chipper, torpid and solemn were poorly understood within the group. Further discussion yielded a general belief that this may be due to the translation from German where these emotions may offer a more unique meaning. Other emotions such as outstanding, exuberant and overwrought were believed to be covered by a different descriptor with a clearer application. Thankful, vital and quiet were understood to be emotions connected to receiving food rather than how you would feel about the food itself and as such would not match the requirements for the study.

What was removed				
Positive	Neutral	Negative	Undefined	
Aroused	Quiet	Overwrought	Dazed	
Charged	Solemn	Tired	Thankful	
Chipper			Torpid	
Exuberant			Vital	
Lusty				
Outstanding				

Figure 42: The emotions from the GFEE list which were removed from the list used in this survey.

The below figure contains suggested additional emotional descriptors found in literature that were believed to be missing from the original GFEE list. These were again sorted into confidence tiers based on the number of participants that agreed that the descriptor would be used to describe food. The participants were asked to confirm that they understood the definition of each emotion and that they believe these to be unique additions.

		Participants 1	thoughts on the suggeste	ed additions to the GF-EE list		
Definite Keep	Кеер	Probably Keep	Unsure	Probably Remove	Remove	Definitely Remove
Amused	Alarmed	Ecstatic	Hopeful			
Angry	Angst	Optimistic	Joyful			
Anxious	Apathetic	Panicked				
Aversive	Frightened	Sad				
Concerned	Pensive	Shame				
Curious	Stressed					
Fearful	Timid					
Guilty						
Hesitant						
Interested						
Suspicious						

Figure 43: Confidence tiering of the additional suggested emotions by the focus group participants.

Finally, the participants of the focus groups were asked to provide their own thoughts on any further emotions which may be used to describe food that had not already been covered. These were openly discussed as a group in order to determine a consensus before participants were asked to discuss and determine as a group whether the emotions that they have suggested were positive, negative, or neutral in order to maintain the functionality that the GFEE list provides. These details are summarised above.

Participants suggested additions:				
Positive	Neutral	Negative		
Enticed	Satiated	Dirty		
Passionate	Anticipation	Unappealing		
Desirable	Intrigued	Greasy		
Pleased	Distracted	Pressured		
Appealing	Hunger	Disgusted		
Satisfied	Confused	Apprehensive		
Appetising	Sociable	Worried		
Superior		Vulnerable		
Healthy				

Figure 44: Categorisation of the additional suggested emotions by the focus group participants.

The finalised emotions list was put together as a combination of all of the above efforts during a meeting of the project supervisory team. Figure 45, shows the complete list, colourised as per the confidence tiering. Exhilarated was included after a lengthy discussion despite it being below the confidence threshold for inclusion due to the feeling that it would offer something unique to the list that may be of particular importance to the study on insects-based food consumption. The belief was that if the focus groups were framed around a novel food such as this, this emotion may be more significant.

Final list after further review				
Positive	Neutral	Negative		
Нарру	Hesitant	Uneasy		
Excited	Curious	Dreadful		
Refreshed	Surprised	Fearful		
Relaxed	Strange	Anxious		
Interested	Apathetic	Guilty		
Amused	Timid	Angry		
Strengthened	Unique	Suspicious		
Good	Intrigued	Aversive		
Content	Confused	Concerned		
Motivated	Sociable	Greedy		
Terrific	Anticipation	Sickened		
Invigorated	Satiated	Bad		
Optimistic		Dissatisfied		
Ecstatic		Bored		
Well		Disappointed		
Energetic		Stressed		
Impassioned		Alarmed		
Cheery		Angst		
Hopeful		Frightened		
Joyful		Pensive		
Exhilarated		Grossed out		
Enticed		Shame		
Pleased		Irritated		
Superior		Panicked		
Healthy		Sad		
Pasionate		Inferior		
		Pressured		
		Disgusted		
		Apprehensive		
		Worried		
· · · · · · · · · · · ·		Vulnerable		

Figure 45: The final list included within the survey, organised into their emotional categories. Coloured highlighting represents the previous tiering lists.

4.4.2 Survey

The full survey can be viewed in appendix C. In total there were 519 responses to the survey, 207 responses were filtered due to being incomplete, leaving the total responses considered as results to be 312.

Survey question 1 was a confirmation of agreement to participate in the survey for ethical purposes. Survey questions 2-6 defined the survey participants as 72% female, 26% male with the remainder describing themselves as non-binary. 82% of the participants were from the UK. The largest age group was 21-29 making up 47% of the responses. Nobody was below the age of 18, 26% were 18-20, 12% were 30-

39, 8% were 40-49, 4% were 50-59 and 2% were over the age of 60. When it comes to education, a single participant had no formal higher education, 49% of participants had completed A-levels, 24% had finished their bachelor's degree and 27% had a post graduate degree. The responses from those whose work, or education are food related made up an even 50% of the responses. Of the 312 participants, 17 came from countries that regular consume insects.

Survey question 7, (Do you believe we will need a greater diversity of animal protein in the UK?) found that over 70% (226) of the participants believed that the UK needs to diversify its animal protein supply while 86 participants did not.

The results (Fig 46) show that participants recognise that the UK will be affected by a futureprotein shortage, although the perception of how much the country could be affected is not consistent.

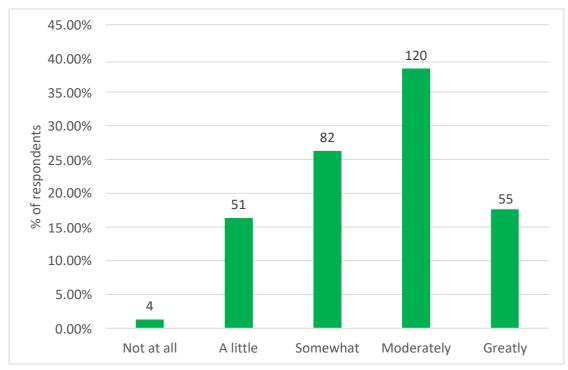


Figure 46: Survey question 8. "There is a prediction that there will be a shortage of protein in the world in the future. How much do you think this will affect the UK?" (n=312)

Nearly all of the participants had at least a little knowledge of edible insects with justunder half of all of the participants claiming some understanding. Confirmation of this result through requesting participants to describe the benefits of consuming insects and insect based food products without looking it up did not yield any abnormal answers which needed to be discarded from the study.

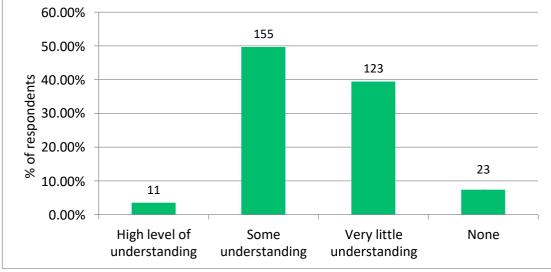


Figure 47: Survey question 9. "How would you describe your knowledge of edible insects?" (n=312)

The majority of the participants had witnessed edible insects either as part of TV shows (75%) or on the internet (74%). Print media was found to be secondary to this with 28% of participants reporting seeing insects in newspapers, 22% in magazines and 18% in books. Only 7% of respondents reported not having seen insects in any form of media. The "other" responses primarily consisted of those who have read research papers on the topic, or had witnessed them in person at trade shows, conferences or as retail advertisements.

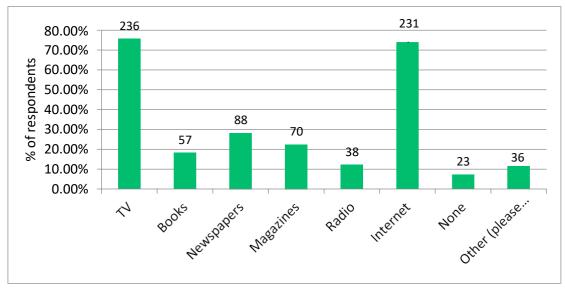


Figure 48: Survey question 10. "Have you seen edible insects in any of the following media? Select all that apply." (n=312)

Over 50% of the participants report seeing both positive and negative portrayals of edible insects in the media. However, an additional 39% report strictly positive portrayals.

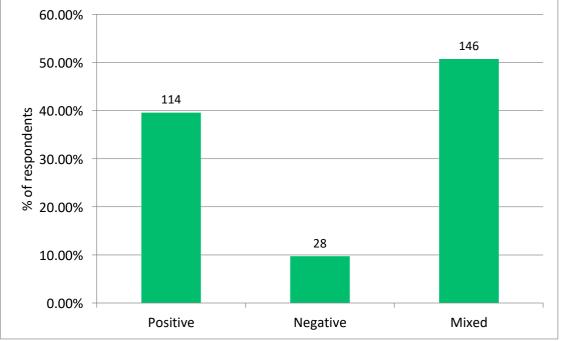


Figure 49: Survey question 11. "If you have seen edible insects in the media, was their portrayal positive or negative?" (n=312)

Question 12 qualitatively queried the nature of the benefits of consuming insects or insect-based foods as a means of confirming the validity of the results of question 9. One third of the participants of the study had previously consumed edible insects (Question 13) and 63% of the participants expect to eat edible insects in the future(Question 14). Further to this 95% of the participants reported that their home country does not consume insects as part of their regular diet (question 15). The results shown in figures 50-52 demonstrate that a nervous curiosity with an undertone of disgust and revulsion are the dominant emotions towards entomophagy within this participant group as the highest results are for curiosity, intrigue, hesitance, interest, strangeness, and apprehensiveness. The potential for shame and inferiority discussed in literature appear quite minimal in comparison to other emotions.

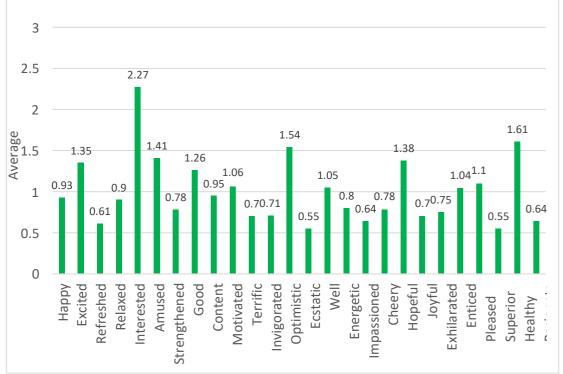


Figure 50: Survey question 16. "Rate 1-5 the following emotions based on what you would expect to feel when trying insects. (Positive Emotions)" (n=312)

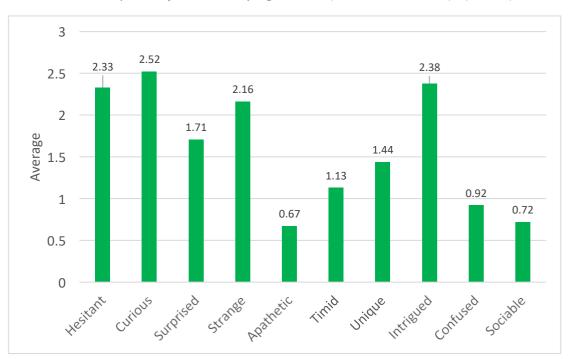


Figure 51: Survey question 17. "Rate 1-5 the following emotions based on what you would expect to feel when trying insects. (Neutral Emotions)" (n=312)

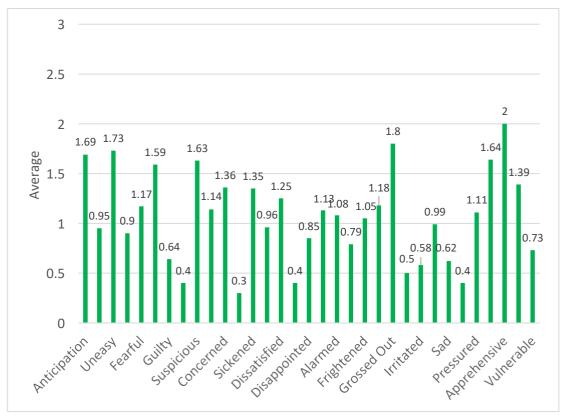
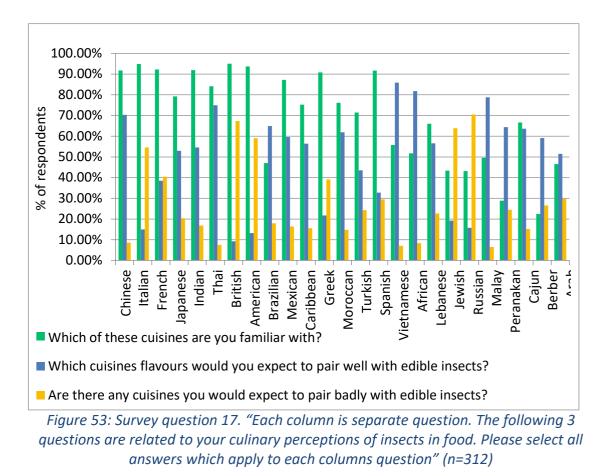


Figure 52: Survey question 18. "Rate 1-5 the following emotions based on what you would expect to feel when trying insects. (Negative Emotions)" (n=312)

The lowest scores for familiarity come from Berber (22%) and Peranakan (29%) cuisines. The highest positive expectations are from Vietnamese (86%), African (82%) and Malay (79%) cuisines while the most negative expectations come from Russian (71%), British (67%), Jewish (64%), American (59%) and Italian (55%) cuisines. Relatively unknown cuisines such as Peranakan and Berber had over 50% of the respondents expecting those to pair well with insects despite their lack of knowledge. Similarly, some cuisines such as British, American and Italian have high familiarity and are expected to pair badly with insects. However, the results for Jewish, Russian and most Asian cuisines do not follow this trend.



Ground, dried powder is the most preferred format of introducing insects into thefood system with this participant group with whole, fresh insects having the least potential acceptance.

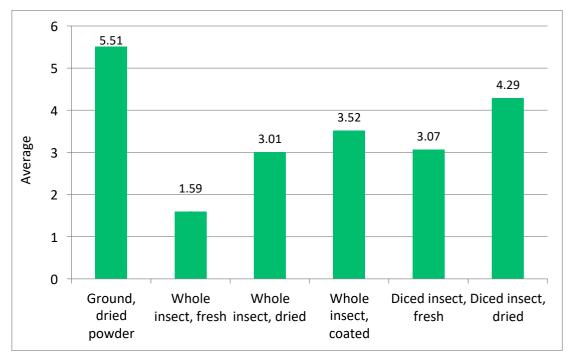


Figure 54: Survey question 18. "Edible insects can be included in a product formulation in the following formats. Please rank these formats in order" (n=312)

The results demonstrate a strong expectation that insects will pair better with savoury flavours with an additional 20% of participants who would be fine with either sweet or savoury.

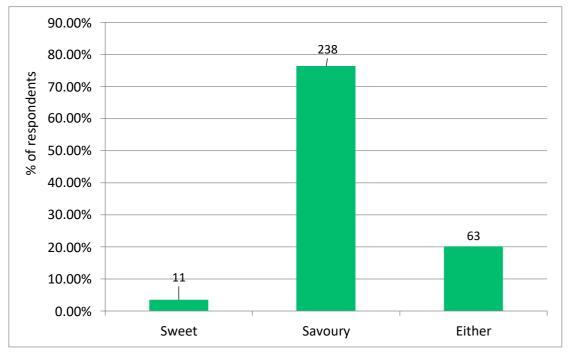


Figure 55: Survey question 19. "Would you expect the flavours that insects could provide as an ingredient to be pair better with sweet or savoury foods?" (n=312)

Insects are expected to perform well in all areas except for food safety. The dominant messages regarding general sustainability and protein content come to the front but the individual aspects of the sustainability and other nutritional aspects are lesser.

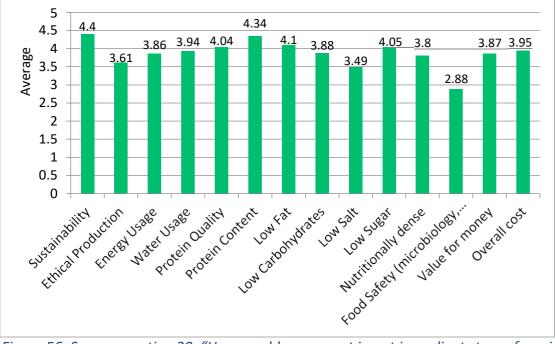


Figure 56: Survey question 20. "How would you expect insect ingredients to perform in the following areas?" (n=312)

The participants of the study generally expect insects to cost less as an ingredient than other products which provide the same attributes although there are a smaller group of participants (29% total) which expect insects to cost the same or more than the direct competitors.

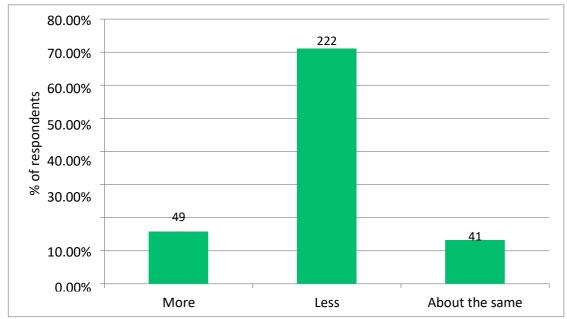


Figure 57: Survey question 21. "Compared to ingredients which provide similar attributes - would you expect insects to cost more or less?" (n=312)

There are similar numbers of participants stating that they would not be willing to consider insects if the price point under any circumstances. The other responses primarily consisted of participants who wanted more information surrounding the sustainability of ecological impacts of insects by comparison to the product it was an alternative for while others focused on the hedonic attributes being important. There were also several suggestions that it would have to be as an alternative for specific foods (shellfish, or meat replacement rather than staple foods).

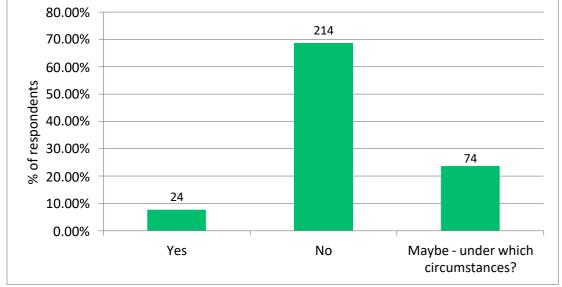


Figure 58: Survey question 22. "Would you be willing to purchase insect ingredients if they cost more than similar products?" (n=312)

For falafel, meatballs and burgers, most people said that they would expect no change (55.13%, 51.28%, 51.92%) with the second highest option being slightly better (22.12%, 25%, 22.76). Wholemeal bread was also mostly no change expected (52.24%) with the second highest result being slightly worse (28.85%). The white products have the lowest scores indicating that these are areas where the participants have strong expectations for the product being worse as a result of the insect inclusions.

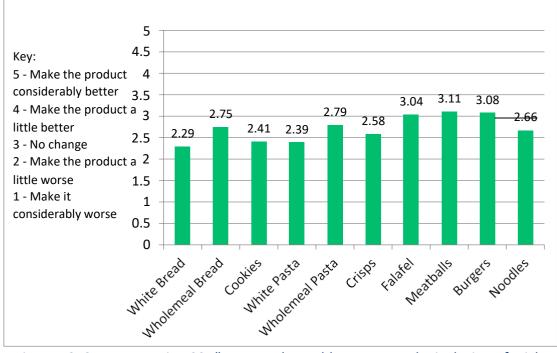


Figure 59: Survey question 23. "How much would you expect the inclusion of cricket

The results of question 24 show that savoury products containing insects would be strongly preferred compared to sweet products although 22% of participants claim they have no expected preference for either.

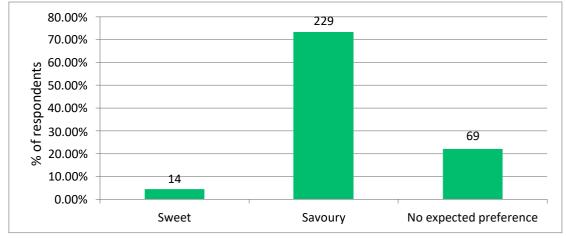


Figure 60: Survey question 24. "Would you expect to prefer products containing cricket powder if they are sweet or savoury?" (n=312)

Health food (82%) and savoury snacks (73%) have the highest expected association with insects with ready meals also yielding above 50% of the responses. There are a few participants who do not believe any of the options presented would be expected to contain cricket powder but aside from this option, sweet snacks have the lowest expectation at 16%. The few other responses proposed pet food, meat substitutes and health supplements as additional categories.

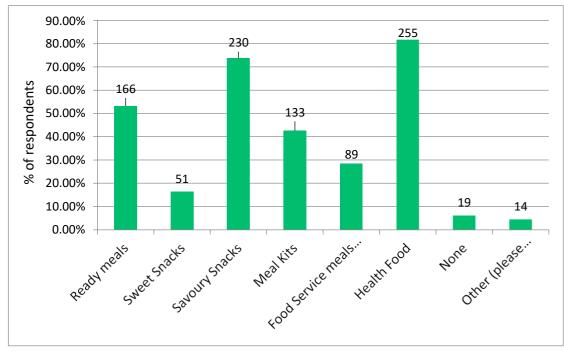


Figure 61: Survey question 25. "What type of products would you expect to contain cricket powder? Select all that apply." (n=312)

Snacks were the option that the highest number of participants associated with the potential for eating insects although main meals were also notably high. There is a clear response that areas such as brunch, supper, dessert, and special occasions are to be avoided due to their low response rates. Similarly, breakfast is another area which was not favoured. The option for not including insects anywhere is the lowest score. The few other responses indicated either more specific niches of the options provided or nutritional supplementation that would fall outside of these areas such as post-workout protein.

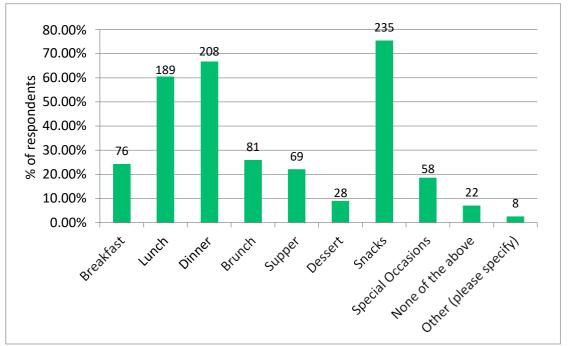


Figure 62: Survey question 26. "What occasions would you associate with the potential of eating products containing cricket powder? Select all that apply." (n=312)

With the exception of breakfast and dessert, over 50% of the respondents believed the inclusion of insects would have no effect on these meal occasions. Dinner and snacks had over 10% of the respondents believing that it could make those occasions better when insects are included.

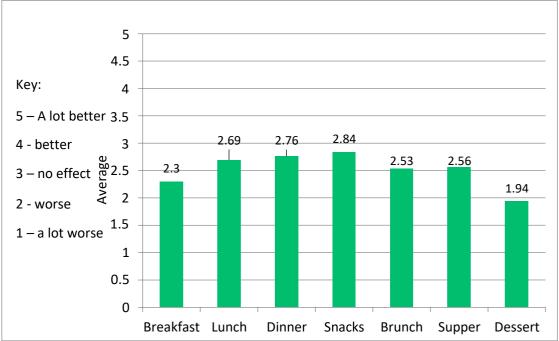


Figure 63: Survey question 27. "If products containing cricket powder were included in the following meal occasions, how would you expect it to affect your enjoyment of the meal?" (n=312)

The highest expectation is for insects' products to be priced as mid-range. Both the highest and the lowest end of the pricing tiers scored very low numbers of responses although the high and low end of the pricing brackets both scored similarly.

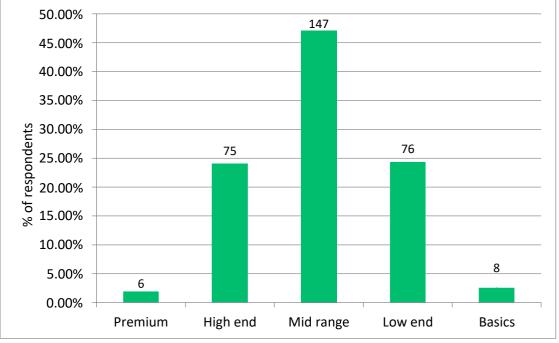


Figure 64: Survey question 28. "How do you expect products containing cricket powder to be priced?" (n=312)

The order of importance demonstrates that the overall sustainability of a food product containing insects to be by far the most important attribute when questioned in this way. Overall nutritional density is less important than the specific nutritional attributes of protein quantity and quality, but ethical production methods and some individual attributes of the sustainability message are more important to the participants than that nutritional density. The remainder of the potential nutritional attributes of a food product are what the participants viewed as the least important areas when considering an insect-based food product.

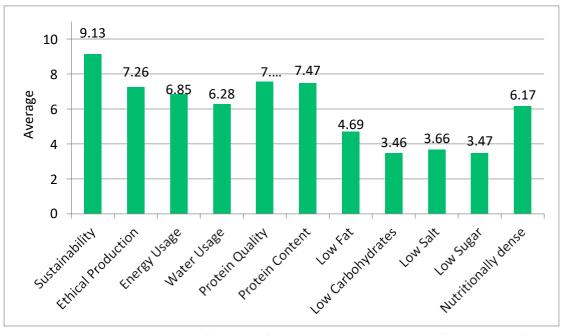


Figure 65: Survey question 29. "Rank the following attributes in order of importance for a product containing cricket powder?" (n=312)

The considerations for appropriate flavour profiles of insects demonstrates that Asian, Indian and Mexican flavours are the most likely to be appropriate with Thai and Chinese being the standouts specifically. British, American and French flavour profiles are expected to be below average.

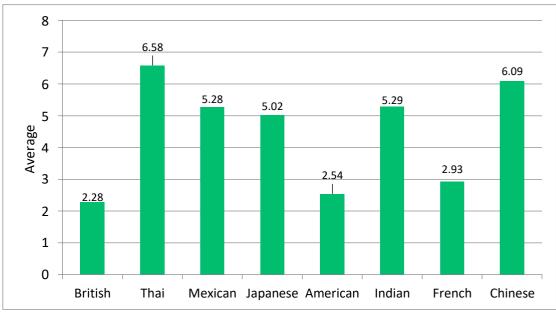


Figure 66: Survey question 30. "Cricket powder is currently used in some retail products with the following flavour profiles, rank them in order of which you expect to be the most appropriate combination" (n=312)

As the following questions are related to the consumption of products, participants were asked whether they had health or dietary choices which would prevent them from consuming insect-based foods (Question 31) were filtered at this stage resulting in 264 participants taking the remainder of the survey.

The purpose of question 32 was to try and use imagery of existing products (See appendix C) to overcome a lack of knowledge towards insect-based foods as it was expected at the time of publication that the participants will have limited knowledge of the area. The weighted average is potentially a little misleading in this case as staples are ranked 1st by 39.77% of the participants with bars having 26.14% of participants putting them first but the remainder of the responses average out to protein bars being more expected. Savoury snacks were still preferred over sweet snacks even when whole insect is clearly visible in the savoury.

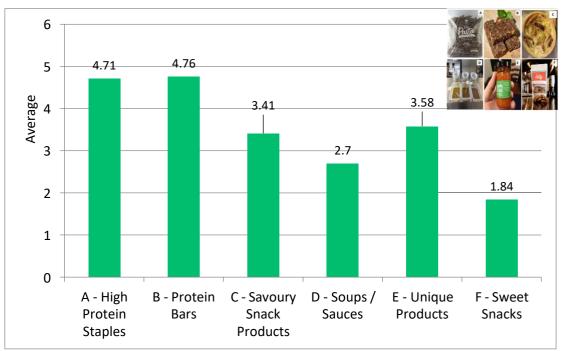


Figure 67: Survey question 32. "Using the above pictures as examples of products within the category, rank these options in order of which matches your current expectations for insect-based foods the most." (n=264)

High protein staples are the most preferred form to consume insects out of the options given with this question with protein bars being a reasonably close second. The remainder of the results averaged out to be similar demonstrating little preference beyond the two prominent results.

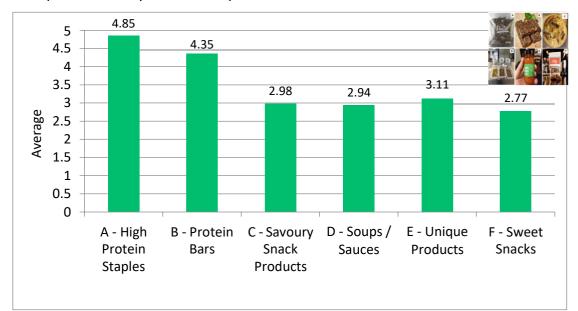


Figure 68: Survey question 33. "Using the above pictures as examples of products within the category, rank these options in order of which you would most prefer to consume if given these as options." (n=264) The picture attached to this question shows insect protein in a visually high-quality staple product by using a restaurant-style plating and professional photography. The results of this question detail that more than half of the participants believe the associated image to detail a product that is better than their current expectations foran insect-based food product, however, more than 30% indicated that this meets their expectations, demonstrating that expectations for some participants are potentially higher than hypothesised.



Figure 69: Survey question 34. "The above picture shows a potential use for cricket powder. Is this what you expected?" (n=264)

The Mean result of this question was that the presented product is expected to tastegood (50.38%).

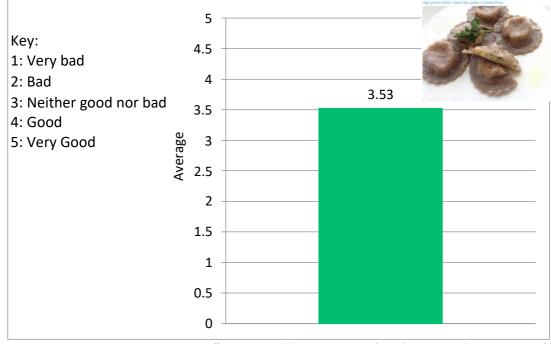


Figure 70: Survey question 35. "How would you expect the above product to taste?" (n=264)

On average this shows little preference for a non-insect protein source over insect powder but considering the individual results clearly favours non-insect as 57.58% ofparticipants placed this 1st compared to 39.39% for insect flour.

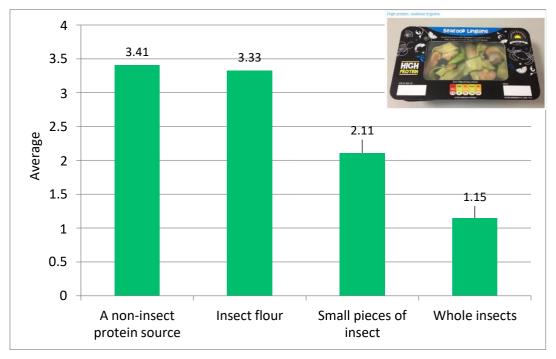


Figure 71: Survey question 36. "The pictured product could be formulated to provide high quality protein utilising the following options, rank them in order of your expected preference." (n=264)

The overall mean result is "good" (42.42%). Almost twice as many people believed the product would be good than those on the negative side of the scale and the neutral option was almost equal to the combined negative results making this positive overall.

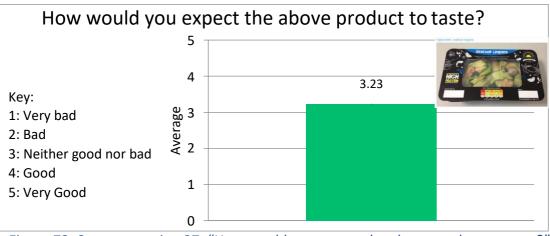


Figure 72: Survey question 37. "How would you expect the above product to taste?" (n=264)

Low visual appeal was responsible for most of the negative answers to question 38 but there was also a similar dislike towards seafood and ready meals. The pasta content, despite that being where the insect is incorporated, was only an issue for 3of the participants. The product design was laid out with the idea that minimal frontof pack information may be better, but this was a negative attribute for 24% of the participants in the study. There are also notable concerns for the presented nutritional scores and the other ingredient choices. The few "other" responses primarily disliked the packaging.

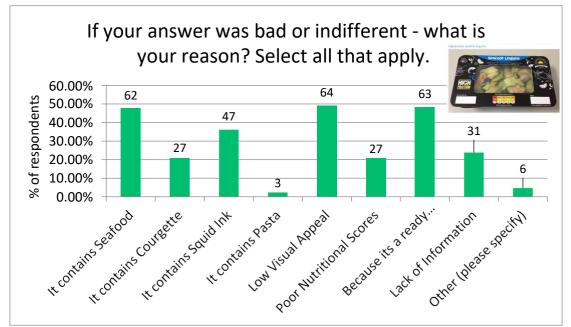


Figure 73: Survey question 38. "If your answer was bad or indifferent - what is your reason? Select all that apply." (n=130)

The mean result for taste, aroma and appearance is that the participants would expect it to be the same (53.41%, 52.27%, 75.76%). However, texture is expected tobe a little worse (47.35%).

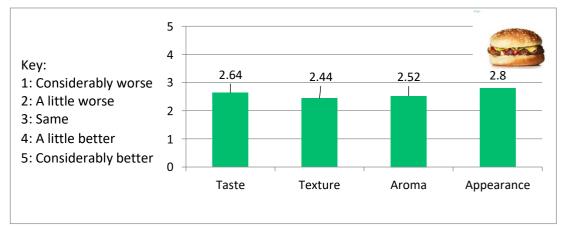


Figure 74: Survey question 39. "Your favourite burger brand has decided to introduce a new burger that uses all the same ingredients as your favourite but replaces a portion of the meat with cricket powder in order to create a higher protein product which uses less meat as part of a sustainability drive. How would you expect is to compare on the following attributes?" (n=264)

The participants stated clearly stated that the for the majority of them, ethical and sustainability attributes that could be gained from using insects as a meat reduction product would be enough for them to consider trying the new version of the burger in this scenario. Although, 19% of the participants were a strict no and the remaining 8% would consider it under specific circumstances. The additional requirements suggested were nutritional quality, specific product types and the hedonic attributes.

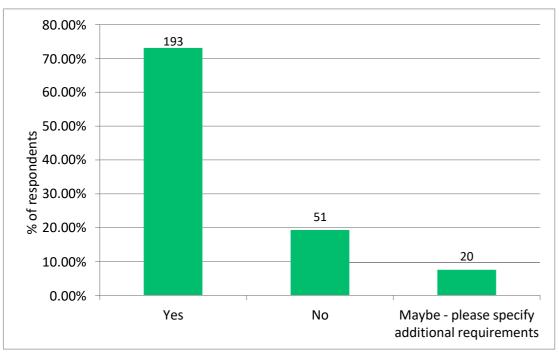
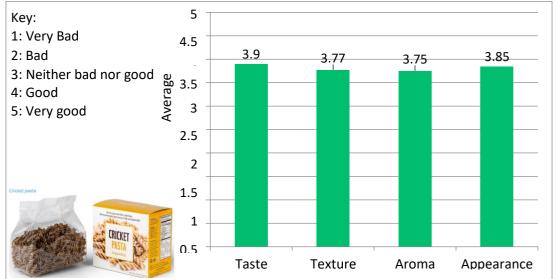


Figure 75: Survey question 40. "Would the potential ethical / sustainability benefits of meat reduction using insects be enough for you to consider trying the new product?" (n=264)

The mean result for all attributes was "good" with around 20% answering "verygood"



for each attribute

Figure 76: Survey question 41. "This is a product you can find for sale in the UK. Please rate how you expect it to score on the following attributes once prepared." (n=264)

The average result fits squarely into the "a little worse" category but the mean for each answer was "about the same".

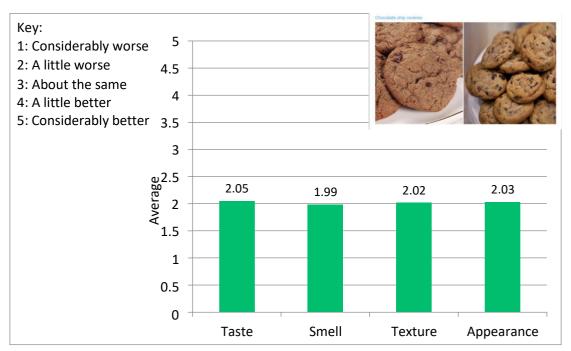
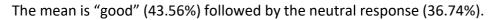


Figure 77: Survey question 42. "The two cookies above are produced using the same recipe except the one on the left has had cricket flour included. What effect do you expect this to have on the following attributes." (n=264)



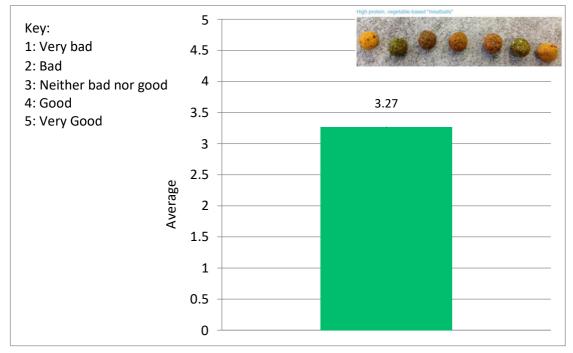


Figure 78: Survey question 43. "How would you expect the above product to taste?" (n=264)

The largest response was that participants expect the presented product to be worseas a result of the insect inclusion (47%), however, 44% of the participants claim that it was not a factor in their response and a further 9% expected the product to be better as a result.

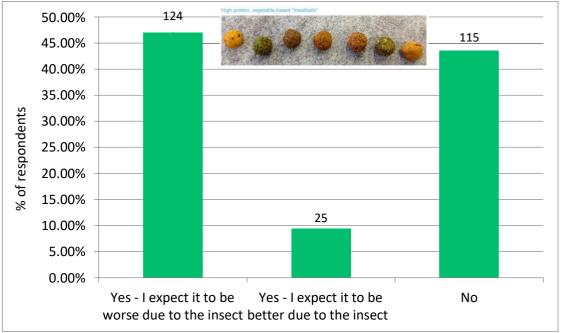


Figure 79: Survey question 44. "Considering your answer to the above question - does the potential inclusion of cricket flour influence your answer?" (n=264)

The results show that participating in research can influence people's conscious perceptions of insect-based food products. Over half of the respondents to this survey state that it has made their expectations higher due to what they have beenpresented.

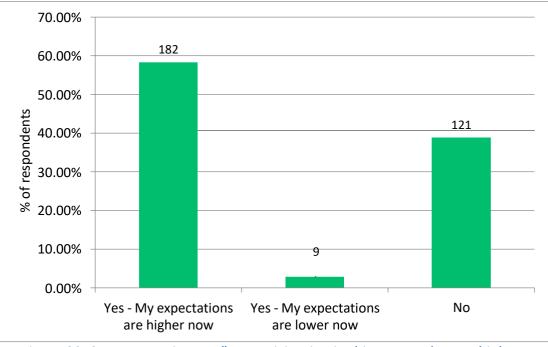


Figure 80: Survey question 45. "By participating in this survey, do you think your expectations for insects as ingredients has changed?" (n=312)

4.5 Discussion

The convenience sample (staff and students at the university) used within this survey is not representative of the demographics of the UK population, so this could be considered more of a snapshot of the entomophagy target market, with a focus on millennials which forms a large part of the survey's demographic. UK gender population is close to 50/50, but nonbinary is unaccounted for by official statistics. Age demographics are more uniformly spread too with the 18-29 grouping for the UK population only making up 16.12%. Similarly, educational levels are likely to be more diverse when considering the wider population (Ranchin and Valle, 2014).

It is expected that the convenience sample of Biosciences and Veterinary staff or students may have influenced this towards the positive when considering whether the UK will need a greater diversification of animal protein, potentially allowing the negative answer to be viewed as a more significant figure. This could be interpreted two ways; one option could be that there is the strong belief that we should diversify away from animal protein, while the other is that our current sources of animal protein are enough. Either way, this result is concerning because it means that the messages by organisations such as the FAO, of this being a dire need, are not reaching some of the populace. When this answer to question 7 is taken in context with question 8, there seems to be the belief that the predicted protein shortage will only have a limited effect on the UK, meaning that diversification is not as immediately necessary for us, when compared to other countries (Figure 46). A commonly held belief is that due to the buying power of the UK market, we would be able to easily import products, if necessary, in a time of shortage. The key problem that this presents though, is that if the participants truly believe there is low risk of us being affected by the predicted protein shortages described by the WHO and FAO, then there would be little need to find alternatives. As the results of question 9 and 10 demonstrate, knowledge of edible insects appears to be growing, with the vast majority of responders to the survey stating that they have seen reports on them in the media (Figure 47 & Figure 48). It is a positive signal for the promotion of entomophagy that more than half of the participants have some knowledge about edible insects in general, as more familiarity is one of the ways to overcome neophobia. With this sample being mostly students, the large internet response was expected, but the notable activity from other sources of mainstream media could indicate growing interest in the subject within the UK, reaching beyond that media's typical demographic. It is unfortunate

for entomophagy development that those media representations are still providing mixed messages, as this is not conducive in getting more people to be willing to purchase and consume insect- based foods. The negative lingers psychologically far more than the positive, simply because the potential negative represents a risk that the positive potential of insect-based foods has a difficult time overcoming (Tan, Verbaan and Stieger, 2017).

Understanding how knowledge of entomophagy is distributed in this way potentially demonstrates how other demographics could be reached where the results are low. As for television or internet, progressing those sources will involve ensuring that their messages are positive, thus encouraging people to try, rather than dissuading them. Mixed responses to how the media portrayed entomophagy is to be expected, as one of the most common ways participants could have viewed insects would be as part of the challenges on television programme, 'I'm a Celebrity, Get Me Out of Here', that generally displays consuming insects as a negative process that is meant to be feared and initiate disgust responses. At the same time positive documentary segments have become more common too, but even these tend to lean heavily on the "weirdness" in order to promote the idea, which may not be good in the long term as this method could drive singular sales that are solely to satiate the curiosity.

The majority of participants were from the UK, where insects are not part of the typical diet. However, one third of responders to the survey had tried edible insects. Further to this, the majority expected to eat insects in the future, which does throw some positivity towards these results. There is the potential that the convenience sample may be expecting to try insect-based foods due to other ongoing research programmes at the university, but this also could be taken as an indication of acceptance of this future protein source. Earlier academic work that has asked similar questions found that a lot less of their populace had previously tried edible insects, and even less were, in principal, willing to eat (Tranter, 2013). This could either be people accepting the necessity of edible insects or being more willing to try due to becoming more familiar over time (Tranter, 2013).

The responses to expected emotional response (Figure 50-52) are interesting, as overall thehighest average responses were for the neutral emotions, but literature that has broached this subject previously suggests that when actually faced with trying insects, emotions strongly swing one way or the other (Gmuer *et al.*, 2016). This suggests that expected emotions may differ from those felt when people are put in the situation of actually trying insects. The results of this study demonstrate that negative fear and disgust responses are expected to be secondary to a cautious curiosity which suggests that people may be willing to consume insects but need some convincing. Such interest, optimism and hope demonstrate that the curiosity is driven by positivity, rather than thrill-seeking as suggested in some literature (Durst andFAO Regional Office for Asia and the Pacific., 2010). The feeling of being healthy while consuming insects is a positive sign for nutritional based marketing. Low responses for inferiority and shame disprove a hypothesis based in literature that consumers of insects may harbour concerns over being judged for their actions by their peers (Shelomi, 2015).

Flavour pairing is a way of approaching the subject of food appropriateness in a way that would be familiar to the participants. To this end some of the responses do demonstrate the given hypothesis that, unfamiliar cuisines would get high positive responses for the pairing. This is clearly shown by Berber, Peranakan, Brazilian and Malay whereas the opposite; high familiarity with low expected pairing, is true for British, American and Italian cuisines (Figure 53). This hypothesis does not seem to be a catch-all though, as Russian and Jewish cuisine have a low familiarity score but a highly negative expected pairing. Cuisines that commonly consume insects such as Thai and Mexican also score highly for a positive pairing, which is likely due to the fact that most of the surveyed population has at least a little knowledge of edible insects. These results are confirmed in a later question where currently available flavour pairings are ranked in order of appropriateness. Participants expected Thai to be the most appropriate combination and British the least. This suggests that Asian flavours could be a good initial route to developing acceptance and that inclusion in home cuisine could be a valid route towards acceptance and the overcoming of bias towards entomophagy. This series of questioning yields results which will be extremely valuable in marketing entomophagy to the UK. The results indicate that the hypothesis of familiarity being an inverse to positive pairing expectations, is not absolute but may hold some merit. On the face of it, the hypothesis that unfamiliar flavours are what is expected to pair well with insect- based foods holds up with a few exceptions. While some psychology suggests that familiar flavours might be a way of easing people into consuming a novel food, in the case of British flavours for British people, this is clearly expected to be bad (Tan, Berg & Stieger, 2016).

In line with most literature on the subject, the participants have a clear preference

for dried and ground insects (Figure 54) (Caparros Megido *et al.*, 2014; Lensvelt and Steenbekkers, 2014; Tan *et al.*, 2016). It seems to be that the concern towards the visual aspect of insect-based foods is consistent in the western world. The strong preference for dried, over fresh, regardless of the format is an interesting area of the psychology which should be further explored. The expectation of poor texture in this case could be a large factor alongside the visual impact.

Participants had high expectations for the qualities of insects (Question 20), particularly as far as general sustainability, protein content, protein quality and sugar content goes, however, further work needs to be done to get the general public to understand what sustainability actually is. While the standard messages of sustainability and protein come through, some of the other positive attributes of insects could be improved upon, particularly the low carbohydrates and the nutritional density derived from the micronutrient content (Figure 65). There seems to be a false expectation that insects would be low in fat but this is definitely not the case, unless the fat is removed as part of the processing. The distinctly low score for food safety is concerning as, if people are expecting the foods containing insects to be unsafe, no amount of positive hedonics, sustainability or ethics is going to help overcome that. This is a clear area that needs to be publicly addressed in marketing campaigns and media. Care must be taken though, as sometimes even positive messages regarding safety can have the opposite effect of what is intended. This is because food safety is typically assumed when it comes to a commercial food product, so having to explain that something is safe causes people to question that assumption (Mitchell et al., 2015). When participants were later asked to rank these attributes in order of importance for a product, overall sustainability is the most important attribute by a fairly wide margin (Figure 65). The individual sustainability attributes that cricket powder can bring to a product are, in theory, less important than the amount and quality of the protein. General nutritional density of the product is also less important than the protein it provides, which suggests that potential benefits associated with fibre, micronutrients, and other aspects that cricket powder products could provide are of lesser importance. While health focussed products tend to go through cyclic marketing, rotating between them being low in salt, low in sugar, low in fat or low in carbohydrates based on changes to government legislation and consumer desires, these values drew the lowest scores by a considerable margin. They could be used to promote

entomophagy when consumer interest in that area is at its peak, but this result would suggest other methods may work better.

Price is a significant factor in the acceptance of insects as a food source, as currently insect powder is very expensive to purchase compared to other powders that provide similar nutritional attributes. The majority of participants expected insects to cost less, and they are generally unwilling to consider them if they would cost more (Figure 57 & Figure 58). The participants in this work appear to view insects as 'lesser'. This is a demonstration of the bias which must be overcome in order to successfully make insects a main-stream protein source and demonstrates that price is likely to be a hard cut off point in terms of creating willingness to buy insect-based ingredients and products. It is a significant viewpoint as this attitude is one of a few that has been proven to be the same in practice as in theory (Hurgobin, Le Floch and Lemercier, 2020).

When considering specific products, the responses to Falafel, meatballs and burgers were that the majority of participants believed they would either be unchanged, or would be made slightly better by including the cricket powder (Figure 59). This appears to be a positive response to the idea of using cricket powder for meat replacement purposes, lowering the amount of meat in a product but using the cricket powder to maintain, or increase, the nutritional properties of those products. The low score responses to the inclusion of cricket powder to things like white bread and white pasta could be due to the expected visual change to this type of product as that has already been discussed as a potentialissue to overcome.

The flavour of insect ingredients is expected to pair best with savoury foods (Figure 64). In addition, there is a very strong preference for savoury products that contain cricket powder (Figure 60) which is interesting, as the bulk of the products available so far in the UK are sweet protein bars (Engstrom, 2020). The expectation of contradictory flavours is an example of negative hedonic expectancy. Producing explicitly sweet products for this consumer base would be unlikely to yield much consumer support, as the products are expected to be bad. In the US there has been a lot of development of sweet products containing cricket powder, such as cookies and shakes, whereas the countries that are leading the entomophagy movement in Europe have focused heavily on meat replacement. This ties in well with the previouspositive responses towards the idea of burgers, meatballs and falafel type products being made using cricket powder adding further weight to the

idea that this may be the way to attract new consumers towards the idea of eating insect-based food products. The protein bars fall in a niche between health food and sweet snacks, so the results appear contradictory. However, when protein bars are presented as a separate option, they score highly, suggesting that participants more strongly link them to the health food category. Health food had a significant positive response as an area where people expect to find products containing cricket powder, along with savoury snacks and to a lesser extent, ready meals (Figure 61). The high response towards health food is expected, as the primary thing insects have been marketed for is their protein content and high protein foods form a significant portion of development in that area (Engstrom, 2020). Based on these results it would seem that products made from insects would benefit from a health focus. It might be possible to combine these areas and consider healthy snacks as a way to promote entomophagy.

When considering meal occasions where the inclusion of cricket powder may be the most appropriate, snacks continue to dominate the results but with additional high response rates for lunch and dinner (Figure 62). The results for lunch and dinner would lend itself to the previously discussed idea of using insects as a form of meat replacement or through including them into the carbohydrate-based staples to improve the nutritional properties of those products. Also following the previous train of thought, desserts scored particularly low in this instance confirming again that this should be an area to avoid.

A series of questions used imagery (Appendix C) as a way to provide context and overcome a lack of knowledge towards insect-based foods when discussing them (Figure 67). The images purposefully included some whole insect applications to determine how that may affect the results when contrasted against similar questions. From these examples, it appears consumers expect insects to be made into high protein staples such as pasta or noodles, as well as protein bars first and foremost. Although the scores average out to be close together, the staples have more than 45% of participants ranking them first, indicating that this is the most strongly expected product type. The results for the whole insect product are indicating that the line drawn against whole insects could be overcome depending on the product type. However, the preference for powder is clear, so it would be harder to convince people that whole insects are better. In combination with previous results, this demonstrates that people are likely to group the protein bars into the

health food category, rather than the sweet snacks, despite them falling into a niche between both areas. This goes some way to explaining their expected preference. High protein staple foods are a good potential as it could be used for lunch and dinner occasions which also scored highly and can easily accommodate Asian flavours.

The Image attributed to question 34 was taken as a representation of a visually highquality product using techniques from restaurant-style plating and professional photography. Based on the results, it would seem that visual appeal on its own is likely to be a big factor in acceptance as the results indicate that this style of product is better than what most participants would expect from something containing cricket powder (Figure 69). This creates a difficult dilemma. Images like this could be used to raise expectations of insect products as demonstrated by the positive result to the question, potentially generating a greater willingness to try and willingness to buy initially. However, if the product that is bought, or tried, does not then meet or exceed those expectations, that consumer is unlikely to be willing to give insectbased foods a second chance due to the single negative experience. At this current time, the development of foods that contain insects is in its infancy in the western world. Compared to foods which have had decades, or even millennia, to develop organically, it is likely to prove difficult to be able to rapidly develop something that can meet or exceed the hedonic properties of those foods without greater exploration of the physical properties of the insect ingredients that are being used. Although this work typically happens through the development procedure in food production facilities, restaurants or even home-cooks experimenting, it could potentially be expedited through experimental food science as long as the results of these studies are distributed in a way that they could be understood by the layperson.

While previous questions demonstrated some negative bias towards insect ingredients, when the participants are asked their preference for a protein source for a high protein ready meal, there is little difference between the averages for a noninsect source and insect flour (Figure 71). Nearly 40% of the participants put the insect flour as their first choice, which further suggests that at least a reasonable portion of the population may attribute some value towards insect protein sources. Over 90% of participants responded that whole insect is the worst of these options, providing further confirmation that consumers really do not want to have that visual or expected texture profile thatthose options could yield in a product.

When considering a pasta product that was commercially available in the UK, cricket the scores for expected hedonics are considerably higher than those provided for the burger (Figure 76). This could mean that a burger is a product considered poor anyway, when compared to pasta, regardless of the addition of the cricket powder. If this result is to be believed it would demonstrate further strong evidence that the staples are a better target for the inclusion of cricket powder than the meat reduction / meat alternatives, which was one of the original goals of the developmentof insect-based foods set by the WHO (van Huis *et al.*, 2013). The mean for all attributes on the pasta was "good" with around 20% of participants answering that they believe it to be "very good". Whether this level of positive hedonic expectation would be enough to translate to WTB and WTE on a regular basis is unclear. It would likely still require other attributes, such as price to be appropriate.

Asking a similar question but framing it as a comparison between a cookie with and without cricket flour yields that most people believe it to be made "a little worse" from the inclusion of the cricket (Figure 77). This adds further evidence towards the theoretical dislike of sweet products made using insect ingredients, regardless of the context and adds further weight towards expected poor texture being one of the more prominent expected reasons for disliking insect-based foods.

The final question of the survey provides a clear demonstration of a topic touched on earlier. The majority of people are approaching insect-based foods with absolutely no context, so whatever is shown to them or told to them as part of that process can be highly influential in their future attitudes. Due to the framework put in place here, and the examples given, the majority of participants from this study now have higher expectations for insect-based food (Figure 80). An unfortunate side effect of this is that they could be inspired to go out and try some of the existing products and find that their heightened expectations are not being met, which could put them off trying insects in the future. Overall, it would be considerably harder to entice someone who has previously had a negative experience, compared to someone without any experiences at all.

Due to the nature of the study, the demographics within the participants are generally too similar generate any meaningful discussion points. There was some

suggestion that males are more accepting, as demonstrated by broadly higher scores while maintaining similar ratios on all of the rating questions aside from a few notable differences. Participants under 30 years old had a slightly stronger positive emotional response across the board than older individuals. Cuisine familiarity is much higher in over 30's but the under 30's had much stronger opinions about whether something will pair well or pair badly. The over 30's were more likely to accept ingredients in either sweet or savoury compared to the under 30's, who more strongly specify savoury. The under 30's group valued sustainability and ethics over the health claims favoured by the over 30's group. As highlighted by literature, level of education seems to have little impact on thoughts and feelings towards entomophagy.

Participants who had previously consumed insects believed that a greater animal protein diversity is required in the UK. Unsurprisingly, they all claimed to have at least a little knowledge of edible insects. Interestingly, 21% more of the participants who had previously consumed insects expect to consume insects in the future, suggesting that these people either had good experiences or the hypothesis related to poor product quality limiting the uptake of entomophagy does not hold up with this group. The demographic who had previously consumed insects claimed far more culinary knowledge than those who had not suggesting that they are potentially more adventurous eaters. The largest differences between the two demographics are for savoury snacks, food service and ready meals. However, when it came to the appropriate meal occasions, there was a notable increase in those believing lunch is an appropriate time to consume insects. In addition, less of those previous consumers are willing to accept insects as part of a dessert.

4.6 Conclusion

Whole insects are arguably the most nutritious and sustainable format that entomophagy could be introduced as, but this work, and all other literature on the subject, has found that consumers in the western world struggle to accept this even when whole insects are utilised in a familiar product. Similarly, the results of this work contradict the belief within some academic literature that incorporating whole insects into familiar products is the best way forward as the participants categorically rejected the idea of a preference for familiarity and whole insects. Currently, the insect ingredient industry has pushed the idea of dry powdered insects as the consumer-friendly way forward and this work demonstrates that the potential UK consumer base agrees. The primary options for using consumer expectations as a way of promoting entomophagy lean towards meat replacement, staple foods, or savoury snacks, all with a health food twist. Pricing may be within the category appropriate for the product, but it should aim to be less than equivalent products, as a draw to potential purchasers. Initial consumption appears to be a barrier to overcome, as after that point there is more receptivity to eating insects, and emotional expectations, become more positive. Education and visually high-quality products can help to overcome this barrier, but the actual hedonic qualities of the product must not be overstated, or there is the risk of alienating the consumer entirely due to that poor experience. Asian flavours appearto be the best way forward, as it seems that they are inherently associated with insects, regardless of the participants knowledge base.

Based on these results, it is difficult to confirm a single product type which would be the most appropriate for promoting entomophagy by using consumer expectations. The potential options that could combine the expectation for Asian flavours and the categories of products that the participants favoured, while also remaining cheap enough, would most likely fall into the carbohydrate staples such as bread, pasta or noodles. Main meals could utilise insects as part of those staples, or as the sauce that goes with them. After scoring so highly, it is also worth considering sports nutrition products, as the high protein, combined with other aspects of useful nutritional properties provided by crickets, could lend itself quite well to this category. The downside with this is going to be the price, as the cricket powder is likely the most expensive ingredient, and this type of product will require higher quantities than a staple food. As for meat replacement, while it scores highly, this is an area where there is currently heavy development due to the rapid expansion of the vegan market. As such it would be difficult to compete for consumers with a meat replacement product that is functionally replacing one animal product for another in a market is likely going to prefer plant-based options. The greatest future potential lies with finding a unique product that can only be made with insects, but this development option also offers the greatest risk. Development time is going to be considerably longer when considering experimenting from scratch, as opposed to creating a product that already has defined boundaries and methods to create it commercially. As such, this is likely a task for another study. Ultimately, whatever is made must be high enough quality to compete with other products within that category, and drive repeated sales in order for it to be successful at being a way to promote entomophagy. As such, the next step will be to run brief trials for each of these options, from which a choice can be made about which to develop as a conclusion to this study with the ultimate goal to be to learn as much as possible about the practical applications of cricket powder in product development.

5.1 Introduction

What the world needs from insects can be divided between the needs of the developed world and the developing world, but according to the WHO paper on the future prospects of food and feed security, the hopes are that one follows the other. However, it is clear that both can benefit from including insects in the food chain (van Huis *et al.*, 2013). The goal of promoting entomophagy in the Western world is an increase in the sustainability of protein sources and an opening up of discourse on appropriate foods for dealing with malnutrition by overcoming our biases towards the novel foods being presented as potential solutions. The work described in this chapter explores how insects could be incorporated into foods that are already familiar to consumers in the western world thereby maximizing the likelihood of acceptance.

Insects once offered a great sustainable protein source for countries now suffering from malnutrition and it is believed that our interventions in these countries have promoted the unsustainable, unhealthy diets of the developed world, which are then delivered via food aid which some countries have become reliant on to survive (DeFoliart, 1999). Overcoming our bias and helping return those countries to their traditional insect consuming diets would improve nutrition where it is needed, and if at the same time, the Western diet is made more sustainable through a protein switch, even if partial, would also be an improvement.

One of the issues surrounding development of insect-based foods is that it is a proactive development being treated as though it is reactive. While malnutrition is already prevalent in the world, it is being discussed in the western world as a future problem due to initiatives such as the 2050 goals. This mentality changes what products are developed and how they are marketed. "Eat this because it may save the world in the future" or insects being a "protein of the future" has less impact with a consumer than using insects to solve the current issues (Mitchell *et al.*, 2015). The problem is those current issues of malnutrition suffer from a different issue, the distance away from sources of food production (van Trijp and van Kleef, 2008). As presented in the introduction to this thesis, entomophagy provides opportunity here due to the limited environmental requirements to farm insects successfully.

environment through processes like deforestation to create farmland. The developed world stands to benefit from insects as it seeks to diversify its proteinsources because conventional animal farming is unlikely to meet our protein needs for much longer (Westhoek et al., 2011). In short, introducing entomophagy here seeks to be a profitable way of increasing the sustainability of our food chain. Whereinsects are enjoyed in their natural state, insects are steadily getting more expensive, however, it is believed that processed insects will continuously get cheaper to produce through economy of scale and developments in production efficiency, making them a valuable resource as similar ingredients with a high protein content are expensive and usually unsustainable (Müller et al., 2016; Berger et al., 2018). Therigorous processing that commonly used protein supplements have to undergo are harmful to the environment and more consumers are looking for sustainable alternatives (Zhong, 2017). Insects as a protein source require very little processing to reach similar protein values which could make them a preferable alternative to some consumers (Román, Sánchez-Siles and Siegrist, 2017). The other side of sustainability and health focused foods are that they are often very profitable due to those claims fetching a premium price (Mintel, 2020).

Ultimately what is needed for insects to be successful in the developed world is a high quality product that delivers on desirable hedonic properties and nutrition because this demonstrates value above and beyond what can be demonstrated by the production of the raw ingredient.

Initiatives that utilise entomophagy to improve on malnutrition need to consider appropriate combinations of species, cultural preferences, production systems and dietary preferences for greatest benefit. In this sense, crickets may not be the best solution for everywhere as it is a western idea of insect farming that has heavily focused on cricket powder production (Nadeau *et al.*, 2015). If a product is developedwith this potentiality in mind, the insect proportion of a recipe may be interchangeable for something more appropriate and it would still have a similar effect but the implication of swapping between insect types has had limited study. The potential implications on nutrition are presented in chapter 2. The increase in nutritional quality through the additional protein, essential fatty acids, vitamins and minerals insects can provide when incorporated into products, could be highly valued in some areas of the world. The countries investing in insects across Europe have mostly focussed on meat replacement (Tan, van den Berg and Stieger, 2016). However, if the results of the survey are considered, there should be other appropriate options for an adequate commercial introduction of insects to the UK. As the results of chapter 4 demonstrate, despite unfamiliarity, consumers have defined expectations for insect-based foods, and these expectations must be met and exceeded in order for a product to have a chance at acceptance in the western world. Further to this, exceeding the standards set by conventional food products would allow for the potential of an insect-based food being commercially successful. A lower hedonic rating and lower consumption intentions are often the result of having no taste history and neophobia prevails as perspective consumers are very unsure about insects (Tan, van den Berg and Stieger, 2016). This means that products are generally going to be assumed to be bad and the consumers will be scared of adopting them until they can see clear tangible benefits in doing so. A preference for a food cannot be formed before the first taste experience so a lot of effort must be put into creating something that consumers are willing to try (Tan, van den Berg and Stieger, 2016).

There appears to be a process to developing acceptance and the first stage of that seems to be developing insect-based foods which will lower the barriers to initial tasting as this will begin to breed familiarity within the consumer base (Tan, van den Berg and Stieger, 2016). From there, insect-based foods not only need to succeed in encouraging further willingness to try but those products also need to be comparable with conventional food products with similar attributes if they are to be successful and accepted by the consumer base (Tan *et al.*, 2016). Current commercial uses of insects only appear to value its nutritional properties and little consideration has been given to its sensory properties. In part, this explains why there has been some difficulty in driving commercial acceptance for insects as the products made with insects have adulterated food products with insects which are viewed as not appropriate by the consumer base (Tan, Verbaan and Stieger, 2017). Lack of availability, high prices, limited knowledge, and the social constructs surrounding insects are the other aspects limiting its uptake (Tan, Verbaan and Stieger, 2017).

Utilising familiar products or flavours to promote insects has the potential to promote a willingness to taste but also could evoke a sense of disgust if the consumer believes that product to be contaminated by the insect's presence (Tan *et al.*, 2016). In general, adulterating a familiar product with cricket powder appears to be a direct trade between quality and nutrition (Osimani *et al.*, 2018). Adulterating a familiar product with insects for a nutritional benefit can help to create more positive

expectations as the known positive expectations of that product off-set the unknown attributes of the insect ingredient (Tan, Tibboel and Stieger, 2017). However, it is still generally going to be less appealing than the original, at least until those unknown attributes are confirmed through tasting. Utilising insects in a familiar product in this way also risks participants being more disappointed with the flavour due to their expectations being that the product is closer to the original than it ends up being, and a disappointing experience will result in a greater decrease of future intentions as those fears of a negative experience are confirmed. In order to get the most acceptance out of using a familiar carrier product, the insect and the carrier must be deemed as an appropriate pairing (Tan, van den Berg and Stieger, 2016). Limited academic research has been focused on how the functional properties of insects can influence product quality. It is understood that chitin when separated from insects can affect the textural properties when added into a food matrix (Benjakul et al., 2001). As such, it may be a fair assumption that the chitin present within the exoskeleton may have some effect on a product's textural quality, but it isunclear how much those differences would affect consumer enjoyment and acceptance. Academic studies of product development are limited, but there are some suggestions that some processing methods may yet yield superior products to the conventional counterparts. Mechanical forces such as in extrusion could potentially improve the digestibility of insect protein however there was a notable quality change demonstrated by decreased porosity and the lower overall expanded size when compared to a control (Azzollini *et al.*, 2018). Pasta or similar products may well be a positive product carrier for insects as the protein in pasta is considered to be low quality due to limited amounts of some essential amino acids, particularly lysine which insects could improve on (Duda *et al.*, 2019). Additionally, incorporation of cricket powder into pasta reduced cooking losses and water absorption while increasing firmness (Duda et al., 2019). These changes, particularly the decrease in cooking loss, are indicative of a higher quality product. This appears to be something unique to insects as the inclusion of plant proteins have previously been demonstrated to increase cooking loss in pasta products (Nielsen, Sumner and Whalley, 1980). However, optimal cooking time increased from 6 minutes to 7 minutes which is a minor decrease in convenience and when tasted, consumers noted a pronounced flavour change, but the acceptance remained comparable with the conventional product (Duda et al., 2019).

For insects to be accepted into the western world, they must be a commercial

success as commercial availability allows for familiarity which is currently the biggest barrier to insects as a food source. The lack of familiarity is currently breeding wildly variable expectations that are often negative, resulting in the lack of acceptance. As such, their existence within retailers would normalise their existence as a food source and provide opportunity for consumers that are willing to try. Repeated exposure has the potential to stimulate liking of a highly novel productover time due to a steady increase in familiarity (van Trijp and van Kleef, 2008). In this sense insects appear to be stuck in a vicious cycle of media exposure, as highlighted in chapter 4, without mainstream availability creating frustration and confusion for potential interested consumers. This issue has partly stemmed from unclear legislation which has had to be refined so that insects fall under the guidelines for a novel food (Marberg, van Kranenburg and Korzilius, 2017). This lack of legal clarity has created confusion for manufacturers preventing the impact big food producers would have on the industry but also permitted small companies and start-ups to create and sell a wide range of insect-based foods without defined insect-specific safety regulations. Consideration was given as to which types of products meet the criteria developed through the survey in chapter 4. From this, carbohydrate staples such as pasta, meat replacement and unique products are the areas that stand out. Products intended for consumption as snacks or at main mealtimes as well as those suitable to utilise an Asian flavour profile should be given priority. Overall quality needs to be high; pricing needs to be able to be cheaper than similar products and it needs to be high in good quality protein. The sustainability aspects of the cricket powder also need to not be sullied by unsustainable ingredients as that would go against the purpose of utilising insects. Although it is still worth trialling due to its popularity as a food source, bread may not be a suitable use for insect-based food products because 2 of the bacteria identified within samples in chapter 3 (Bacillus licheniformis and Bacillus subtilis) are causes of ropy bread. It would likely cause bread to reach the point of spoilage quicker than normal which is not acceptable to consumers. The only way to prevent this is if powder is processed specifically to remove these organisms (Osimani *et al.*, 2018). Meat substitution is an area with its own set of negative perceptions as a lack of familiarity and people's perceptions of the skill required to prepare those productsstill hamper complete acceptance (Schösler, Boer and Boersema, 2012). While that negativity is decreasing it still needs further development and education in order to become completely accepted by everyone. Combining the issues that insects bring into product development with an area that is beginning to overcome similar issues

would not be ideal for the promotion of entomophagy until meat replacement becomes completely established. It could be possible to utilise a product category that is not explicitly tied to meat but fulfils the same function on the plate for this purpose though.

5.1.1 Noodles as a viable option

While it is valuable to test a range of options, the results of the survey present noodles as a prominent option for the promotion of entomophagy both in the developed and the developing world. The typical person who consumes instant noodles regularly are males aged between 20 and 49 years old. This grouping of people has significant crossover with those suggested to be most accepting of insects (Park et al., 2011). There is also correlation between some areas of high proteinenergy malnutrition and high consumption rates of ramen noodles. This occurs primarily in southeast Asia (World Health Organisation, 2004; World Instant Noodles Association, 2020). While it is difficult to directly attribute this malnutrition to the increasing consumption of ramen noodles it can be argued that there is the potential for that to be the case due to the lack of good quality nutrition provided by this meal choice. As such, incorporating insects into noodles has the potential to provide a dietary improvement in these areas without the need for a change of routine. In the western world, the noodles containing insects could be presented as a premium product due to the same increase in health and nutritional benefits provided through the inclusion of insects, potentially solving the issue of commercial viability.

Noodles are believed to have originated in China around 5000 BC (Hou, Kruk and Center, 1998). However, ramen style noodles are a relatively recent invention as they were first manufactured in 1958 by Nissin Foods, Japan (Gulia, Dhaka and Khatkar, 2014). Ramen, being a relatively recent creation, should yield fewer negative emotions when considering food appropriateness due to the limited time it has had to generate a cultural attachment when compared to food products with a longstoried history. A large proportion of Asia's wheat is consumed as noodles and the proportion of that which is eaten as instant noodles is the fastest growing, accounting for steadily more of the continent's dietary energy and nutrient intake. Due to this there is an apparent decrease in the consumption of rice-based products and an increasing turn towards wheat-based foods (Bronder *et al.*, 2017). Noodles are consumed all over the world but 51% of globally consumed noodles were consumed in China, however, consumption is increasing in the western world (Park *et* al., 2011). In Korea, noodles represent the second largest contributor to nutrition after steamed rice however, noodles are typically high in carbohydrates but low in fibre, vitamins, and minerals. As such, the consumption of instant noodles can lead to intake of excess energy, fat and sodium while simultaneously yielding lower intake of protein, calcium, phosphorous, iron, potassium, vitamin A, niacin and Vitamin C when compared to non-consumers, prompting suggestions that manufacturers should consider making nutritional improvements during new product development (Park et al., 2011). High ramen noodle consumption rates in Asia correlate with some areas of malnutrition because of the limited nutritional elements beyond the carbohydrates. Noodle consumption is also rapidly increasing (500% growth between 2006 and 2011) in other areas of the world with malnutrition such as India (Gulia, Dhaka and Khatkar, 2014). An average packet of noodles contributes between 35% and 95% of the WHO's recommended daily salt intake which creates a growing health concern as they are increasingly being consumed for all meals in areas of malnutrition due to low income (Farrand et al., 2017). Ultimately, instant noodles are the most important convenience food consumed globally (Gulia, Dhaka and Khatkar, 2014). Pricing of the noodles could be acceptable with insects despite insects currently being an expensive ingredient because premium noodles often utilise other expensive ingredients to improve quality aspects.

A procedure for conducting bench scale trials on noodles was outlined by Kim et al (1988). However, there are some variations in this procedure presented in literature that are highlighted as ways to improve quality. Hou (1998) suggests that after ingredients are mixed, the dough crumb is rested for between 20 and 40 minutes before they are brought together in order to allow for water to disperse evenly throughout the mixture. After the initial sheeting, the dough is transported through an enclosed environment where temperature and humidity can be accurately controlled in order to relax the dough before being put through rollers to reduce the thickness to the desired point and then cut (Hou, Kruk and Center, 1998). Most types of noodles are then part cooked by boiling in order to begin the process of gelatinising the starch before being coated with oil to prevent them sticking together. Drying the noodles can be done by hot air which takes between 5 and 8 hours, or deep fried to produce a kind of instant noodle. Deep frying is typically preferred by the consumer due to the fatty taste and mouthfeel that comes with the increased oil content absorbed during frying however, air drying does result in a significantly

healthier product (Hou, Kruk and Center, 1998). Frying to dry noodles typically takes place at between 140°C and 150°C for around 2 minutes. This reduces the water content to under 10% and provides a distinctive flavour. This kind of noodle rehydrates quickly as the frying leaves behind a porous structure due to the rapid removal of water. Alternatively, the noodles can be air dried for about 30 minutes at 80°C after steaming. Air dried noodles are often made thinner in order to keep this process quick (Kim, Freund and Popper, 1988). Noodles that are fried to dry absorb between 15 and 20% oil by weight during that process. However, a higher protein noodle has been shown to absorb less oil (Gulia, Dhaka and Khatkar, 2014). Vacuum drying has limited testing but has been linked with producing a higher quality noodle (Hou, Kruk and Center, 1998). In a technical sense, when cooking a noodle for trials it is considered as cooked when no visible core can be seen when the noodle is broken or cut (Rombouts *et al.*, 2014).

Flour used for noodles is generally hard wheat of high protein content and a high ratio of glutenin to gliadin referred to in the text as "strong gluten". It should also have an ash content of 0.4% or less as this affects the end colouration (Hou, Kruk and Center, 1998). Finer flour particles with low starch damage results in a noodle with better cooking qualities as starch damage can affect the colour, increase cooking loss and cause excessive surface swelling of the noodles (Gulia, Dhaka and Khatkar, 2014). As such, a flour that is designed to be reasonably high in protein content and have a finer particle size like 00 flour that is commonly used in the production of pasta may make for good noodles. Christiano et al (2019) reported 00 flour as having a mean particle size of 71.686µm. Utilising a strong flour results in a firm, more elastic noodle (Shiau and Yeh, 2001). The inclusion of different kinds of alkaline salt had a notable change on the texture of noodles. Typically, this inclusion increased springiness and cohesiveness of the noodles resulting in a higher score for overall acceptance during sensory trials (Lee, Kim and Park, 2011). Kansui, the normal alkaline ingredient used in noodle manufacture, is typically a 9:1 mix of sodium and potassium carbonate although sometimes this name can refer to a sodium hydroxide solution (Kim, Freund and Popper, 1988). The addition of kansui to a noodle dough appears to increase the solid-like behaviour of the dough. However, utilising Kansui results in a large increase in cooking loss but this is believed to have only been a surface issue and does not translate to a poorer quality texture overall when the noodle is consumed (Shiau and Yeh, 2001). The alkalinity also results in a dough that is tougher, tighter and less

extensible while also increasing the water absorption potential, increasing starch paste viscosity and slowing starch gelatinisation (Shiau and Yeh, 2001). The cooked noodles are firmer and require a higher force to break or cut (Hou, Kruk and Center, 1998). Alkalinity increases the temperature at which starch gelatinisation takes place but increases the swelling potential, increasing the optimal cooking time but also increases firmness (Hou, Kruk and Center, 1998). Due to the alkalinity, doughs become tougher and shorter. This texture change is attributed to a change in the sulphur bonding structure within the gluten matrix from sulfhydryl bonds to disulphide (Shiau and Yeh, 2001). Kansui induces the formation of lanthionine and lysinoalanine, decreasing the content of available cysteine and lysinoalanine may be toxic with long term consumption (Rombouts *et al.*, 2014). The yellow colour of alkaline noodles is created when the flavones detach from the starch due to the alkalinity (Kim, Freund and Popper, 1988).

There are 5 key quality characteristics by which noodles are judged during tasting: colour, surface appearance, texture, taste and cooking loss (Rombouts et al., 2014). A high-quality noodle is defined as having a bright colour, clean and smooth surface, firmness, springiness, lack of stickiness, some tolerance to overcooking and a pleasant flavour (Kovacs et al., 2004). The most important attribute for consumer acceptance of cooked noodles is their texture (Gatade and Sahoo, 2015). Alkaline noodles are preferred where they are consumed because of a firmer texture when compared with alternatives such as Japanese Udon (Huang and Lai, 2010). The colour of noodles is of great importance to Asian consumers. The preference is for a bright yellow coloured noodle when considering alkaline noodles while dull, grey or brown noodles are likely to be assumed an inferior product (Kim, Freund and Popper, 1988). However, the brown visual could be indicative of a "healthy" product due to its similar visual presentation to wholemeal products which may be a useful psychological tool for the promotion of insects. From a manufacturer's standpoint, a noodle that can rapidly absorb more water during the cooking period while maintaining the desired texture characteristics it should yield a more desirable product that is also more profitable (Hou, Kruk and Center, 1998).

Improving the quality of noodles has been the subject of quite a lot of research (Nielsen, Sumner and Whalley, 1980; Kruger, Hatcher and Anderson, 1998; Shiau and Yeh, 2001; .조희숙.배경윤 *et al.*, 2008; Widjaya, 2010; Huang and Lai, 2010; Foo *et*

al., 2011; Lee, Kim and Park, 2011; Wang et al., 2011; Rombouts et al., 2014; Gatade and Sahoo, 2015). Various additives have shown potential to improve noodle quality. The effect of an inclusion of table salt is debated as Kim (1988) suggests it makes little difference while Rombouts (2014) states that it reduces the extent of gluten polymerisation during cooking, increases the development time required for the dough, increases elasticity, improves flavour, colour and textural properties but decreases water absorption. Emulsifiers such as fatty esters prevent starch retrogradation, improve the internal structure of the noodle and have some benefit to the cooking qualities (Gulia et al., 2014). An inclusion of phosphates accelerates the formation of the gluten network making the noodles more elastic, flexible and chewy while also allowing for greater water retention, thus improving mouthfeel (Gulia et al., 2014). Enzymes such as transglutaminase increase break strength and firmness of the noodle while also reducing stickiness and cooking loss (Gulia et al., 2014). Antioxidants can also be included in order to retard oxidative rancidity which is the primary method that a noodle product would demonstrate spoilage, particularly if dried (Gulia et al., 2014). In premium noodles, native or modified potato starches are incorporated to provide a springier texture as well as improved cooking properties due to a lower gelatinisation temperature (Hou, Kruk and Center, 1998). Hydrocolloids can be incorporated into noodle recipes in order to improve firmness and rehydration. Stabilisers such as gums can improve rehydration during cooking, decrease fat uptake during frying and modify the mouthfeel in a positive way (Gulia et al., 2014). An addition of guar gum has been demonstrated to increase yield, improve texture, and create a greater resiliency in baked goods (Gatade and Sahoo, 2015). In noodles this improves moisture absorption, stabilises the dough and improves extensibility. However, at higher levels it increases cooking loss (Gatade and Sahoo, 2015). Guar was shown to be useful in noodles that incorporated oil in the recipe by helping it distribute evenly throughout the dough and preventing that oil from interfering with water uptake. The addition of dehydrated wheat protein, also known as vital gluten powder, has been demonstrated to improve texture and eating quality of the noodle when non-gluten containing ingredients make up part of the dry ingredients (Huang and Lai, 2010). Increasing the fat content of noodles reduces their extensibility but also reduced their stickiness due to the formation of an amylose-lipid complex (Gatade and Sahoo, 2015).

5.2 Aims and Hypotheses

The aim of the study is to utilise the results of the previous chapters to develop an alkaline noodle product that improves on the nutrition of conventional alkaline noodles significantly while maintaining the primary quality aspects of texture and colour. Through this development, a secondary aim is to gather an understanding of how known methods can be utilised in the improving the quality of insect-based foods.

The hypothesis is that the cricket powder addition will result in a good improvement to the nutritional quality of the noodles but is also likely to cause a detriment to the product quality that is difficult to recover.

5.3 Materials and Methods

5.3.1.1The development of Alkaline noodles

Production of the baked soda followed the guidelines of Professor Harold Mcgee (2010). This involved spreading an even layer of bicarbonate of soda onto a tray and oven baking for 1 hour at 150 °C (± 5 °C monitored with an oven thermometer). Noodle samples were prepared as follows. Ingredients (see table 14) were weighed out separately. The alkaline ingredients (table 15) were added to the water before being combined with the dry ingredients in the bowl of a Kenwood K mixer (Havant, UK) using the K paddle attachment. The mixer was run on speed 4 while slowly adding the liquid ingredients (alkaline ingredients and water) and left to run for a total of 10 minutes. Dough was roughly pressed together and placed into a sealed container before being refrigerated for a minimum of one hour. Dough was then flattened and passed through a pasta machine (Argos, UK) while gradually decreasing the spacing from 1-5 before being passed through the spaghetti attachment to produce the finished noodles.

Fresh noodles were boiled at 97 °C \pm 1°C for 2 minutes and left to cool at room temperature prior to analysis.

5.3.1.2 Ingredient details

Ingredient	Brand	Producer details
Plain Flour	Sainsbury's own brand	London, UK
Bread Flour	Sainsbury's own brand	London, UK
Kansui	Tung Chun	Hong Kong
Bicarbonate of Soda	Dr Oetker	Leeds, UK
Cricket Powder	Entomo farms	Canada
00 Flour	Sainsbury's own brand, Taste the Difference	London, UK
Salt	Sainsbury's own brand	London, UK
Egg powder	Yourhealthstore.com (via Amazon.co.uk)	London, UK
Guar Gum	Bob's Red Mill	Milwaukie, US
Xanthan Gum	Life Free From	MH foods, Kent, UK
Chia Powder	Linwoods	Armagh, Northern Ireland
Vital Gluten	Bob's Red Mill	Milwaukie, US
Sunflower Oil	Sainsbury's own brand	London, UK
Potato Starch	Bob's Red Mill	Milwaukie, US
Buffalo Worm Powder	Protifarm	Harderwijkerweg, Netherlands
Vegan Blend Protein	My Protein	Cheshire, UK
Brown Rice Protein	My Protein	Cheshire, UK
Whey Protein	My Protein	Cheshire, UK
Pea Protein	My Protein	Cheshire, UK
Algae Protein	AlgaVia	Amsterdam, Netherlands
Soya Protein	My Protein	Cheshire, UK
Rice Flour	Doves Farm	Hungerford, UK

Table 14: Ingredient and producer details for everything used in the noodle formulations

Table 15: pH and molar concentration of the diluted alkaline ingredients (n=3)

	рН	Concentration (M)
Baked Soda Solution	10.28	0.64
Kansui Solution	11.78	0.11

The solutions refer to the baked soda dissolved in 48.5 g of warm water and the Kansui diluted in 29.19 g of water as this is how it is added into the mixture when making the doughs.

Two grades of cricket powder were purchased from Entomo farms, their regular (hereafter detailed as coarse for differentiation) and the fine powder. Unless detailed, all formulations utilised the coarse powder.

5.3.1.3 Initial formulations

The control samples and the standard cricket samples that follow (as described in 5.3.1.3 & 5.3.1.4) were made in triplicate while all testing samples (as described in 5.3.1.5 - 5.3.2.2) were made once. The initial recipes were adapted from two online sources (Chang and McGee, 2015; Ginger and Scotch, 2017). The first utilises the traditional alkaline ingredient used within the production of alkaline noodles in Asia; Kansui. As Kansui is an unusual commodity in the western world, a second recipe was adapted from a publication (no longer available) by Professor Harold McGee & David Chang which advocated heatingbaking soda in order to produce sodium carbonate as a source of alkalinity for noodleproduction.

Kansui Control 143.55 g Plain Flour 50.91 g Bread Flour 72.96 g Water 29.19 g water + 3.24 g Kansui

Baked Soda Control 193.31 g Plain Flour 48.5 g Water 48.5 g Water + 9.69 g Baked Soda

5.3.1.4 Texture trial 1: Setting controls.

Three different sources of commercial noodles were purchased from a local retailer and cooked according to manufacturer's instructions. Samples were chosen to be similar to various attributes of the cricket noodles. Control 1 was a preserved fresh alkaline noodle (Amoy, London, UK), control 2 was a dried alkaline noodle (Oriental Food Shop, Loughborough, UK) and control 3 was a soba noodle (Utaka, Enfield, UK), chosen due to its similar appearance to cricket noodles. These were compared to the control formulations (no cricket powder) above. As commercial control 2 was the sample most similar to the target product, the texture of this sample was considered the benchmark.

5.3.1.5 Texture trial 2: Cricket powder addition.

Using Recipe professor software (Point74, Bourne, UK), the control recipes were adjusted by replacing wheat flour for cricket powder to the point of meeting the requirements to be labelled as high in protein in the UK. Resulting in the following recipes:

Standard Kansui Cricket 131.55 g Plain Flour 38.91 g Bread Flour 72.96 g Water 24.12 g Cricket Flour 29.19 g Water + 3.24 g Kansui Standard Baked Soda Cricket 174.92 g Plain Flour 48.5 g Water 48.5 g Water + 9.69 g Baked Soda 18.39 g Cricket Flour

5.3.1.6 Texture trial 3: Alkali quantity.

Based on the results of the previous trial, this trial tested the impact of an increase in Kansui and a reduction in Baked Soda on the texture of the noodles. Kansui was trialled at 4 and 5 grams while baked soda was trialled at 5g and 7.5g.

<u>4g Kansui</u> 150 g plain flour 44.48 g Bread flour 72.96 g water 29.19 g water + 4 g Kansui

<u>4g Kansui Cricket</u> 131.55 g plain flour 38.91 g Bread flour 72.96 g water 29.19 g water + 4 g Kansui 24.12 g cricket powder

<u>5g Kansui Control</u>

150 g plain flour 44.48 g Bread flour 72.96 g water 29.19 g water + 5 g Kansui

5g Baked Soda Control

198 g plain flour 48.5 g warm water 48.5 g cold water 5 g Baked Soda

7.5g Baked Soda Control

195.5 g plain flour 48.5 g warm water 48.5 g cold water 7.5 g Baked Soda

5g Kansui Cricket

131.55 g plain flour 38.91 g Bread flour 72.96 g water 29.19 g water + 5 g Kansui 24.12 g cricket powder

5g Baked Soda Cricket

179.61 g plain flour 48.5 g warm water + 5 g Baked Soda 48.5 g cold water 18.39 g Cricket Powder

7.5g Baked Soda Cricket 177.11 g plain flour

48.5 g warm water + 7.5 g Baked Soda 48.5 g cold water 18.39 g cricket

5.3.1.7 Texture trial 4: Flour type.

The inference from the category trials was that a higher gluten content may improve firmness. This trial tested that theory by replacing the flour used in the original recipes with a "00" flour with a higher protein content than the plain flour (9.7 g / 100 g vs 13.2 g / 100 g protein content) and only slightly lower than the bread flour (13.4 g / 100 g).

<u>00 Kansui Control</u> 194.48 g "00" flour 72.96 g water 29.19 g water + 3.24 g Kansui

BS 00 Control 195.5 g "00" flour 48.5 g warm water + 7.5 g Baked Soda 48.5 g cold water 00 Kansui Cricket

170.46 g "00" flour 72.96 g water 29.19 g water + 3.24 g Kansui 24.12 g cricket powder <u>BS 00 Cricket</u> 177.11 g "00" flour 48.5 g warm water + 7.5 g Baked Soda 48.5 g cold water 18.39 g cricket powder

5.3.1.8 Texture

trial 5: Comparison to other protein powders & rice flour.

This trial was designed to determine whether the softer texture of the cricket noodles was caused simply by the reduction in wheat flour (and the gluten it contains) or if the cricket powder could be having a different effect. Protein powders and rice flour was incorporated into the recipe replacing the same quantity as cricket powder did previously.

BS 00 Control

195.5 g "00" flour 48.5 g warm water + 7.5 g Baked Soda 48.5 g cold water

BS 00 Cricket

177.11g "00" flour 48.5 g warm water +7.5 g Baked Soda 48.5 g cold water 18.39 g cricket or protein powders or rice flour

5.3.1.9 Texture trial 6: The effect of cricket powder particle size.

The fine grade commercial cricket powder was processed using an Octagon digital OCT-G472-11 sieve shaker (Endecotts, Wimbledon, UK) to determine particle size fractions and then those fractions were used as the cricket powder portion of the recipes to test whether the particle size impacts on the texture of the noodles. The sieve shaker was run at amp 8 for 5 minutes containing 100 g fine powder set on the top layer. In descending order from the top, the sieve shaker contained sieves for 600 microns, 355 microns, 250 microns, 125 microns, 106 microns and 75 microns. Each size fraction was then incorporated separately into noodle recipe.

> BS 00 Cricket 177.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket

5.3.2 Texture trial 7: Drying temperatures.

A range of noodle samples were dried in a l'equipe filterpro dehydrator (Utah, USA) at 35 °C and 70 °C for 24 hours in order to determine whether the drying parameters for these noodles may affect the end product quality once they are cooked. Dried Noodles were boiled for 4 minutes.

> 00 Kansui Control 194.48 g "00" flour 72.96 g water 29.19 g water + 3.24 g Kansui

00 Kansui Cricket 170.46 g "00" flour 72.96 g water 29.19 g water + 3.24 g Kansui 24.12 g cricket powder

BS 00 Control 195.5 g "00" flour 48.5 g cold water

BS 00 Cricket 177.11 g "00" flour 48.5 g warm water + 7.5 g Baked Soda 48.5 g warm water + 7.5 g Baked Soda 48.5 g cold water 18.39 g cricket powder

5.3.2.1 Texture trial 8: The effect of steaming before drying.

As some noodles are sold part-cooked by steaming prior to drying, this trial was

designed to imitate that process by steaming noodle samples using a Thermomix

Varoma module (Vorwerk, Berkshire, UK) in order to be able to control the temperature.

After steaming for 2 minutes the noodles were then dried using the l'equipe filterpro

dehydrator (Seoul, Korea) for 24 hours at 70 °C.

BS 00 Control 195.5 g "00" flour 48.5 g warm water + 7.5 g Baked Soda 48.5 g cold water

BS 00 Cricket 177.11 g "00" flour 48.5 g warm water + 7.5 g Baked Soda 48.5 g cold water 18.39 g cricket powder

5.3.2.2 Texture trial 9: Additions of other ingredients utilised in literature.

A literature search (see chapter introduction) revealed that there had previously been a range of research that considered additional ingredients that could improve the texture of noodle products. These ingredients were included into the baked soda cricket recipe by way of further wheat flour reduction in order to determine what effect they may have on the texture of

the noodles.

BS 00 Cricket

177.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket powder

<u>1% salt</u> 174.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 3g salt

2% salt

171.1g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 6g salt

0.25% Guar

176.36 "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 0.75g guar gum

<u>1% guar</u>

174.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 3g guar gum

0.5% xanthan

175.61g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 1.5g xanthan gum 0.5% salt

175.61g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 1.5g salt <u>1.5% salt</u> 172.6g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 4.5g salt

<u>1% egg powder</u>

174.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 3g egg powder

<u>0.5% guar</u> 175.61g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 1.5g guar gum

<u>0.25% xanthan</u>

176.36 "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 0.75g xanthan gum

<u>1% xanthan</u>

174.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 3g xanthan gum

<u>1% chia</u>

174.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 3g chia powder

<u>5% chia</u> 162.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 15g chia powder

2% gluten

171.1g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 6g gluten powder

<u>1% oil</u> 174.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 3g oil <u>5% oil</u> 162.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water

2% starch

18.39g cricket

15g oil

171.1g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 6g starch

<u>2% chia</u> 171.11g "00" flour 48.5g warm water + 7.5g baked Soda 48.5g cold water 18.39g cricket 6g chia powder

<u>1% gluten</u>

174.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 3g gluten powder

<u>5% gluten</u>

162.11g "00" flour 48.5g warm water + 7.5g Baked Soda48.5g cold water 18.39g cricket 15g oil

<u>2% oil</u>

171.1g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket 6g oil

1% starch

174.11g "00" flour 48.5g warm water + 7.5g Baked Soda 48.5g cold water 18.39g cricket3g starch <u>5%</u> starch

162.11g "00" flour 48.5g warm water + 7.5g Baked Soda48.5g cold water 18.39g cricket15g starch

5.3.3 Analysis

5.3.3.1 Texture analysis

Texture analysis for each trial was carried out in triplicate (technical replicates) on a Stable Microsystems TA XT texture analyser (Godalming, UK) using the pasta firmness protocol AACC (16-50) using a 5 kg load call calibrated with a 2 kg weight prior to use. In this protocol, 5 strandsof each noodle were placed under the Perspex light blade which cuts through the noodleusing increasing force to determine the overall firmness as the amount of force taken to cut through the noodles.

5.3.3.2 Nutritional estimation

Nutritional estimation for the final control and cricket samples was created using Recipe Professor software (Point74, Bourne, UK). Nutritional data that was not a part of the nutritional declaration for the ingredients was taken from the McCance and Widdowson database held within the Recipe Professor Software. Mineral data was taken from the results for oven dried silent crickets from chapter 2 as these are the same species and a similar production process as the commercial sample.

5.3.3.3 Pricing Estimation

The estimation of pricing for each of the final noodle recipe was calculated in the Recipe Professor software using the pricing for the ingredients as though they were purchased from retail sources.

5.3.3.4 Nutritional claims

Nutritional claims were developed through comparison to the requirements outlined in the Annex of Regulation (EC) No 1924/2006, lastly amended by Regulation (EU) No 1047/2012 (European Commission, 2012).

5.3.3.5 Scanning Electron Microscopy

Scanning electron microscopy was utilised to image the microstructure of the dried noodles (Dried at 70C in the l'equipe filterpro dehydrator for 24 hours) in order to see the effect of cricket powder particulates on the physical structure of the noodles. Images were produced on a JEOL 6060LV SEM (Tokyo, Japan) after being coated with gold dust (<15nm) using an argon plasma sputter coater at the Nanoscale and Microscale Research Centre. The noodle was broken to reveal the internal structure (left) and the surface structure (right) on each image.

5.3.3.6 Microbial absence testing

Agar plates were produced following the manufacturer's instructions inside a laminar flow cabinet that had been cleaned with antibacterial wipes and transferred to a cleaned sealed container and refrigerated to ensure minimal risk of contamination prior to work beginning.

Two-gram samples (using the BS 00 Cricket formulation detailed above) of raw noodles, cooked fresh noodles and noodles that were dried at 70C in the l'equipe filterpro dehydrator for 24 hours were diluted in 18ml MRD (Oxoid CM0733) followed by serial dilution down to 10⁻⁵. Samples on PCA (Sigma 70152) were plated using Miles Misra; down to 10⁻⁵ while the samples on MAC, RBC & MRS (Sigma M7408, Sigma 17211, Sigma 30912) were spread plated at 10⁻¹ in triplicate. PCA plates were incubated at 2 different temperatures (25°C and 35°C) for two days both aerobically and anaerobically using sealed containers with Anaerogen patches (Oxoid AN0035) and anaerobic indicator paper (Oxoid BR055). MAC plates were incubated at 35°C for 2 days, MRS was incubated in a low oxygen environment at 30°C for 2 days followed by a further day at 22°C and finally RBC was incubated at 25°C for 5 days before being analysed for growth.

Presence of Salmonella was analysed using standard methodology for determining absence in 25g of sample. Twenty-five grams of each sample was added into 225ml of buffered peptone water (Oxoid CM0509) in duplicate and incubated at 35°C overnight. 0.1ml of each sample was added into Rappaport-Vassiliadis (RV) enrichment broth (Oxoid CM0669) and incubated for 42°C for 48 hours. This was then streaked onto Xylose Lysine Deoxycholate agar (XLD) (Oxoid CM0469) in triplicate and incubated at 35°C for a further 24 hours before being checked for the biochemical signs of presumptive salmonella.

The results are compared to the IFST microbial criteria for foods (Dried foods, to be cooked) in order to define safety (The Instutute of Food Science and Technology, 1997).

5.3.3.7 Colour

A Hunter Lab Colour Quest XE spectrophotometer (Murnau, Germany) was used in the analysis of the noodles colour. Prior to use, the instrument was calibrated using the black and white calibration panels. Samples were scanned three times inside clear plastic pouches, moving the scanning point each time to ensure an average would be representative of the entire noodle.

Example colour images are produced by entering the average L* a* b* values to a paint producing website (E-Paint, 2019). As such, these are only meant as a visual representation.

5.3.3.8 Water activity

Water activity was measured on an Aqualab 4TE (Munich, Germany). To ensure functionality of the machine, pure water was run before and after a set of samples as a control with a reading of 0.95 or lower being cause for recalibration.

5.3.3.9 Statistical Analysis

The statistical analysis of the texture analysis results were carried out by one-way ANOVA followed by a posthoc Bonferroni using Genstat (VSN International, Hemel Hempstead, UK). Lettering at the top of each figure represents the Bonferri grouping results.

5.4 Results5.4.1 Texture Analysis

5.4.1.1 Texture trial 1: Control sample identification

Both of the handmade control samples (Fig 81) required more force to cut through than the commercial controls suggesting they may be too hard at this stage. Statistically, the kansui control noodle is different from the STW and soba noodle controls and the 3 alkaline noodles are similar (BS control, Kansui control, commercial control 2) (P = 0.002). Physically the differences were noticeable as the Kansui noodle was harder to rollout consistently without it breaking up.

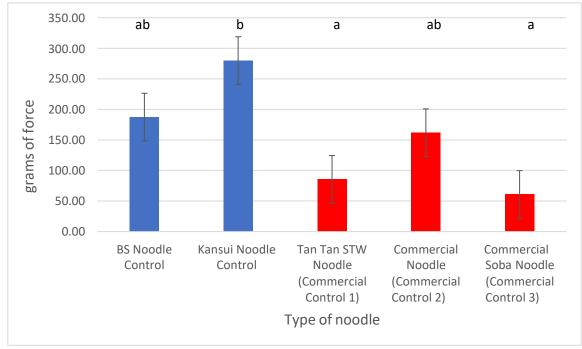


Figure 81: Texture analysis: AACC (16-50) - Average peak force required to cut through cooked control samples (n=3 technical replicates, 2 biological replicates)

5.4.1.2 Texture trial 2: Cricket powder addition

Comparing the results from the control samples made previously, the Kansui noodle with the cricket addition was considerably softer than the control while the baked soda with cricket required much more force to cut through it when compared to the controls. The inclusion of the cricket to the Kansui noodles brought theforce required to cut in line with the commercial samples. Statistically the 3 alkaline controls are similar (P <0.001). The baked soda cricket sample is statistically similar to both of the handmade controls but only the Kansui cricket sample is similar to the 3 commercial controls.

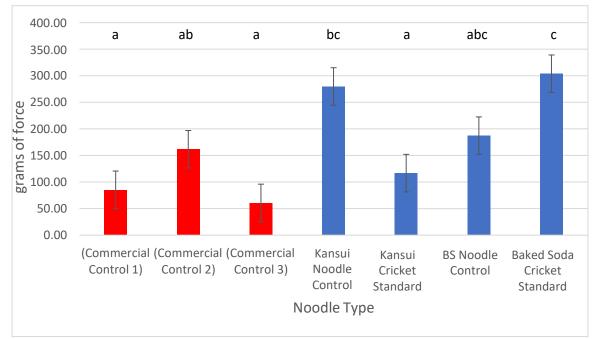


Figure 82: Texture analysis: AACC (16-50) - Average peak force required to cut through cooked handmade cricket noodles (n=3 technical replicates, 2 biological replicates)

Texture trial 3: Alkali quantity

All control samples required more force than commercial equivalents. Increasing the Kansui concentration in the control samples appears to make a difference but little changes in the cricket samples. A decrease in the baked soda (BS) concentration brings the BS cricket sample closer to commercial control 2. The physical texture of the 7.5g BS sample was the easiest to produce uniform noodles from. Lowering the concentration of baked soda in the cricket samples has resulted in those being similar to commercial control 2.

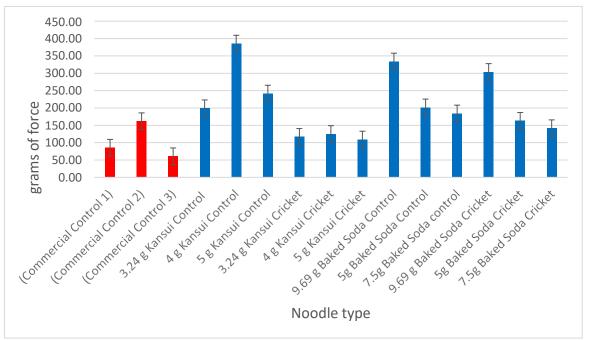


Figure 83: Texture analysis: AACC (16-50) - Average peak force required to cut through cooked handmade noodles, varying concentration of alkaline ingredients (n=2 technical replicates)

5.4.1.3 Texture trial 4: Flour type

A change to a flour with higher protein than the plain flour and smaller particles, is successful in increasing the bite force required to cut the BS noodle however there was a small decrease in the Kansui noodle. Statistically this flour change made it the only sample in this test similar to commercial control 2 (P <0.001).

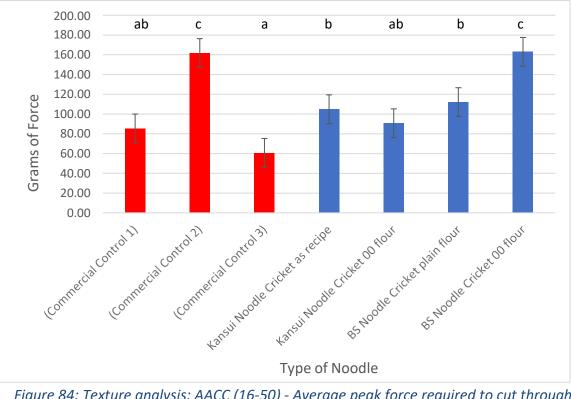


Figure 84: Texture analysis: AACC (16-50) - Average peak force required to cut through cooked handmade noodles, varying the type of wheat flour (n=3 technical replicates)

5.4.1.4 Texture trial 5: Comparison to other protein powders

The higher force required for the rice flour sample shows that the softness of the insect noodles is not simply caused by gluten absence. Cricket appears to perform better than the most of the other protein additions with the exception of buffalo worm. Statistically though, the cricket powder sample is similar to all of the conventional protein samples except for the brown rice (P <0.001). The cricket noodles are texturally different to the buffalo worm samples which are statistically similar to the rice flour sample.

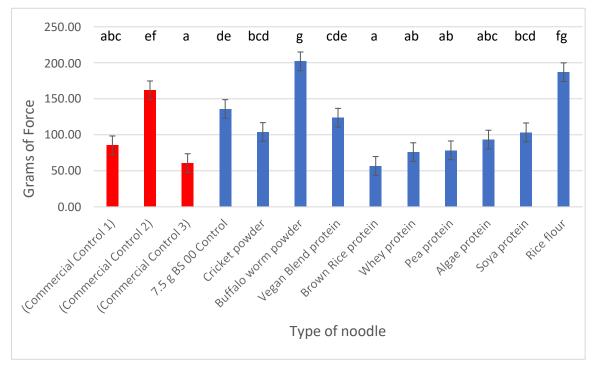


Figure 85: Texture analysis: AACC (16-50) - Average peak force required to cut through cooked handmade noodles, varying the protein ingredient (n=3 technical replicates)

5.4.1.5 Texture trial 6: Cricket powder particle size

The table below demonstrates a wide variation in particle sizes within the cricket powder. In the sample tested, particles under 75 microns made up the largest individual fraction but particles over 125 microns made up over half of the sample. 2.3g of the cricket powder was unaccounted for after the sieving.

Expected particle size	Weight of the powder recovered
(microns)	(g)
600+	0
355-600	0.5
>250 <355	25.1
>125 <250	26.1
>106 <124	5.8
>75 <105	11.6
<75	28.6
Total	97.7

Table 15: Particle size distribution of the fine grade commercial cricket powder

The force required to cut through the noodle does vary based on the particle size of the cricket powder fraction however the results are inconsistent. Increasing the cricket particle size results in a steady increase of the force required for the texture analysis machine to cut through the noodle but there appears to be a limit to this at the top end where there is a decline. Statistical analysis shows that a step change in particle sizes of 100 microns or more is what is required to make a definite difference to the texture of the sample (P <0.001). The fine grade powder shares similarity to the <75 micron powder fraction but the coarse grade doesnot however both commercial powders share a similarity with the highest particle sizefraction (355-600 microns).

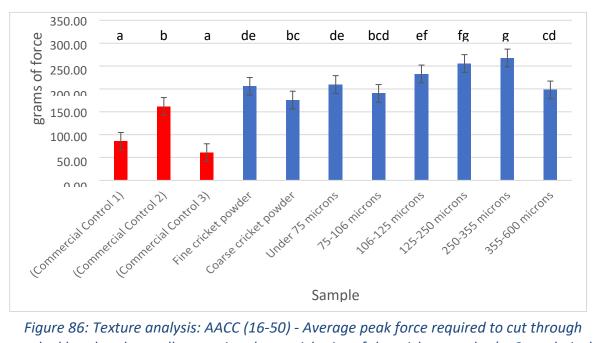
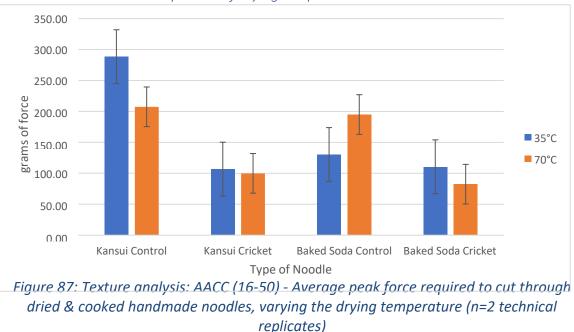


Figure 86: Texture analysis: AACC (16-50) - Average peak force required to cut through cooked handmade noodles, varying the particle size of the cricket powder (n=3 technical replicates)

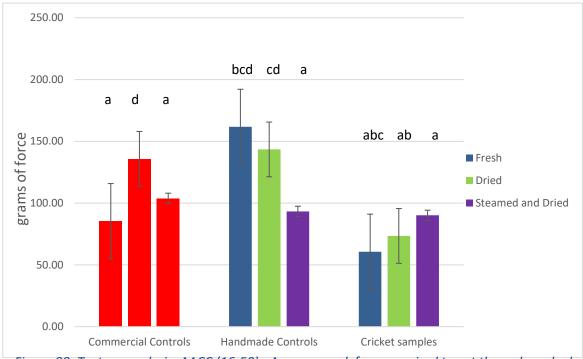
With the exception of the baked soda controlsample, a higher drying temperature made the noodles require less cutting force. The control samples dried at 70C were the most similar to commercial control 2 with the exception of the baked soda cricket sample which was softer. Drying at 35C made the cricket samples similar texturally to commercial control 1 but the differences between drying temperatures in the crickets samples are very small and covered by the potential error.

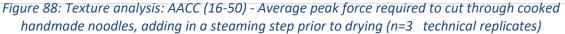




5.4.1.7 Texture trial 8: The effect of steaming before drying.

Steaming prior to drying resulted in softer noodles in both comparisons, although the difference is minimal for the cricket samples. This is confirmed statistically as those noodles which are steamed prior to drying end up only statistically similar to the softer texture of commercial control 1 & 3 (P <0.001). The fresh and dried baked soda control remains the most similar to commercial control 2.





5.4.1.8 Texture trial 9: Additions of other ingredients utilised in literature.

When compared to the 7.5g BS 00 Cricket sample, the addition of salt or egg powder reduced the force required to cut through the noodles, although the amount of salt did not cause much variation. Guar gum, 1% xanthan gum and 5% additional gluten powder increased the force required to cut through the noodles. Additional oil made little difference to the noodles with the exception of the 5% oil version as this required the lowest force of the samples being compared here. The addition of different starch concentrations also caused a decreasing amount of force required to cut through the noodle as the starch quantity was increased. Additions of starch, 2% oil, 2-5% chia powder, 1-2% gluten, up to 0.5% xanthan resulted in noodles that were statistically similar to commercial control 2 (P <0.001). A higher level of gluten (5%) resulted in a noodle that was statistically different to any other sample in this trial.

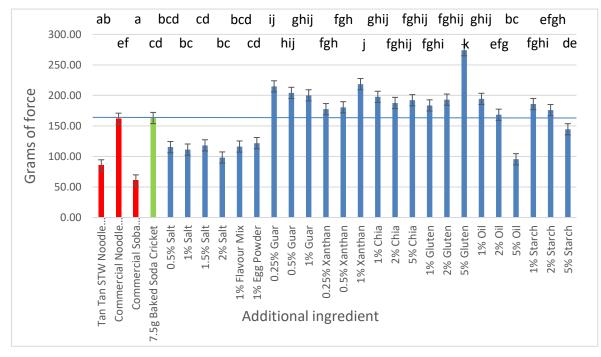


Figure 89: Texture analysis: AACC (16-50) - Average peak force required to cut through cooked handmade noodles containing cricket powder, trialling additives (n=3 technical replicates)

5.4.2 Nutritional Estimations

The use of Kansui instead of baked soda provides less sodium making it healthier nutritionally. The inclusion of cricket powder greatly increases the protein and mineral provision of the noodles compared the controls. The fat content increases with the addition of crickets but not by an amount which would be significant to nutritional health.

	Standard	Standard	Baked Soda	Baked Soda	7.5 ~ 0.0
	Standard Kansui	Standard Kansui	Standard	Standard	7.5g BS 00
	Control	Cricket	Control	Cricket	Cricket
Energy (KJ)	474.02	957.23	934.27	946.63	960.40
Energy (kcal)	111.79	225.94	220.31	223.38	226.64
Fat (g)	0.46	1.42	0.84	1.27	1.34
Saturates (g)	0.07	0.45	0.13	0.37	0.37
Monounsaturates (g)	0.03	0.24	0.33	0.30	0.06
Polyunsaturates (g)	0.20	0.68	0.39	0.60	0.61
Trans Fats (g)	0.00	0.00	0.00	0.00	0.00
Carbohydrate (g)	22.31	40.56	45.56	41.74	40.96
Starch (g)	21.89	39.11	44.65	40.41	39.68
Sugars (g)	0.42	0.78	0.90	0.82	0.77
Fibre (g)	1.01	2.43	2.00	2.32	2.34
Protein (g)	4.10	11.51	6.64	10.09	11.53
Salt (g)	0.00	0.07	2.22	2.27	1.77
Sodium (g)	0.00	0.03	0.89	0.91	0.71
		Miner	als		
Potassium (mg)	42.34	169.85	96.66	153.94	143.23
Magnesium (mg)	10.10	20.63	12.89	17.64	24.28
Phosphorus (mg)	39.09	130.03	70.88	114.62	121.33
Iron (mg)	0.68	1.88	1.29	1.72	1.80
Copper (mg)	0.06	0.62	0.10	0.50	0.51
Zinc (mg)	0.29	1.81	0.39	1.44	1.62
Chloride (mg)	20.19	43.56	52.19	47.24	36.61
Manganese (mg)	0.23	0.54	0.39	0.49	0.56
Selenium (µg)	0.98	5.29	1.29	4.23	4.84
lodine (μg)	0.00	22.72	6.44	19.81	13.98
Calcium (mg)	45.60	95.96	90.21	94.15	95.17

Table 16: Nutritional estimations table of the 4 starting formulations and the final			
formulation (7.5g BS 00 cricket)			

5.4.2.1 Nutritional Claims

The final formulation (7.5 g BS, 00 cricket) would meet the requirements to be a source of phosphorous, zinc, manganese, thiamine, and niacin while also being considered high in copper and protein.

5.4.3 Pricing

The estimated cost for the ingredients in each recipe is as follows:

 Table 17: Cost of the noodles - Retail cost of the commercial controls and the combined

 retail cost of the ingredients for the handmade noodles

Type of noodle	Retail Price or cost of ingredients (Per KG of noodles)
Commercial Control 1	£5.00
Commercial control 2	£5.12
Commercial control 3	£7.00
Baked Soda Control	£0.65
Kansui Control	£0.58
7.5 g Baked Soda Cricket	£1.78
Kansui Cricket	£2.01

5.4.4 Scanning Electron Microscopy

Figure 99A shows a smooth external texture (right) with uniform internal texture (left) whereas Figure 99B shows exoskeletal fragments of the cricket are visible disrupting the structure of the gluten network which is detrimental to the texture of the noodles.

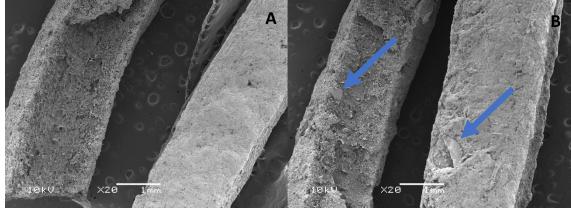


Figure 90: (x20 magnification, 1mm scale bar) scanning electron microscope image of the standard baked soda noodles. A = Control, B = Cricket. Left = internal, Right = External view of the same noodle

The same samples presented in Figure 99 were subjected to higher magnification, micrographs of which are shown in Figure 100. Figure 100A shows the uniform gluten network of the control sample entrapping starch granules. Figure 100B shows frayed protein strands that appear consistently throughout the cricket samples but not in any of the imaged control samples.

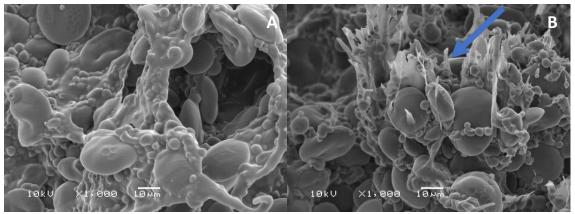


Figure 91: (x1000 magnification, 10µm scale bar) scanning electron microscope image

The frayed protein strands remain visible after further processing (Figure 101).

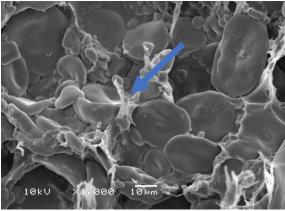


Figure 92: (x1000 magnification, 10µm scale bar) scanning electron microscope image of cricket containing baked soda noodles dried after steaming.

The shards of cricket carapace that are visible appear to create gaps in the structure of the noodles potentially making the noodle more fragile and prone to breakage overall.

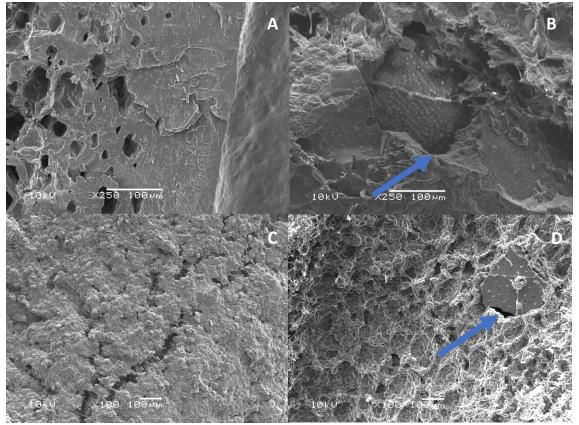


Figure 93: Scanning electron microscope images of cricket containing noodles. A = (x250 magnification, 100 μ m scale bar) steamed kansui control, B = (x250 magnification, 100 μ m scale bar) steamed Kansui cricket (x100 magnification, 100 μ m scale bar) 7.5g baked soda

5.4.5 Microbial absence testing 5.4.6

Lack of growth on MAC agar demonstrates absence of Gram-negative bacteria. Absence on MRS demonstrates the lack of lactic acid fermentative bacteria. Absence on RBC demonstrates that there are no yeasts or moulds present within the sample. Salmonella absence ensures that products comply with legal requirements that food has no salmonella presence within 25g of the product. Total viable counts on PCA yielding below countable levels demonstrates a very minor bacterial presence. These results would demonstrate compliance with the standards outlined by the IFST for conventional noodle products.

Table 18: Results of the microbial absence testing of the cricket containing baked sodanoodles (n=3 technical replicates)

	Raw	Cooked	Dried
MAC	Absent	Absent	Absent
MRS	Absent	Absent	Absent
RBC	Absent	Absent	Absent
Salmonella	Absent	Absent	Absent
PCA 25C	Below countable levels	Below countable levels	Absent
PCA 37C	Below countable levels	Below countable levels	Below countable levels
Anaerobic PCA	Below countable levels	Absent	Below countable levels

5.4.7 Water activity

The drying process is successful in lowering water activity to below the levels required to prevent microbial growth within the noodles.

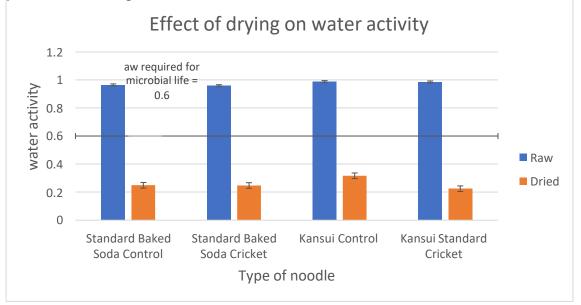


Figure 94: Water activity of fresh and dried noodle samples

5.4.8 Colour

The addition of crickets clearly affects the colour of noodles, changing them from a

lighter almost yellow colour to a dark, dull brown/grey.

Table 19: Colour comparison between control and cricket containing noodle samples.

Noodle	L*	a*	b*	Colour
Kansui Control	58.66	0.85	14.48	
Kansui Standard Cricket	35.3	3.11	5.68	
Baked Soda Control	53.01	0.31	13.91	
Baked Soda Cricket	36.85	2.49	4.03	

5.5 Discussion

The steps taken in the textural analysis section of this research demonstrates that it is possible to produce noodles where the primary hedonic attribute (texture) can be considered similar to good quality commercial products when tested in ananalytical setting is a positive step forward as the majority of the other hedonic attributes of noodle dishes are derived from how the noodles are prepared and what other ingredients they are served with. However, there is still a way to go and more testing that can be done.

As alkaline noodles are preferred in some areas of Asia due to their chewiness, comparing the noodles produced in this research against other types of noodles of a softer texture provided grounding for comparison (Figure 81). The cricket noodles needed to be similar to commercial control 2, and generally not as soft as the other 2 commercial controls. Meeting that criteria with a handmade control demonstrated that it was possible to produce good quality noodles with the ingredients and facilities available in bench scale trials. The first addition of cricket powder revealed that it would not be a simple task as this addition resulted in a textural change (Figure 82). The baked soda cricket sample became very hard and difficult to work while the kansui variant became softer. This difference is possibly due to the difference in composition of the alkali's. Kansui is a mix of potassium and sodium carbonates while the baked soda is just sodium carbonate. There is also the small difference in pH and concentration that may be a reason for the differing results (Table 15). With that result in mind, determining if the quantity of alkali in the formulation made a difference to the texture revealed that this is a key change which can be made to make the texture comparable to the commercial samples (Figure 83). As highlighted during the introduction, Kansui is not a common ingredient to the western world so the baked soda as an alternative would be more readily available and producible here in a simple way that would not affect the "natural" perception of the noodle. In addition, buying Kansui in the UK is expensive and the noodles need to remain similar in cost in order to be commercially viable. Further to this, baked soda as an alternative appears to produce cricket powder noodles that are closer to the commercial control when using the texture as the metric. Lowering the baked soda in the formulation from the 9.69 grams used in the initial recipe to 5 grams or 7.5 grams resulted in cricket samples that were statistically similar to commercial

control 2. These changes are likely to be introduced by the changes of alkaline concentration within the dough as there would beless available to induce the different bonding structure within the gluten network. By changing the type of flour used it was believed that the quality of noodles could be further improved from their base formulations. A change in the flour from plain flour to 00 flour did result in an increase in the force required to cut through the baked soda noodles but not the Kansuias statistical analysis revealed that only the baked soda that used 00 flour (Figure 84).

When making noodles that included other high protein powders, the results for the cricket were statistically similar to most of the other protein powders but not to buffalo worm or rice flour. The primary physical difference between the cricket powder and the buffalo worm that could cause this difference is the buffalo's worms thinner and softer chitinous exoskeleton (the significance this component is highlighted during microscopy). Another factor may be the composition of the powder as the buffalo worm powder is higher in fat than the cricket powder used (29 g / 100 g of fat in the buffalo worm vs 17.5 g / 100 g in the cricket). There may also be differences in the processing of the powder that yielded this result such as the particle size of the powder. The difference to rice flour provides evidence that the textural change triggered by the inclusion of cricket powder is not simply the by-product of the decrease in available gluten proteins as the rice flour also provides no gluten proteins (Figure 85). The small differences between the proteins tested and the fact that the brown rice protein noodle is different to the rest suggests that the functionality of the protein may be responsible. The particle size of the cricket powder provides a clear change in that up to a point, a higher particle size of the powder increases the force required to cut through the noodles. The differences between the samples are small but statistically significant (Figure 86). The important part of this result for the manufacturers of the powder is that the milling process they use does not need to result in absolutely uniform powder to provide similar results as the fine powder yielded noodles similar to those that only contained the smallest particles. The drying methodology used within the study yielded no consistent results that could be used to determine whether one drying temperature was better than another (Figure 87). Frying to dry would be worth trialling to see if anything is learned about the quality of noodles

produced but as the aim of the product is to focus on nutrition, a high fat content as a result of that process would not be conducive to the aims of the project. Steaming prior to drying resulted in softer noodles which is expected as the cooking process has already been started during the production process and the noodles are then cooked again afterwards when preparing them to eat (Figure 88). The interesting result is that the change in texture is quite large in the control sample but minimal in the cricket samples. This difference may result from the differing physical properties such as the hydration capacity of the wheat flour when compared to the cricket powder but withoutfurther examination of the samples this is only speculation (Berton *et al.*, 2002; Stone, Tanaka and Nickerson, 2019).

Testing various additives resulted in several that could be used to ensure that the cricket noodles meet the textural qualities of other alkaline noodles (Figure 89). There appears to be optimal quantities or even limits to the quantity that these additives could be included in order to gain the benefit they provide to the texture though. For example, the smaller additions of powdered gluten could be useful to replace what is lost through the process of swapping wheat flour for cricket powder but adding too much made the noodles much harder to cut through than the commercial control samples. The literature surrounding an addition of salt suggested that it would either make little difference or be an improvement, however in this case it made the noodles significantly softer which is theorised to be a detriment to the product quality. A further note to this success, texture was only measured in one way and although that is the standard way of analytically defining similar products, it does not overwrite the findings a conclusive sensory study would have. As literature has demonstrated that the denser texture is what is favoured by consumers of this type of noodle it may be a possibility to use the findings discussed above to make noodles which are favoured hedonically over commercial control 2, by optimising the recipe to achieve that texture. However, when considering using the additives in particular, the marketability of such things for a western consumer would have to be considered as "naturalness" may be one of the key marketable attributes of a high protein noodle that uses insects over some of the other types of high protein powders. One previously studied additive that was not tested

during this study could be interesting though. As presented during the introduction, transglutaminase provides structure through crosslinking cricket proteins. However, as the final testing reached the point where the product was texturally similar to the control samples, it is unlikely this is necessary, and the ingredients list should be kept as short as possible for marketing purposes as this maintains that "natural" appeal. Antioxidant ingredients may be worthwhile considering for the noodles because fat rancidityis likely to be the primary factor that determines shelf life in dried noodles due to the quantity of polyunsaturated fatty acids in the cricket powder (see results from Chapter 2).

A further downside to the incorporation of cricket powder is the dramatic colour change of the noodles when compared to the control samples (Table 19). The colour was highlighted as an important aspect for Asian consumers of alkaline noodles who are expecting a bright yellow colour. It is possible that the colouration of these noodles may be acceptable though as the colour of the cricket powder noodles are reminiscent of whole grain products or the soba noodles which could help convey a healthy perception. Although modifying the colour of insect powder during production forms a branch of research that is in progress so this may change in the future (R. Janssen *et al.*, 2017).

The goal of improving the nutrition of alkaline noodles through the incorporation of insect powder is achievable (Table 16). The noodles are designed specifically to meet the criteria for a high in protein claim, but this formulation would also allow for several beneficial mineral claims with several others sitting just below the level required to communicate this to the consumer on the label. From a commercial standpoint, it may be worth slightly modifying the recipe further to bring those other micronutrients up to relevant levels. Research into the vitamin content of insects is still very limited but the previous studies into this area would suggest that valuable quantities of vitamins are provided by the cricket powder (Kouřimská and Adámková, 2016). If there are useful quantities of vitamins present, this would add further value to both consumers and to aiding with malnutrition.

However, there is a trade-off to be considered when it comes to which alkaline ingredient is used in the production of these noodles. As baked soda contains high levels of sodium, it may pose a health risk if consumed in large quantities. Kansui does not have the same risks but it is more difficult to acquire, costs more, and does not appear to produce the same quality of noodles as the baked soda does. Although this was not tested in the study, there are likely limits to which the replacement of wheat flour can take place due to the practicalities of needing the gluten that the wheat flour provides in order to form the structure of the noodle. At the level required to reach a "high in protein" labelling claim, the texture was already being affected compared to the control and as such other methods of mitigating that textural difference had to be considered. It is possible that through the use of the strategies outlined in the textural analysis, other fortifications in addition to the cricket powder could be considered to further benefit both the commerciality of the noodle to a western consumer and also provide further benefit to the malnourished. An overlooked side effect of making this style of noodle is that the protein from the cricket powder is potentially solubilised by the high pH of the alkaline ingredients (Zielińska, Karaś and Baraniak, 2018). While this has been noted to not affect the functionality in a way that is likely to have changed the noodles characteristics, this may result in differences in the digestibility of the protein (Zielińska, Karaś and Baraniak, 2018). Whether this is a positive effect through increasing digestibility and making the protein more available during digestive transit or if the formations of those other amino acids presented in the introduction would decrease the nutritional potential would need to be tested.

The ingredient costs are less than half of the retail prices for other fresh high protein noodles (£4.17 per kg) so there should be potential for profitability after considering production, packaging and transportation costs (Morrisons, 2020). They would cost more than conventional noodles but potentially less than others that are sold on health virtues such as high protein (Table 17). This estimated pricing is based on the retail pricing of the ingredients though so there will be economies of scale to consider during manufacturing that would bring the price down considerably.

As highlighted by the texture study replacing wheat flour with powdered cricket appears to be a trade-off of nutritional gain for product quality loss as the wheat flour provides functional properties that the insect powder lacks. However, as the change in texture from replacing wheat flour with a protein addition is seemingly greater than that caused by rice flour, it suggests that the protein addition affects functionality more than simple gluten loss. Under electron microscopy (Figure 90-93), it would seem that the inclusion of insects causes some structural differences. Where larger pieces of carapace end up in the noodle matrix, the gluten strands do not seem to form normally, when directly compared to the standard noodle samples which could be one of the reasons the dried noodles were brittle. The theory proposed through discussions with experts in food microscopy at the NMRC is that this could be caused byboth parts having similar electromagnetic charges causing them to repel, preventing thebonds and ultimately leading to the softer texture. The views of the frayed protein strands highlighted in figures 91 and 92 as well as the gaps visualised around fragments of cricket exoskeleton in figure 93 would support this idea.

The microbiology results confirm the safety of the noodles (Table 18). Interestingly they appear to have less microbial growth than the insect powders tested during chapter 3. This could be linked to the alkaline ingredients used during the noodles production but also the additional cooking steps (boiling) involved in making the noodles ready to consume. Even though the drying methods used in this study require further optimisation, they achieved the goal of reducing the water activity of the noodles below what would be able to support microbial growth. This ensures that the cricket noodles should have a long shelf life like the conventional version.

5.6 Conclusions

Ultimately, the end result were noodles that are comparable to commercial equivalents in texture when produced simply during bench scale trials. However, despite this success, the colour may be a further barrier when considering product development using cricket powder. This may make them less desirable in a retail setting currently, but when presented in food service, the toppings decide the majority of the important quality aspects of the finished food and would often disguise that colouration.

The noodle from an analytical point of view seems viable but it is uncertain how much emphasis consumers would place on the colouration of the noodle. It is entirely possible this would make the attempt moot, but this could only be answered with extensive consumer trials. The important message here is that the study shows that incorporating the powdered crickets does affect the physical qualities of food products but there are ways of adjustment that can be used to bring them in line with commercial samples. Similar studies would need to take place for the other products being used to promote entomophagy in order to find ways of making quality improvements to the pointthat they are comparable hedonically.

Good product quality is going to be hard to achieve without research. Conventional foods have developed over a long period of time to the point we have an almost innate knowledge base that we draw from when producing those foods as well as vast resources available on every media platform. This doesn't exist yet for insects and shoehorning the powder into existing product types by direct replacement needs careful thought as the result is almost always different, due to the mechanical and chemical properties of the insects in comparison to the ingredient they are replacing. Not all product types are going to be suitable, and more work needs to be done to find out what the limits are for consumers when trading hedonic quality for nutritional quality.

Chapter 6: Overall conclusions

The aim of this project was to evaluate all aspects of incorporation of insects into the human diet, with a particular focus on what may be acceptable in high-income countries. This started with a detailed study of the impact of production methods (of a variety of species) on nutritional value and safety. The attitudes and likely behaviour of consumer towards products containing insects was then explored before exploring the impact of incorporation of insect powder on the quality of food products. In this chapter the results of each study are integrated to explore the overall challenges and opportunities that incorporation of insect-derived ingredients may offer in terms of improving the sustainability and nutritional value of our food systems.

Chapter 2 investigated the impact of processes on nutritional quality and the primary hypothesis heat treatments would have the largest effect. The results showed that this hypothesis was accurate although the effect was minimal. A detrimental change to the fatty acid profile was the biggest notable effect with a clear indication that this was through high temperature oxidation as the change did not take place in the vacuum oven or the other methods. As drum drying did not have a similar effect, the length of drying time, or the remaining moisture, are likely to be key factors. Some changes to the protein and amino acids were also observed in the oven samples that could be attributed to Maillard with that likelihood increasing as there were also changes to colour and sugar content. The impact on these factors was small compared to that of the species of insect and even when the taxonomy was similar this was the dominant factor when looking to produce food powders from insects.

Each species of insect tested during the study were high in protein with a good mix of essential amino acids when using the WHO/FAO/UNU metrics as a comparison. The high values for crude fibre, polyunsaturated fatty acids and minerals elevate the value of these samples from beyond a simple protein powder to a food source that is more valuable as a complete food. Nutritional differences were noted between the powder of the same species of insect (*Gryllodes sigilatus*) from two different production facilities demonstrating that the feed and rearing environment also likely plays a role as the stage of the development for each sample was the same.

The focus of chapter 3 was the safety of the cricket powders with the hypothesis being that the processing involved in the production of the cricket powder would leave the

initial levels of microflora quite low, but that any surviving microflora could yield pathogenic bacteria. This chapter also considered the potential toxicity of minerals due to literature highlighting insect's bioaccumulation. The hypothesis surrounding this was that the content of the powder itself would not be beyond the safe limits for minerals, but specific diets may pose a risk if a high level of insect powder was introduced to it. Hot drying processes did contribute to the decrease in microflora and the samples would meet the safety standards laid out by the IFST and FASFC. Pathogenic bacteria were found when the microflora of the commercial samples were DNA identified, indicating that the hypothesis is true overall, as these organisms are very difficult to process out of food products. The mineral toxicity hypothesis also holds true as none of the samples had a mineral that reached the upper limits, but the copper, aluminium, and zinc in particular, could be a concern for diets of individuals who may consume large amounts of powdered insects as a form of protein.

Overall, this chapter maps out the impact of the processing steps on the microflora as well as highlighting what the risks could be if care was not taken when utilising the powder as an ingredient for a food product. Although the methodology used when producing cricket powder did produce a powder that was safe to consume using the metrics provided by the IFST and FASFC, there were key areas of concern. The sample taken from the commercial cricket powder was found to contain 2 relatively novel bacteria which highlights the importance of more work being carried out to understand the microflora of insects.

The questionnaire used in Chapter 4 aimed to understand the expectations which may be attached to the idea of eating insect-based foods. It had several hypotheses attached to it, including that emotions surrounding fear and disgust would be the most prominent, people would assume unfamiliar cuisines are the most appropriate for the utilisation of insects due to inherent biases, dry fine powders will be the most appropriate way to consume insects and that insects in general will be expected to be result in poor quality foods.

The first of these was not entirely accurate as the dominant emotions were curiosity with fear being more represented as apprehension and nervousness which still appear relatively positive. Disgust was secondary to these. The hypothesis suggesting that bias may influence the expected cuisine pairings appears to be more nuanced than expected. There are elements to indicate that this may be true due to the relatively high expected appropriateness of obscure cuisines such as Peranakan but this may be more influenced by participant knowledge as they chose regions that typically do consume insects. On the flip side though, cuisines with a high familiarity were rated very low for appropriateness further adding to the evidence for this hypothesis to hold up. As predicted, dry powders were preferred to alternative formats of consuming insects but as this has a large amount of literature backing, it was not unexpected. Indications that the participants believed insect-based foods to be inferior to their traditional counterparts were apparent in questions surrounding pricing and specific food products, demonstrating that this hypothesis is accurate and one of the key barriers to insect acceptance.

The promotion of entomophagy may not require a product that ticks every box, just enough to make it valuable enough to consider comparable or better than the currently available options. There are clear red lines drawn by the participants of the survey on price and format of the insect-based foods with further insight into what products may be appealing. Inherent biases towards insects were notable in some of the questions but the questionnaire results showed that there was potential to utilise these biases in a positive way. Although this was not the path that this study took in chapter 5, an alternative approach could see the product development segment leaning into the expectations and biases and creating completely novel insect-based foods utilising the unfamiliar flavours that were expected to pair better than what is typically being tried now with insects. This may even fit better with some of the psychology models that suggest that using familiar flavours would be harder as consumers already have clear expectations on how products that use those will perform. The fact that the participants primarily met the idea of consuming insects with curiosity over fear and disgust is a positive that indicates there may be less of an issue with the initial willingness to eat if a product targets the correct market.

Solely focusing on the sustainability of insects for marketing purposes only really works if the products are almost identical. Developers of insect-based foods need to do their best to ensure that price, hedonic attributes, the nutritional benefits, the commercial viability, and the sustainability are all, at least, comparable to any conventional alternatives that are in the market if they are to be successful beyond the generation of singular purchases derived from curiosity. The perceived issues of safety need to be openly discussed as there are clear ways of ensuring that insect-based foods are as safe as any other. While it is possible to prove food safety effectively, as insect-based foods must follow the same rules as any other food, talking openly about safety is a double-edged sword as it brings it to the forefront of peoples thought process. So, it is going to be hard to overcome that low safety expectation from the public. Campaigns to this end such as stating that insects are safer, due to the lack of biological crossover, feel out of place as it's not commonplace for any other food to present this information. As such this further enforces insect-based foods as being different when the goal is to normalise.

The theory that the Western consumer may take a generalistic approach to trying insects for the first time may present interesting opportunities as this allows for the products to be presented in a different way compared to the assumption that consumers will be inherently neophobic when approaching the idea of entomophagy. A generalistic consumer is one who will weigh up both products and take home what appears to be the best one so presenting the whole story, from ethics and sustainability to the natural nutritional benefits that insects provide presents them a powerful message that should be difficult to ignore. The products can be marketed towards curiosity rather than using fear as this sets a negative tone for the experience. Long term commercially viable products need to present as good, rather than a difficult challenge to overcome as some insect-based products have been marketed previously. It should be fine to present the idea as new or novel without the idea of it being a scary process to try for the first time. The information being presented with this approach focuses on describing how these products are made better through the inclusion of insects and how the important attributes that consumers enjoy, remain the same. The final part of the study, described in Chapter 5, attempted to demonstrate that cricket powder could be successfully incorporated into a product (noodles) which is highly familiar and acceptable to 'Western' consumers. The hypothesis was that the addition of cricket powder would provide a valuable nutritional increase but at a detriment to quality that is difficult to overcome. This hypothesis appears accurate as the nutrition of the final product was improved over the controls due to the additional protein, minerals and unsaturated fats but additional steps were required to make the

texture similar to the commercial sample. This chapter concludes that it is possible to make good quality food products from insects that should appeal to multiple audiences if formulated correctly. The noodles are not perfect, as the darker colour induced by the addition of crickets may result in a lowered consumer appeal, but the primary aspect of quality noted in literature; the texture, can be matched to similar commercial products. Further studies into consumer acceptance in that area may reveal whether the nutritional benefits of such a product may overcome any aversion to this aspect of their quality. If they would be accepted as aviable option, they should be suitable for helping some of the areas of malnutrition around the world as they are superior nutritionally, could be produced at a price point comparable to other noodle products and they utilise ingredients that those areas of Asia are familiar with.

There are also key findings within the product development that leave this study with open questions. The effect of the chitin shell on the gluten structure of noodles is a visible defect under microscopy that needs to be understood in order to be overcome. It may be as simple as milling the powders finer so that it uniformly blends with the wheat flour without affecting structure but that work to demonstrate this needs to be done. The noodles are effectively a blank canvas which could utilise almost any flavour profiles which were expected to pair well with insects and utilise other ingredients in the meal to generate hedonic qualities that would be appealing to a Western consumer base.

The ideal way forward for insects would be to find a product which is unique and can only be made using insects. This would potentially generate and go some way towards normalising seeing insects as a food as the current feeling from consumers surrounding insects is that there are already better conventional alternatives to what insects are being utilised for. In the end though this research shows that it is difficult, but not impossible, to meet the needs of both the developed and developing world within a single product. A long-term solution could be to focus on making the best products for both situations individually, but the process would be slower as a result, as it would take time for the Western world to accept the new products before they would then consider making appropriate products for the areas of malnutrition which could benefit the most from the inclusion of insects. Presenting the idea in this way, as one solution for both sides allows the process to proceed together rather than as separate issues which may then branch off to be idealised for each community. This way of thinking about the problem could switch the messages surrounding malnutrition from reactive to proactive. As a scientific community it is possible to predict that the Western world may suffer from protein shortages in the future without interventions, but communicating that now through providing alternatives such as insects reframes the issue with a sense of urgency. Messages like the 2050 goals unfortunately present the average person with a distance to overcome and 30 years feels like a long time, whereas presenting this idea as a solution to a current problem now, would add emphasis to the importance of entomophagy

6.1 Strengths, limitations and weaknesses of the study and potential areas of future study.

Although this study is limited to a few of the 2000 potential insect species available for food use, it covers some of the species considered to be the most acceptable to a Western audience. Other insects, including larvae are considered elsewhere and may be among the first to gain approval for commercialisation.

The primary weakness of this study is the limited biological replication, as this limits the conclusiveness of some of the results presented and prevents the discussion around species x drying method interaction. Further replication would be required in order to make definitive conclusions.

Another limitation of this study is that it was not able to fully evaluate the nutritional content. The amino acid analysis is missing tryptophan and as such the results are not strictly able to be confirmed as meeting the figures laid out for a good quality protein. Vitamins are another important category of the nutritional analysis that is missing from this work. As vitamins are likely to be highly affected by the processing methods, these details remain important to determine.

The participants of the survey were not representative of the population as a whole. The convenience sample of students and staff may have biases towards sustainable foods due to the campus's fields of study primarily focusing on food. The age range of the participants are also highlighted as being more accepting to new experiences such as novel foods.

Despite these limitations, the study represents a broad analysis of the impact of both insect species and preparation methods on nutritional value and microbiological safety. Much of this is likely to be broadly applicable to other species. It is all clearly demonstrated that insects (in the form of powder) can be incorporated into food recognisable by the consumer which may represent an early step in promoting more wider consumption. As such the work in this thesis demonstrates considerable potential for the use of insect powder as an ingredient for food consumption in the West and for aiding in malnutrition where it is needed in the world.

One obvious area of future study would be to undertake further direct consumer

acceptance studies by actually getting them to try insect containing foods. Blind sensory studies to confirm that the quality of the noodles is similar to the commercial controls would be a logical next step as this would be the only real way to confirm that the noodles are of acceptable standards organoleptically.

Another potential way in which the worked could be expanded could be to widen the range of foods produced. Perhaps exploring the use of insects in meat replacement products. Widening the species' of insects used would also be beneficial to the knowledge base as a whole.

6.2 Final Conclusion

This work acts as a demonstration that through careful production and development, insects represent a sustainable food source, while simultaneously improving the available nutrition. The concerns over safety can be addressed through careful processing, but it may prove more difficult to alter people's perceptions of the safety of insects as a perception of risk will be a hard barrier to consumption for a lot of people. The way forward appears to be to work with the inherent biases. Make use of them and design products that incorporate them and reframe them as a positive. Clever product design can appeal to both worlds, aiding in malnutrition where it is needed, while simultaneously being a commercially viable, and sustainable, product in higher income countries, where malnutrition is not the primary focus.

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Appendix A: Figures used to calculate protein content

Sample Name	Average Nitrogen %
BADD	11.40
BAFD	11.41
BAOD	11.31
BAVD	11.12
BLDD	10.42
BLFD	9.66
BLOD	8.69
BLVD	9.48
Comm	9.86
HDD	12.03
HFD	12.63
HOD	12.90
HVD	12.69
LDD	9.46
LFD	9.31
LOD	9.33
LVD	9.38
QDD	10.68
QFD	10.41
QOD	10.81
QVD	10.22

Table 21: Nitrogen content of each sample

These were the nitrogen figures used to calculate estimated protein content by using the x6.25 conversion factor.

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mg/100g	В	Na	Mg	Р	S	K	Са	Cr	Mn	Fe	Со	Cu	Zn	Se	Мо	K:Na
BADD	n.d.	252.76	68.07	668.95	483.98	738.51	146.72	0.08	4.13	7.38	0.01	4.93	16.63	0.11	0.03	10.85
BAFD	n.d.	286.26	78.61	735.47	522.87	837.93	157.03	0.04	4.48	7.57	0.01	5.12	16.83	0.12	0.03	10.66
BAOD	n.d.	279.94	77.15	736.87	523.08	823.01	168.14	0.04	4.47	6.62	0.01	5.16	17.65	0.12	0.02	10.67
BAVD	n.d.	270.64	73.46	722.32	519.81	804.66	151.76	0.02	4.39	6.57	0.01	5.24	16.69	0.12	0.03	10.95
BLDD	n.d.	349.88	75.29	713.96	496.79	936.59	124.18	0.07	3.68	13.87	0.01	1.95	19.52	0.05	0.04	12.44
BLFD	n.d.	322.80	75.37	671.59	424.74	891.67	102.35	0.04	3.50	9.04	0.01	1.70	17.81	0.05	0.04	11.83
BLOD	n.d.	344.66	75.22	693.04	442.86	928.68	107.64	0.04	3.50	9.51	0.01	1.76	18.65	0.04	0.04	12.35
BLVD	n.d.	319.39	74.01	704.76	465.48	890.02	114.67	0.02	3.62	14.45	0.01	1.83	20.53	0.05	0.04	12.03
Comm	0.10	333.29	97.48	823.58	459.35	1084.58	204.03	0.06	2.35	9.06	0.01	6.66	17.80	0.05	0.05	11.13
HDD	n.d.	312.44	83.58	781.88	506.04	846.31	114.95	0.11	3.95	7.90	n.d.	3.78	24.62	0.04	0.02	10.13
HFD	0.02	401.85	92.42	899.98	611.21	1065.23	202.47	0.14	4.61	8.67	0.01	3.49	44.62	0.06	0.07	11.53
HOD	n.d.	349.02	81.06	813.56	535.70	931.51	194.26	0.02	4.07	6.68	n.d.	3.11	27.65	0.05	0.03	11.49
HVD	n.d.	331.61	79.19	797.22	521.62	887.55	187.96	0.01	4.07	6.80	n.d.	3.15	28.58	0.05	0.03	11.21
LDD	n.d.	160.25	88.84	695.61	441.01	977.54	164.27	0.31	0.98	11.24	0.01	2.20	9.44	0.02	0.04	11.00
LFD	0.04	131.88	75.04	601.39	382.82	863.40	96.50	0.03	0.67	5.99	0.01	1.93	7.94	0.02	0.02	11.51
LOD	n.d.	138.45	79.40	630.02	391.36	899.78	97.45	0.04	0.67	6.51	0.01	2.22	10.57	0.02	0.03	11.33
LVD	0.03	125.59	75.45	610.11	380.41	846.92	98.80	0.03	0.64	6.19	0.01	2.18	9.98	0.02	0.02	11.23
QDD	0.16	364.55	99.66	839.62	496.61	1105.13	265.73	0.42	5.97	11.67	0.01	2.01	18.66	0.06	0.05	11.09
QFD	0.07	380.89	111.75	868.52	537.04	1013.11	178.36	0.72	5.86	14.60	0.07	1.95	137.50	0.05	0.18	9.07
QOD	n.d.	376.13	91.91	834.62	503.96	983.29	173.68	0.01	5.69	6.57	0.01	2.58	19.57	0.05	0.02	10.70
QVD	n.d.	335.70	88.65	814.16	485.33	914.66	167.26	0.02	5.61	5.83	0.01	1.88	19.81	0.05	0.02	10.32
RNI	1	1600	300	550	n.a.	3500	700	0.2	5	6.7	6E-07	1.2	9.5	0.075	0.3	
SUL / TUL	9.6	2300	400	2400	n.a.	5000	1500	10*	12.2	17	1.4	10	25	0.45	1	

Table 22: Overview of the nutritionally relevant mineral content by ICPMS (mg/100g sample weight)

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mg/100g	Ti	Li	Be	AI	V	Ni	As	Rb	Sr	Ag	Cd	Cs	Ba	TI	Pb	U
BADD	0.16	n.d.	n.d.	3.69	0.01	0.09	0.03	0.42	0.27	n.d.	0.01	n.d.	0.12	n.d.	0.06	n.d.
BAFD	0.19	n.d.	n.d.	4.81	0.01	0.04	0.03	0.47	0.27	n.d.	0.01	n.d.	0.12	n.d.	0.01	n.d.
BAOD	0.13	n.d.	n.d.	3.13	0.01	0.06	0.03	0.46	0.44	n.d.	0.01	n.d.	0.10	n.d.	0.00	n.d.
BAVD	0.11	n.d.	n.d.	3.28	0.01	0.15	0.03	0.45	0.25	n.d.	0.01	n.d.	0.13	n.d.	0.01	n.d.
BLDD	0.06	n.d.	n.d.	3.19	0.01	0.07	0.07	0.59	0.42	n.d.	0.01	n.d.	0.11	n.d.	0.01	n.d.
BLFD	0.04	n.d.	n.d.	2.60	0.01	0.06	0.06	0.56	0.31	n.d.	0.01	n.d.	0.10	n.d.	0.01	n.d.
BLOD	0.06	n.d.	n.d.	2.77	0.01	1.43	0.06	0.58	0.33	n.d.	0.01	n.d.	0.10	n.d.	0.02	n.d.
BLVD	0.06	n.d.	n.d.	4.76	0.01	0.21	0.08	0.57	0.39	n.d.	0.01	n.d.	0.12	n.d.	0.01	n.d.
Comm	0.10	n.d.	n.d.	4.88	0.03	0.09	0.01	1.02	0.29	n.d.	n.d.	n.d.	0.10	n.d.	0.01	n.d.
HDD	0.07	n.d.	n.d.	0.81	0.01	0.06	0.02	0.67	0.35	n.d.	n.d.	n.d.	0.14	n.d.	0.01	n.d.
HFD	0.07	n.d.	n.d.	1.92	0.01	0.10	0.01	0.53	0.52	n.d.	n.d.	n.d.	0.15	n.d.	0.02	n.d.
HOD	0.07	n.d.	n.d.	3.96	n.d.	0.04	0.01	0.46	0.50	n.d.	0.01	n.d.	0.10	n.d.	0.02	n.d.
HVD	0.05	n.d.	n.d.	2.29	n.d.	0.06	0.01	0.45	0.49	n.d.	n.d.	n.d.	0.10	n.d.	0.03	n.d.
LDD	0.05	n.d.	n.d.	0.68	n.d.	0.23	n.d.	0.18	0.27	n.d.	0.01	n.d.	0.11	n.d.	0.02	n.d.
LFD	0.02	n.d.	n.d.	0.43	n.d.	0.10	n.d.	0.12	0.16	n.d.	0.01	n.d.	0.07	n.d.	0.01	n.d.
LOD	0.03	n.d.	n.d.	0.46	n.d.	0.12	n.d.	0.13	0.15	n.d.	0.01	n.d.	0.06	n.d.	0.01	n.d.
LVD	0.03	n.d.	n.d.	0.90	n.d.	0.18	n.d.	0.12	0.16	n.d.	0.01	n.d.	0.06	n.d.	0.01	n.d.
QDD	0.10	n.d.	n.d.	3.54	0.01	0.25	0.04	0.47	0.50	n.d.	0.01	n.d.	0.37	n.d.	0.02	n.d.
QFD	0.08	n.d.	n.d.	2.50	0.02	0.47	0.04	0.48	0.44	n.d.	0.01	n.d.	0.19	n.d.	0.02	n.d.
QOD	0.06	n.d.	n.d.	0.73	n.d.	0.21	0.04	0.46	0.43	n.d.	0.01	n.d.	0.15	n.d.	0.01	n.d.
QVD	0.05	n.d.	n.d.	0.98	n.d.	0.07	0.03	0.43	0.41	n.d.	0.01	n.d.	0.15	n.d.	0.01	n.d.
RNI	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
SUL / TUL	n.a.	n.a.	n.a.	8.5	n.a.	0.26	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.1	n.a.

Table 23: Overview of other mineral content by ICPMS (mg/100g sample weight)

Appendix C: Survey Layout, question purpose and justification for inclusion.

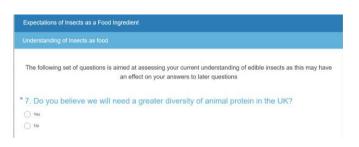
The question numbers presented in the results and discussion section do not align for the numbers presented in the survey below due to those results referring strictly to the analysed questions with reference to the subject. As such the agreement to participate, demographic questions and the prize draw are numbered in the full version but not counted in that way in the results section.



The first page of the survey was used to set out ethical considerations, cover requirements dictated by the ethics process, set expectations for what is required and confirm that the reader is willing to participate after the introduction.

emographics Quest	ons	
2. How would y	ou identify your gender?	
Male		
Female		
Non-Binary		
3. What is your	age?	
17 or younger		
) 18-20		
21-29		
30-39		
40-49		
50-59		
60 or older		
4. What is the h	ighest level of education you have completed? €	
4. What is the h		
5. Is your work		
5. Is your work	•	
5. Is your work ves No	•	
5. Is your work	•	
5. Is your work ves No	•	
5. Is your work ves No	•	
5. IS your work Yes No If yes-Please describe	or education food related?	
5. Is your work ves No	or education food related?	

The above are the basic demographic questions asked in the survey. They are used in order to break down the survey results in order to create further discussion points by comparing the different demographics where appropriate to the question.



As the primary reason for utilising insects within the diet would be creating protein diversity, this question establishes the base line for that need within the UK. Literature establishes that people predominantly agree in theory that it is needed but find in practice that people do not understand how the lack of protein diversity will affect them in economically rich countries.



As with the previous question, this is aimed at establishing the base line for determining whether people would understand why insect protein may be important for the future of our food security.

```
* 9. How would you describe your knowledge of edible insects?

High level of understanding
Some understanding
Very little understanding
None
```

As insects are being researched on the campus and have appeared in various media formats, this question was felt to be useful as a potential method of creating demographics based around prior knowledge of the subject area. Further to that, it serves the purpose of demonstrating whether the insect consumption is being popularised to the wider populace beyond those that research it.

TV Books Newspapers Magaines Rado Internat Noss Other (please spectr)	10. Have you	seen edible insects	in any of the f	ollowing media	a? Select all t	hat apply.
Newsgapers Mapatines Radio Radio Internet None	TV					
Magzines Fado Fado None	Books					
fado trianet None	Newspapers					
Internet	Magazines					
None	Radio					
	Internet					
Other (please specify)	None					
	Other (please specif	9				

As above, appearance of entomophagy in media demonstrates a growing interest and popularisation which may affect the results of later questions due to how they had been represented in those media formats.

```
      11. If you have seen edible insects in the media, was their portrayal positive or negative?

      Peaker

      Negative

      Mixed
```

Both positive and negative media had been witnessed personally but it is important to establish whether one or the other type of media is more dominant as this may affect the participants expectations and perceptions of insect consumption, as a result, influencing answers within the survey.



This question is used as a qualifier for question 9. For example, a participant claiming a high level of knowledge should be able to provide a some of the notable benefits of consuming insects. This allows for the removal of erroneous sets of results.

) Yes			
) No			
no, would you be wi	lling to try insects?		

This data is an important comparison to literature. It is expected that insect consumption rates should be growing over time as they have been promoted for several years now. This question can also serve as a demographic separation as having previously consumed insects – expectations are going to be routed in that experience.

* 14. Do you expect to eat edible insects in the future?

A positive response to this question demonstrates willingness to try at the very least. This may also be interpreted as whether the participant thinks they may end up consuming insects out of necessity rather than desire. This question would be considered a technological expectation as it is asking about the product category as a whole rather than individual product types or attributes.

* 15. Does you	r home countr	y eat insects	as part of thei	r regular diet?	
Yes					
O No					
If Yes, where are you	trom?				

As the campus that the survey is being directed to has a significant number of non-home students from Asia, this group may influence the results if their home country is a common consumer of insects such as Thailand.

notional response t	o insects					
e following questions insect or insect base	d food product.					
 Rate 1-5 the sects. 	e following em	otions base	d on what yo	u would ex	pect to feel w	hen trying
	None	Weak	Moderate	Strong	Very Strong	Overwhelming
lappy						
believe						
tefreshed						
telaxed						
sterested						
mused						
trengthened						
bood						
Content						
lotivated						
emfic						
wigorated						
ptimistic						
static						
ell						
nergelic						
spassioned						
heery						
opetul						
iyful						
hilarated						
nliced						
eased						
uperior						
eathy						
isionate						
esitant						
urious						
spased						
range						
cathetic						
mid						
nique						
nique trigued onfused						

Satiated						
Uneasy						
Dreadful						
Fearful						
Aminus						
Guilty						
Angry						
Suspicious						
Averatve						
Concerned						
Greedy						
Sickened						
Bad						
Dissatisfied						
Bored						
Disappointed						
Stressed						
Alarmed						
Angat						
Frightened						
Pensive						
Grossed Out						
Shame						
Initaled	0	0	0	0	0	0
Panicked						
Sad						
Inferior						
Pressured						
Disgusted						
Apprehensive						
Worried						
Vulnerable						

Emotional expectations are the crux of the survey. The expected emotions are influential when consumers consider trying a product for the first time. This format, while long and potentially tedious for participants, it allows for much more information to be garnered from the results. The emotions can be categorised between positive, negative and neutral to get an overall feel for how their potential emotional state would be when approaching the consumption of insects. The individual emotions can also be analysed and considered to create further theory on how to develop insect-based food products to minimise those negative expectations and improve on the positive.

xpectations of ed	ible insects as an ingredient		
	imn is separate question. The ptions of insects in food. Plation		
	Which of these cuisines are you familiar with?	Which cuisines flavours would you expect to pair well with edible insects?	Are there any cuisines you would expect to pair badly with edible insects?
Chinese			
Italian			
French			
Japanese			
ndian			
hai			
Intish			
merican			
irazillan			
fexican			
aribbean			
ireek			
foroccan			
Turkish			

Spanish		
Vietnamese		
African		
Lebanese		
Jewish		
Russian		
Malay		
Peranakan		
Cajun		
Berber		
Arab		

This question was designed to test the theory that as insects are unfamiliar cuisine, people will associate insects with the unknown and believe that that is how insects would be best consumed. At the same time, it is believed that the opposite expectation would exist, that cuisines that people are familiar with would be perceived as poor quality if insects were included. In addition, this could act as a steer for the later product development work as the ultimate goal is to match or exceed a consumer's perception. This question is asking for implicit expectations of the consumer as a comparison is being made between the cuisines.

	vorst	k these formats in order - with 1 yielding the best expected result and 6 beir
11	•	Ground, dried powder
11	•	Whole insect, tresh
П	\$	Whole insect, diled
ŧ	\$	Whole insect, coated
	•	Diced insect, tresh

This question has been asked a lot in literature and always yielded the same result, ground dried powder. This poses the question in an alternative format in line with the questionnaire theory outlined in the introduction, as it is normally just a pick one option question which limits the potential discussion of the results. It is also an important question to use as a comparison to literature as this survey is taking place in the UK, where limited formal research has taken place on insects. This is a situational expectation as no defined product is mentioned.

```
* 19. Would you expect the flavours that insects could provide as an ingredient to be pair
better with sweet or savoury foods?

O Breat

Savoury

D Eller
```

According to the available literature, the answer to this question appears to differ around the world so it is important to understand and contrast this answer to those given in literature as it can be used to steer future product development. This is another implicit expectation as comparisons are being made mentally between the presented options.

	Very poorty	Poorty	Average	Well	Very well
Sustainability					
Ethical Production					
Energy Usage					
Nater Usage					
Protein Quality					
Protein Content					
ow Fat					
.ow Carbohydrates					
.ow Salt					
.ow Sugar					
Nutritionally dense					
Food Safety (microbiology, oxins)					
alue for money					
Overall cost					

The purpose of this question was to provide examples of potentially positive attributes that could be attributed to insects and establish what the participants believed were the dominant attributes. Establishing this would shows which areas are being focused on in media coverage by those participants who have seen edible insects previously and also demonstrate those attributes which need to be highlighted more. This question deals with static performance expectations, as it considers the attributes rather than the product itself.



Establishing price expectations is not a subject that has been discussed often in literature but food psychology states that this is one of the most important values when considering the initial willingness to buy. It is one of the few aspects which translate almost directly from theory in questionnaires to actual purchasing practice. Again this is a question regarding the static performance expectations of insect-based foods.

2. Would you be willing to purchase insect ingredients if they cost more than similar roducts?
Yes
No
Maybe - under which circumstances?

This question works with the previous one to establish boundaries. The theory is that currently insects are seen as a lesser option so should be cheaper. As this is not currently the case, insect powder is fairly expensive compared to conventional powdered proteins, confirmation would demonstrate that work needs to focus on this area in particular if insects are to become popularised.



* 23. How much would you expect the inclusion of cricket powder as an ingredient to affect the flavour of the following products.

	Make it considerably worse	Make the product a little worse	No change	Make the product a little better	Make the product considerably better
White Bread					
Wholemeal Bread					
Cookies					
White Pasta					
Wholemeal Pasta					
Crisps					
Falafel					
Meatballs					
Burgers					
Noodles					

The range of products used as examples for this question are foods that have been demonstrated in media, retail or academic literature from around the world. Understanding this expectation is important as it is typically the hedonic expectancy that drives initial product purchases. If it is entirely believed that a product that includes the cricket powder is going to be uniformly worse, it makes achieving sales an uphill struggle for the producers. This question deals with actual products but as it is asking for a comparison to existing product this question so it would fall under implicit expectations.

```
* 24. Would you expect to prefer products containing cricket powder if they are sweet or
savoury?

    Sweet
    Benowr
    Ne wpeckd pathwnce
```

This question acts as a confirmation for question 19 while providing a slightly different variation as such it would also be questioning the implicit expectations that the participants have for insect-based foods. Combined they make it clear which type of product is likely to be more preferred. It is an important distinction because while the literature from European countries that are making an effort to popularise insects demonstrates a strong preference for savoury products, the majority of what is available are sweet.

* 25	5. What type of products would you expect to contain cricket powder? Select all that
ap	oply.
	Ready meats
	Sweet Snacks
	Savoury Snachs
	Meal Kits
	Food Service meals (restaurants / cafe's ect)
	Health Food
	None
	Other (please specify)

Establishing expected product category allows for the demonstration of consumer boundaries to where insects can be included and also show where development could have the most positive impact. This would fall under interpersonal expectations as it is questioning how the participants expect to interact with insect-based foods.

* 26. what occasions would you associate with the potential of eating products	containing
cricket powder? Select all that apply.	
Breakfast	
Lunch	
Dinner	
Brunch	
Supper	
Dessert	
Snacks	
Special Occasions	
None of the above	
Other (please specify)	

As above, meal occasions are another area where consumers may set boundaries for a novel ingredient. For example, special occasions may be primarily linked to indulgent foods and as insects are viewed primarily as a health food, they do not fit in with those desires during that occasion. Similar to the previous question, this would also be questioning interpersonal expectations.

Lunch			
Dinner			
Snacks			
Brunch			
Supper			
Dessert			

This is an alternate way of asking question 26, providing both a confirmation question to ensure that results are accurate but also providing further details through the rating system. This follows the same line of questions on interpersonal expectations.

```
* 28. How do you expect products containing cricket powder to be priced?

Premium
High end
Motrange
Levied
Basics
```

By asking this question in this format, it links the pricing issue to a retail setting, asking the participants to categorise. Asking this further qualifier would permit the situation that while the participants believe that insect based products should be cheaper – they still fit into a higher priced category due to health foods in general being premium products. This question is an explicit expectation as it asks about the product directly.

H	\$ Sustainability
Π	\$ Elhical Production
H	\$ EnergyUsage
8	\$ Water Usage
ii.	\$ Protein Quality
Π	\$ Protein Content
II	\$ Low Fat
11	\$ Low Carbohydrates
П	\$ Low Sait
	\$ Low Sugar

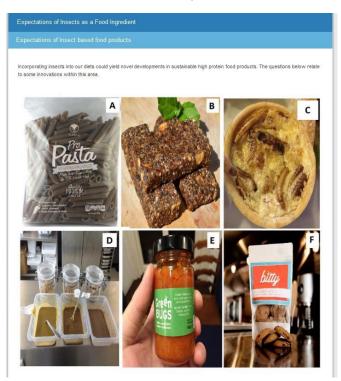
This importance of ranking the nutritional and sustainability aspects of insect-based foods lies in this information can be used to steer information surrounding insect-based foods. The aspects which are shown to be important can be displayed prominently in the limited space available for messages like these on packaging. All of these messages may be important to the overall story of insect-based foods but it is a necessity to be able to pick and choose those that are important to consumers as it is this type of thing that elevates a product above competitors that provide similar functional or hedonic properties.

	i bein	g the most appropriate flavour combination
II	\$	British
H	\$	Thai
II	•	Mestcan
11	\$	Japanese
II	\$	American
	\$	Indun
	\$	French

Appropriateness is a relatively new area of food psychology focusing on the hypothesis that if the combination of food flavours are not viewed as appropriate for the product type then there is automatically a negative hedonic expectancy generated which is difficult to overcome. Therefore, ensuring that products using insect ingredients do not generate this negativity could be an important step in ensuring willingness to buy and willingness to try.

* 31. Do you have any dietary restrictions or lifestyle choices which may prevent eating insects? (edible insects are an animal product which have been linked v similar allergen restrictions to shellfish)	
○ Yes	
O Na	
If yes, please give details	

As the following section of questioning deals with hypothetical situations surrounding the consumption of insect based foods, this question acted as a qualifier for being able to complete those questions because it would be impossible for, vegans for example, to provide accurate answers related to the consumption of animals.

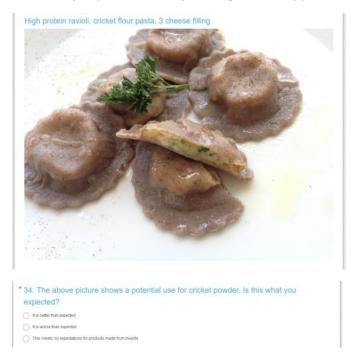


		order of which matches your current expectations for insect based foods the he most, 6= the least.
	\$	A - High Protein Staples
1	\$	B - Protein Bars
E	\$	C - Savoury Snack Products
	\$	D - Soups / Sauces
	\$	E - Unique Products
	•	F - Sweet Snachs

This question deals with the participants explicit expectations. The examples were real world products designed to demonstrate what these categories could look like for the participants who do not have any reference point on which to base an answer. This ensures that all participants are answering from an even playing field rather than utilising their own past experiences and biases towards those product categories in order to provide their answer. The question itself is aimed at gathering further understanding about what type of product should be developed in order to promote insect-based food products to a wider audience.

		order of which you would most prefer to consume if given these as options.
ne n	nost, e	6= the least.
H	\$	A - High Protein Staples
II	\$	B - Protein Bars
H	\$	C - Savoury Snack Products
	\$	D - Soups / Sauces
	(a)	E - Unique Products

Qualifying question 32 in this way allows for the potential demonstration of the theory above, that the expectation towards the type of product would yield something that the consumer is more likely to purchase or try when given the opportunity.



Again, this question is asking for explicit expectations towards a specific insect-based food product. The image is designed to show an insect-based food product that is visually higher quality than what they may have seen of insect-based food products previously. The questions

questions and show to the participants that a high-quality product is possible when using insects as an ingredient.

		ove product to taste?		
Very Bad	Bad	Neither Good nor Bad	Good	Very Good

Taste is the primary hedonic expectancy that people base their purchasing or trying decisions on. Linking this to the above image is aimed at demonstrating whether visual quality of the product may be enough to overcome the negative association of edible insects.



The product shown within this image does contain cricket powder but the only place this is demonstrated is in the ingredient description on the reverse of the packaging. It was designed as a way to demonstrate that it is potentially possible to normalise insect consumption by creating product that sell the benefits of insects without making the insect content the primary point of market differentiation.

llowing options, rank them in order of your expected preference.						
1	\$	A non-insect protein source				
1	\$	Insect four				
E	\$	Small pieces of insect				

When creating a product like this, the high protein label could be generated by a wide range of different options. This question when asked in literature has always been asked in a single answer format which limits the information that can be gathered. In addition framing it around a product may provide additional context to the participants that is lacking from previous work, ultimately yielding more accurate answers. This question would be considered technological expectations due to it discussing how the products could change.



As a contrast to the restaurant-style plating of the ravioli product, ready-meals are generally viewed as low quality products. This question is meant to provide a comparison point to question 35 based on how the products are presented despite them being a similar category as they are both pasta products. As this question deals with a particular attribute of one product, the question falls under static performance expectations.

If your answer	was bad or indifferent - what is your reason? Select all the	hat apply.
It contains Seafood		
It contains Courgette		
It contains Squid Ink		
it contains Pasta		
Low Visual Appeal		
Poor Nutritional Scores		
Because its a ready meal		
Lack of Information		
Other (please specify)		

This question is a confirmation of the above hypothesis, that the poor perception may be related to how the product is being presented.



This question was designed to provide a hypothetical scenario as a frame of context on which to base expectancy decisions related to basic hedonic attributes. This type of scenario is what is expected to happen in a real setting if insect consumption becomes more commonplace. Using the results of this question it could be determined whether this is a viable route for introducing insects to a wider audience. This would fall under situational expectations.



According to food psychology, this question is predominantly answered as yes. People ultimately like to believe that they would make the good ethical decision when put into that situation, but it has been repeatedly demonstrated that the intention does not match up with the action. Despite this, it is still an important question to ask as it demonstrates the view that these attributes are at least important to a consumer when comparing like for like across a category. Ultimately, if this is answered in line with previous literature this means that if the insect-based food products match the hedonic expectancy and the cost of the products they are alongside, the sustainability benefits may then be enough to generate a willingness to purchase. As this is questioning a particular attribute and asking if the participant would be willing to purchase based on that attribute, this falls under interpersonal expectations.

score on the following attributes once prepared.									
	Very Bad	Bad	Neither good nor bad	Good	Very Good	I do not consum animal product			
Taste									
Texture									
Aroma									
Appearance									

This question was aimed at addressing the hedonic expectancy of the pasta product to see if this type of product may be a way forward for promoting insect-based foods. This would be an implicit expectation question.



* 42. The two cookies above are produced using the same recipe except the one on the left has had cricket flour included. What effect do you expect this to have on the following attributes.

Considerably worse Allite worse About the same Allite before Considerably Effect
Tester

Tester

Considerably worse O

A similar question to those asked about the pasta products but this time asking the participant to compare two products from a different category that they have a visual context for. This is a situational, technological expectation question.

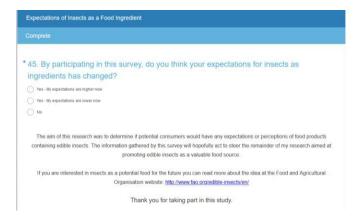
As a contrast to the question which asks how much a participant thinks that the insect component impacts the product, this question is asking them to rate their overall expectation of taste. This contrast is important as it is entirely possible to believe that the inclusion of insects could have a detrimental effect on the product, but the product could still be good.



As above, framing the taste expectancy around a product image allows for the contrast between them to develop a discussion on product type. As before, when dealing with expected hedonics, this would fall under static performance expectations.



The description and the image was absent of direct confirmation of insect contents, however, it is understood that this may be assumed given the rest of the framework of the survey. This question is designed to understand if the environment that the insect-containing product is an important influence while also confirming whether the participants would accept insects within this type of product. This changes the dynamic of the question towards situational expectations.



This survey may well be some participants first interaction with insect-based food products. As such it is important to understand how that interaction may influence their decisions surrounding entomophagy in the future. If conducting research in this type of format is damaging the potential for entomophagy when it is deemed so important by bodies such as the FAO, another way should be considered in the future. At the very least it may demonstrate that absolute care needs to be taken when designing things like this as first impressions may be all you get.

Appendix D: Survey participant information sheet and email

Hello,

I am seeking participants for an anonymous online survey on the expectations for insects as a food ingredient. The survey should take approximately 30 minutes of your time.

Please see the attached information sheet if you require any further information.

You can access the survey here: https://www.surveymonkey.co.uk/r/DKVTGPL

Your participation would be greatly appreciated as the results of this survey will form a significant part of my PhD thesis on "Promoting entomophagy as a response to food insecurity: Overcoming psychological and sensory barriers"

At the end of the survey there will be an option to enter your email address for a chance to win one of two £50 Amazon vouchers as a means of thanking you for your participation. Your personal contact information will not be used for any other purposes than contacting the winners of this draw.

Thank you, Robert Murdock



UNITED KINGDOM · CHINA · MALAYSIA

Division of Nutritional Sciences, School of Biosciences

Participant information Sheet

Study Title: The expectations of insects as a potential food ingredient

Name of Researchers: Robert Murdock, Professor Andrew Salter

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take the time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet. Thank you for reading this.

What is the purpose of the study?

The purpose of the study is to gain understanding of what expectations people in the UK may already have towards consuming insects. The online survey can be conducted entirely at your own leisure. There will be a variety of questions and question types covering basic demographic types, your current understanding of insects as food, the potential for insects as an ingredient and finally some questions on insects within food products.

Do you have to take part?

You are under no obligation to take part and may withdraw your participation at any time and without giving any reason. This would not affect your legal rights.

What do I have to do?

Click the link that you have been provided (or request it if you have not received it) and answer the questions. The survey should take around 30 minutes of your time to complete.

The Prize Draw

At the end of the survey is an optional prize draw to win one of two £50 amazon vouchers. If you do decide to enter your email address here, it will have no baring or affiliation to the results of the survey. The data will be kept separately in a password protected folder and only used for the purposes of making the drawing and informing the winners.

What are the possible disadvantages and risks of taking part?

We greatly appreciate that you will be giving up your free time in order to help us with this study. As an online participant in this research, there is always the risk of intrusion by outside agents, i.e., hacking, and therefore the possibility of being identified. These risks have been minimised to the best of our ability.

What if there is a problem? Who can I complain to?

If you wish to complain about the way in which the research is being conducted or have any concerns about the research then in the first instance please contact Robert Murdock

(sbxrm5@nottingham.ac.uk). If this does not resolve the matter to your satisfaction then please

contact Professor Andrew Salter (Andrew.Salter@nottingham.ac.uk) or contact the School's Research Ethics Officer, Dr Kate Millar (tel. 0115 9516303 email kate.millar@nottingham.ac.uk

Will my taking part in the study be kept confidential?

All collected information during the course of this research will be kept **strictly confidential**, stored in a secure and locked office within a password protected database at the Division of Nutritional Sciences, Sutton Bonington Campus, University of Nottingham. The survey is entirely anonymous by design. All research data will be kept securely for 7 years. After this time it is disposed of securely. During this time all precautions will be taken by those involved to maintain confidentiality.

What will happen to the results of this research study?

The results of the survey will be used as part of a PhD thesis and may be published in a peer reviewed journal.

Who is organising and funding the research?

The research is organised and funded by The University of Nottingham.

Who has reviewed the study?

The study has been reviewed by the departmental ethics committee headed up by Dr Kate Millar.

Further information and contact details?

If you require any further information please contact Robert Murdock (email: sbxrm5@nottingham.ac.uk)

Thank-you for considering taking part in this study. Please keep this information sheet for your reference.

Appendix E: Results for the qualitative survey questions.

Please note, accessing these results was only possible in the unfiltered version so the results presented below will include participants who were not included in the main results sections due to not completing the survey.

Respondents	If yes - Please
	describe
1	prepare practical classes for Biosciences classes
2	Studying towards BSc Food Science
3	nutrition
4	vet
5	Food Science degree
6	Brewing science
7	Studying food science
8	PhD in Nutritional Science
9	vet student
10	Animal Nutrition
11	Animal Nutrition
12	PhD in Brewing and Sensory Science
13	PhD student Food sciences
14	The majority of my study is agriculture based
15	PhD Agriculture and Food Security
16	PhD in Ruminant nutrition
17	first year food science degree
18	Nutrition degree
19	nutrition
20	Student dietitian
21	research into food borne zoonoses
22	Security and quality checks via vets at abattoir
23	Masters degree of nutrition and Dietetics
24	I study food science + nutrition
25	Biotechnology, so related to food security
26	Nutrition within veterinary medicine
27	Food science undergrad student
28	Veterinary Public Health lecturer
29	I'm currently studying microbiology and work part time as a food
	handler for a large retailer.
30	Vet
31	Ubdergraduate Nutrition and Dietetics degree
32	Nutrition degree

Question 5: Is your work or education food related? If yes, please describe.

as an alternative protein source 34 Dietetics student 35 Animal nutrition 36 Dietetics 37 Agriculture 38 Biotechnology 39 Work in the division of nutrition at the university 40 MD Doctor so aware of food in the sense of allergies. Also for advising others of healthy eating 41 Nutritional sciences 42 Animal Sciences 43 Food science lecturer 44 MSc in Food Production 45 MNutr- Dietetic Student 46 MSc in food science 47 r and d 48 Animal nutrition to produce food products for human consumption 49 food science 50 Microbiology 51 My education isn't food related, but I support sensory and brewing courses 52 MSc Nutritional Science 53 MNut Nutrition and Dietetics 54 Brewing 55 Agricultural research 56 nutrition 57 Nutrition and Dietetics 58 Nutrition BSc <t< th=""><th>33</th><th>Animal Science student but delved into greater issues of global food security, human development and even touched specifically on insects</th></t<>	33	Animal Science student but delved into greater issues of global food security, human development and even touched specifically on insects
35 Animal nutrition 36 Dietetics 37 Agriculture 38 Biotechnology 39 Work in the division of nutrition at the university 40 MD Doctor so aware of food in the sense of allergies. Also for advising others of healthy eating 41 Nutritional sciences 42 Animal Sciences 43 Food science lecturer 44 MSc in Food Production 45 MNutr- Dietetic Student 46 MSc in food science 47 r and d 48 Animal nutrition to produce food products for human consumption 49 food science 50 Microbiology 51 My education isn't food related, but I support sensory and brewing courses 52 MSc Nutritional Science 53 MNut Nutrition and Dietetics 54 Brewing 55 Agricultural research 56 nutrition 57 Nutrition and Dietetics 58 Nutrition PhD on the role of fats in obesity 59 Nutrition BSc 60 researcher on ethics		
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37 Agriculture 38 Biotechnology 39 Work in the division of nutrition at the university 40 MD Doctor so aware of food in the sense of allergies. Also for advising others of healthy eating 41 Nutritional sciences 42 Animal Sciences 43 Food science lecturer 44 MSc in Food Production 45 MNutr- Dietetic Student 46 MSc in food science 47 r and d 48 Animal nutrition to produce food products for human consumption 49 food science 50 Microbiology 51 My education isn't food related, but I support sensory and brewing courses 52 MSc Nutritional Science 53 MNut Nutrition and Dietetics 54 Brewing 55 Agricultural research 56 nutrition 57 Nutrition and Dietetics 58 Nutrition PhD on the role of fats in obesity 59 Nutrition BSc 60 researcher on ethics in research	35	Animal nutrition
38 Biotechnology 39 Work in the division of nutrition at the university 40 MD Doctor so aware of food in the sense of allergies. Also for advising others of healthy eating 41 Nutritional sciences 42 Animal Sciences 43 Food science lecturer 44 MSc in Food Production 45 MNutr- Dietetic Student 46 MSc in food science 47 r and d 48 Animal nutrition to produce food products for human consumption 49 food science 50 Microbiology 51 My education isn't food related, but I support sensory and brewing courses 52 MSc Nutritional Science 53 MNut Nutrition and Dietetics 54 Brewing 55 Agricultural research 56 nutrition 57 Nutrition and Dietetics 58 Nutrition PhD on the role of fats in obesity 59 Nutrition BSc 60 researcher on ethics in research	36	Dietetics
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40 MD Doctor so aware of food in the sense of allergies. Also for advising others of healthy eating 41 Nutritional sciences 42 Animal Sciences 43 Food science lecturer 44 MSc in Food Production 45 MNutr- Dietetic Student 46 MSc in food science 47 r and d 48 Animal nutrition to produce food products for human consumption 49 food science 50 Microbiology 51 My education isn't food related, but I support sensory and brewing courses 52 MSc Nutritional Science 53 MNut Nutrition and Dietetics 54 Brewing 55 Agricultural research 56 nutrition 57 Nutrition and Dietetics 58 Nutrition PhD on the role of fats in obesity 59 Nutrition BSc 60 researcher on ethics in research	38	Biotechnology
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51 My education isn't food related, but I support sensory and brewing courses 52 MSc Nutritional Science 53 MNut Nutrition and Dietetics 54 Brewing 55 Agricultural research 56 nutrition 57 Nutrition and Dietetics 58 Nutrition PhD on the role of fats in obesity 59 Nutrition BSc 60 researcher on ethics in research	49	food science
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 58 Nutrition PhD on the role of fats in obesity 59 Nutrition BSc 60 researcher on ethics in research 	56	nutrition
59Nutrition BSc60researcher on ethics in research	57	Nutrition and Dietetics
59Nutrition BSc60researcher on ethics in research	58	Nutrition PhD on the role of fats in obesity
	59	
61 Working with Crops - Oilseed rape	60	researcher on ethics in research
	61	Working with Crops - Oilseed rape
62 PhD student Agriculture and Environmental science	62	
63 I am PhD student in the division of Nutritional Science.	63	-
64 Currently doing a nutrition degree.	64	Currently doing a nutrition degree.
65 studying a nutrition and dietetics degree	65	
66 BSc Nutrition	66	
67 PhD Agriculture and Food Security	67	PhD Agriculture and Food Security
68 bioscience	68	
69 Microbiology	69	Microbiology
70 Nutrition degree		
71 HE Administration	71	
		Veterinary medicine relates to the health of animals as food producers
73 Increase yield	73	

74	animal nutrition
74	academic
75	Food Microbiology
	BSc Nutrition student
77	
78	Dietetics/nutrition
79	Veterinary Degree - Food Animal Production
80	Degree in food science and nutrition
81	Studying a food science degree
82	Farm animal medication - meat quality
83	Studying BSc Nutrition
84	Currently studying a Dietetics and Nutrition degree
85	dietitian
86	Food Science
87	Linked to agriculture
88	I have an HND in Hospitality Management but don't work in catering
89	Dietetic student
90	dietetics at uni
91	Studying Nutrition Degree
92	U
93	PhD Sensory and Brewing Science
94	Microbiology
95	Enabling Innovation project - SME
96	Nutrition and Dietetics course
97	Studying undergraduate Nutrition BSc
98	livestock production
99	Nutrition and Dietetics
100	Brewer
101	Lecturer
102	PhD Nutritional Sciences
103	Veterinary surgeon so was involved in production of food from
	traditional livestock sources, eggs milk and meat. Also import and
104	export of animal products.
104	Food Science Undergraduate
105	Microbiology
106	Food Science BSc
107	Food Science BSc. Food science and Nutrition
108	
109	Veterinary medicine - animals and animal produce entering the food chain
110	Veterinary
111	Food microbiology
112	teacher
113	Environmental Biology (plant science)
114	Agriculture, food industry communications
115	Dietitian
110	

116	PhD Student in Food Science
_	Bsc Nutrition
118	BSc. Food Sciences, PhD in Coeliac Disease
119	Uon agriculture
120	Biotechnology Plants
121	Teach into Food Science degrees
122	Veterinary medicine
123	Dietetics
124	HND in catering. Do not work in food-related area.
125	Food Science - University
126	Nutrition
127	
128	Crop research
129	Farming - Striving to feed the world
130	Agriculture
131	Agricultural business management degree
132	Studying MNutr nutrition & dietetics
133	Nutritional Biochemist
134	Food Science
135	agriculture
136	Veterinary Medicine
137	Animal nutrtionist
138	I'm working on my PhD in plant science, my research is related to food
	security
139	Plant sciences
140	crop biofortification
141	Crop Biofortification
142	veterinary
143	Veterinary
144	Currently studying BSc Food Science
145	Food Science and Nutrition at university, and a job as a chef in a pub
146	Sensory PhD
147	Crop biotechnology
148	Studying for a degree in Food Science
149	Veterinary Medicine
150	Dietetics and nutrition
151	Dietetics MNutr
152	Technician in Food, Nutrition and Dietetics
153	I work on structural properties of food components
154	Veterinary medicine
155	Food Science and Nutrition
156	elephant mineral requirements
157	Plants and Croos for food
158	Nutritional Biochemistry
159	Studying Nutritional sciences msc

160	Food microbiology
161	Farm animal vet
162	Crop science
163	Studying animal science degree
164	Some teaching
165	Plant Sciences
166	PhD in Food Science
167	I learn about human and animal nutrition and feeding across all three
	years.

Question 10: Have you seen edible insects in any of the following media? Select all that apply. Other:

Respondents	Other (please specify)
1	Focus group re-this study
2	live, I have eaten insects
3	L
4	In shops
5	Social media: Instagram/ facebook etc
6	Ν
7	science museum
8	Podcasts
9	Research papers. Plus shop advertisements (In south east Asia)
10	Real life demonstration
11	Discussion
12	in the flesh - at exhibitions, in the food hall and talking to students
13	Real live in my country
14	Lectures during bachelor
15	market
16	For sale as novelty ediable gifts in airports
17	Word of moth
18	School lessons
19	Podcasts such as Talknerdy
20	Parents
21	Seminars
22	Lectures
23	Assembly in school
24	Guest lectures
25	Talks at University symposium
26	University undergraduate course
27	Journals
28	exhibition
29	places like Food Matters Live
30	Wageningen University website
31	Parents
32	Workshop and Project team

33	University lectures
34	Presentation
35	Uni lecture- global food security
36	I'm completing my dissertation on protein content of Meal Worms!
37	from my experience work with locusts in my country as people eat it
38	Talks and discussions
39	Scientific papers
40	Food security lectures
41	Articles
42	animal feed advertisments
43	Conferences
44	on campus student presentations

Question 12: Without looking it up, please describe the benefits of consuming insects and insect-based food products.

Respondents	Responses
1	Renewable source; high protein content. Easier to produce and maintain compared to cattle/farm animals
2	High protein levels
	Less agricultural footprint, less water, gas emissions etc
3	more protein per gram than mammal or bird. Less harmful gases emitted in insect production.
4	Low environmental impact vs normal meat production
5	Very high protein levels, meaning less is needed to be consumed to still recieve the same amount of protein
	Low water consumption required by the insects during farming
	They require much less land space compared to other protein sources during farming
	Low in fats?
6	provide protein when there won't be enough
7	easy to produce
8	 The possibility of producing insects on a high scale over a lower landmass with less inputs ie power, heating etc, therefore reducing our environmental impact and benefiting the planet
	 possibility to enable developing countries to have a source of protein
	- reduction in meat products being eaten
9	More sustainable source of protein
	Widely available
10	High levels of protein, low costs of production at large scales, high availability

-	
11	They are high in protein, and they have a low water demand
12	Good protein source with good amino acid profiles. Rapid to produce due to short lifecycles.
	Can be in powder form so easily incorporated as a food ingredient.
13	low carbon emissions, low production costs
14	There are rich protein inside the insects
15	Cheap, sustainable and highly efficient source of high quality
10	protein.
16	There are a lot of insects in the world so there is a large supply and
	they are a source of protein.
17	Presentation, food shops/ grub cafe
18	A high density of protein for realtively low cost to produce.
19	high protein content, little cost
20	high protein content
	low production costs
	low carbon footprint
	no animal welfare issues
21	Higher rates of reproduction
	More efficient and less wasteful production than other common
22	animal products easy to get hold of
	good source of protein
23	High protein levels?
0.4	More environmentally friendly
24	Alternative protein source with high conversion efficancy but may have anit-nutritional factors and have a challenge of consumer
	perception as whole insects.
25	Less cattle & other animals being killed. Less calories & fat comes
_	with the insect protein
26	Less animal suffering. Massive resource being unused. Cheaper in
	the long run due to scale of industry vs traditional meat industry.
27	Eating insects is more sustainable than other animal products.
28	Less space and energy/resources to grow them than e.g. cows,
20	good source of protein?
29	may help to improve the nutritional need
30	high protein, more environmentally friendly due to less land mass needed compared to animals
31	high protein source, fast generation so quick to produce without big
	environmental impact
32	High protein, low production space, high throughput, low inputs and
	high outputs. Environmentally considerate.
33	it will include the required added vitamins needed in a balanced diet
34	Good source of protein

57	Good amino acid balance. Not in short supply.
58	more efficient source of animal protein.
50	
	lower environmental cost.
	tasty.
59	Alternative protein to mammals
60	Insects contain more protein per mass compared to some
	traditionally consumed species. Insects are also somewhat easier
	to breed and have lesser impacts on the environment (space,
04	resources etc) as compared to current meat sources.
61	- can be easily farmed for production
	- has a different taste compared to other meats
	- possibly a high source of protein
	- the whole insect can be eaten as a light snack
62	- the whole insect can be eaten as a light snack Greater protein efficiency in terms of land and energy required.
02	creater protein emotories in terms of land and energy required.
	Reduced environmental impact.
63	Short generation time, cheap production, high protein
64	They are in plentiful supply and high protein source.
65	High protein, readily available, readily harvestable and flexible as a
	substitute
66	Sustainable, healthy form of protein
67	It would mean we can get protein from a more sustainable source
68	High protein content
	More sustainable than meat
69	They contain high protein that is more digestible in the human body.
	They are lower in fat than other meat products.
70	high protein, easy production - and less ethical questions vs red
	meat/poultry/fish
71	High in protein
72	They are an alternative source of protein to livestock that have a
73	lower environmental impact I do not know enough about this subject to comment.
73	
/4	protein
	cheap
	less risk of zoonosis? food hygiene
	sustainable
75	More sustainable food production
	Using less resources that ruminants and having less greenhouse
	gas production
	Using less space

76	Easy and more eco-friendly, less zoonotic risks
77	Insects are cheap and relatively easy to farm, can be integrated
	with other kinds of farming (e.g. crop farmers catching pests that
	damage their crops), low carbon relative to the protein yield.
78	Cheap protein, easy to farm, good feed conversion ratio, good nutritional profile
79	Their food is our waste so good for environment and less land is
	needed to sustain them
80	Reduction in the need for animal based protein, better for the
	environment, equally bioavailable?
81	Low fat source of high quality protein
	Can produce a lot of high protein food which is more cost- and
	energy efficient than producing animals and plants for food
	Less environmental impact than producing and transporting aninal
	and plant sources of food
82	Insect food products can be high protein sources that are more
	sustainable (less land use, less greenhouse grass emissions, not
	impacting wild populations of animals etc) than meat/seafood and a valuable contribution to feeding the world's population
83	good source of protein and a sustainable source.
84	High protein content - of nutritious value to humans. Easy to farm
04	as high reproductive capacity and small size. These both relate to
	the environmental and sustainability benefits. Additionally, less of a
	moral barrier exists in comparison to consuming mammals - seen
	as less sentient (both by me personally and the public)
85	Easily bred. Vast amounts, small input. More sustainable, less
	environmental impact. Ethics- deemed as non sentient or less so
86	cheap
87	Excellent source of protein, high fibre content, good amino acid profile.
88	Protein rich, not such a burden on the environment
89	High protein food
	Sustainable
	Low energy production
90	Cheap source of protein
91	Fast growth time
	Cheaper
	Less impact on the planet
92	Less impact on the planet Abundanct
92	cheaper more sustainable source of high quality protein
94	I would have said mainly as an alternative protein source as they contain high levels of protein and are relatively easy to mass
	produce.
95	source of protein
55	

00	
96	animal welfare
	better for planet probably
97	Conserving the enironment
98	Reduced load on the environment
99	Protein
100	Cheap.
	High protein content.
	Availablity in tropical regions.
101	High protein in insects. healthy alternatives to fatty meats.
102	high in protein
103	Decreased co2 footprint in production. Easy to produce. High
104	protein compared to their size. Good source of protein.
104	
	Sustainable production possible.
105	Less greenhouse gas emissions A protein source that can be produced using lower quality feed and
105	potentially more efficiently than animals.
106	novel - quirky - faddy (I think these may be attractive to certain
	people - I am not sure if we should class them benefits)
	in theory consuming edible insects could be a way to process waste
	foods.
	possibly a source of protein, though whether it is a good source
107	with a high biololigcal value I am not so sure. Insects contain protein
107	They are an alternative source of protein.
100	It can be an alternative food as a protein source
110	source of protein, easy to grow and environmentally more friendly
	than farming animals
111	Lean source of protein
	Rapid development
	Low costs for breeding/less need for.infrastructures
	Low environmental impact
112	Insects represent a good and cheap source of protein with the
440	same nutritional value as any other animal protein
113	High Protein
	Widely available
114	cheap source of protein

A A F	They are plantiful, and ach provide a yery kink ratio of protein
115	They are plentiful, and can provide a very high ratio of protein against area.
116	Protein which is derived from methods which aren't detrimental to
	the environment
117	alternative source of protein
	easy to grow
	special taste
118	Protein of insects
119	nutritional value
120	cheaper
121	 sustainable source of nutrition which will help with global food security. Potential benefits for IBD and IBS.
122	Insects contain proteins, vitamins, minerals that might be crutial for our health
123	Higher protein levels
	Higher abundance
	Higher abundance
124	high protein source
125	With a veterinary background, and talking to colleagues from the
	food industry, I understand insects can be quick source of proteins
	(compared to current poultry, porcine and beef industries) with a
126	much lower carbon footprint. Would assume there is less environmental impact from consuming
120	insects than other forms of animal protein.
127	More efficient way of producing animal protein than conventional
	lifestock
128	I understand they're high in protein and low in fat so they're super healthy.
129	1. the feed to product ratio is higher in insects, higher efficiency in
	production?
	2. require small space and can raise in large bulk
	3. feed on large range of materials
	4. high in protein and low in lipid
130	High protein content, low space requirements, cheap
131	healthy and sustainable consideration
132	More output per tonne of feed
133	Other essential beneficial micronutrients
134	Maybe high protein even in small quantity
135	Less water consumption and less space requirements with higher
136	protein per Kg. Cheaper source of protein than what we commonly refer to as
130	animal protein (beef chicken etc). Doesn't take a lot of space to
	make a greater amount and doesn't require as much water and
	food either. Perhaps a more environmentally friendly approache.

137	A valuable source of protein which would probably be less resource
400	hungry to produce than traditional meat production
138	good quality protein, doesn't have harmful effects to the
120	environment as the other animal protein sources They can subside on waste material, highly nutritious and can live
139	in dark places so highly energy efficient.
140	High in protein, better for the environment, cheaper and more
	sustainable.
141	lean, clean protein.
	Far greater efficiency of conversion of feed, fuel and water to
4.40	human protein than regular meat.
142	They are beneficial because they have a smaller carbon footprint
	than other animal based protein sources. They are more efficient to produce.
143	Highly sustainable due to short life cycle.
145	
	High levels of protein per mass of insect.
144	Alternative source of protein; more sustainable for the planet and
	healthier (probably) for humans
145	cheaper to produce
	enough protein content
146	No idea
147	More economical production of protein
148	High protein, easily sourced, low farming/husbandry requirements
149	Reduced carbon footprint, opportunities to use insect proteins as food additives (emulsifiers etc), new novel ingredients
150	Easy to produce en mass
	Complete protein source
151	Cheap, protein, efficient to produce, economical to produce, less
	emmissions from production.
152	- Cheap
	- Nutritious
	- Nutimous
	- Ubiquitous
153	High protein source
	They're quick to produce and have high reproductive rates
154	Less land use
	Lower impact on Climate Change than other forms of protein
	Health (guess)
	Cost (Guess)
155	Supposed to be highly nutritional and less damaging to the
100	environment than meat products
156	high protein, good supply

157	High protein in comparison to amount, cheaper to produce, and easy to produce in bulk
158	unparralleled conversion of water and resources to protein, tiny surface area required to cultivate/breed
159	Lots of protein, much better for the environment as require less
	water and land
160	Using protein from insects instead of animals - reduce carbon emissions. More sustainable source of protein. Reduce waste and reduce the amount of feed required to feed animals.
161	High in protein, readily available, easy to produce, lower carbon emissions than more traditional meat produce.
162	High source of protein.
	more sustainable than animal products
	cheaper than animal products
163	Less pressure on the supply of other animal food products.
164	Less water consumption per kg protein compared to other animal protein.
	Easy to grow with high stock numbers in small areas; easy to grow and 'harvest' compared to other animals.
	Can have many nutritional benefits.
165	Good source of protein easy to harvest.
166	They could be cheaper alternative of other animal products such as meat, chicken, lamb etc because they are rich in protein, mineral and fibre.
167	high protein intake
	less land and resources needed to keep large amounts
	less 'welfare' needed to be taken into consideration
168	High protien low fat sustainably produced and intesive
169	It's another source of protein for us to consume, probably quite nutritious and I imagine low in fat and high in taste.
170	need small amount of space to produce a high level of protein
170	I understand that it's a source of protein that might be better for the
	environment than raising cattle
172	source of protein
	provides essential amino acids
	doesn't require much input of human resources
173	lower environmental impact than mammal and fish based protein sources
174	Higher protein concentration
	Less resources required for the same biomass production
175	They are an alternative source of protein, allowing us to get it into a
	diet in a more sustainable way

170	
176	Will help solve world food crisis particularly with a growing population
177	Low input, in comparison to what we eat at the moment. More sustainable?
178	Pest control, alternative protein source, low cost production
179	High protein, easier to farm, less greenhouse gases
180	Lower carbon footprint, more sustainable, less 'bad' fat, can be
	cultivated
181	cheaper to produce
182	They breed quickly and don't take up lots of space
183	Additional protein source; easy to farm
184	High protein level for minimal land use/resources in comparison to traditional foodstuffs. Easy to incorporate into different conventional meals.
185	Good protein source
186	easy access as they are everywhere in the world Full of protein
187 188	High protein, cheap, low emissions None known
189	Nutrients such as iron and calcium. It can also give you protein.
190	good source of protein. Low cost in breeding them
191	sustainable protein production with less water consumption
192	faster production
193	lean form of protein, abundant
194	High protein content, extremely good food conversion ratio
195	higher in protein than other animals - farm animals
196	High protein content, quick to rear, many are primary consumers, can be fed on waste matter
197	Highly available and accessible
	High in protein
	Apparently quite palatable in flavour
	Lessens the negative impact on the planet if used to reduce current animal protein consumption
198	They're easily produced
199	Other sources of protein in our diet
200	Easier to produce, less environmentally damaging, requires less
200	work // food etc to be produced
201	I don't know. But feeling disgusting
202	High in protein
	Low fat
203	Very avalible
	Quick and easy to grow
	cheap to produce

	efficient to grow
204	Good environmentally - requires much less water and energy to
205	produce large quantities of insect protein
205	High in protein
	Low in fat
	Fiber
206	They will be able to provide the essential source of protein needed
	whilst reducing the impact and demand of meat which may not
207	always be available and also causes environmental issues. High Energy, high protein, relatively healthy
207	Low fat, easy to produce, cheap
209	High nutritional protein source with functional roles.
	Some insect products can be used to mimic functions say foam
	structures and stabilities which their typical source of these
	functions eg eggs would require more resources to produce.
210	Variety in diet
211	high abundance of insects everywhere
	accute acteb in bick numbers
	easy to catch in high numbers
	low emissions from farming and less farm space needed
212	More efficient than current sources of protein i.e. produce more
	protein relative to feed given.
213	Another form of protein, reduces demand, reproduces quickly, and lots available
214	Low cost protein source, reduces issues with animal welfare.
215	high protein, more economical,
216	Would result on less demand on other sources of protein which are
210	putting a strain on global food security, especially in a growing
	population.
217	Easy and cheap to grow compared to animals
	Less welfare concerns
	Can be grown in bulk to feed the increasing population
218	They are a good source of protein/g
	Better for the environment as they don't require a lot of feeding or
	space to take care of (and possibly less CO2 emission?)
	They have little taste so can be used to recreate any flavour (eg.
	flies can be used for beef burgers and possibly another meat
	product).
	They are everywhere, it's unlikely we will have a shortage any time
0.10	soon
219	Higher protein content saves space and is much cheaper compared
	to normal protein sources such as cattle.

220	Protein
220	High protein source which is cheap
221	High protein comparable to proteins from conventional sources
	Contain important other nutrients e.g. micronutrients
	Good for the environment as it is alternative protein source
223	Less space and waste produced per gram of protein produces
224	High protein
225	more efficient protein production
226	higher protein content per serving than beef and reduced environmental impact
227	Cheap source of protein which results in a lesser production of greenhouse gases and other negative environmental impacts associated with meat production.
228	- high protein
	- able to priduce alot of them quickyl
229	Source of protein. Low cost?
230	Source of protein but with a lower environmental impact due to reduction in resources required for farming and harmful outputs of farming livestock such as greenhouse gases
231	An interesting taste experience
232	source of protein and less environmental impact than meat
233	Good protein source, easily produced (low energy input, quick, cheap), less demanding welfare needs.
234	Nutrition
235	Less harmful to the planet to farm
	Fewer ethical issues
	They're abundant
236	Take us less land and have a lower carbon footprint
237	Protein, ecofriendlier and high micro nutritional value
238	High in protein, sustainable source
239	good source of protein
240	High in protein, less resources to farm, less water to produce than meat
241	High protein value product and calcuim source with minimal energy input for high feed conversion effiency. Unknwon anti-nutritonal and processing factors
242	High protein sources, plentiful
243	nutrients
244	Lower impact of production on the environment compared to traditional sources of protein.
245	High protein content. Reduction of reliance on traditional farming.
246	High levels of protein
247	- High in protein and healthy fats

	- Low in carbs
	Take up loss space to produce then current enimal products
248	- Take up less space to produce than current animal products Act as an alternative protein source that can be quickly harvested.
	They would otherwise go to waste so are key for a growing
249	population. They are more sustainable e.g. use less water and land to produce
249	per kilo. Good nutrition, lots of proteina and nutrients
250	they have a high amino acid count and are more digestible than
	some plant sources due to the composition of their amino acids.
	also it is tied in with global food security as it provides an alternative source of protein.
251	Insect protein contains high levels of essential amino acids and
	they are beneficial to health.
252	Readily availible
253	sustainable, nutritious, cheaper,
254	Very available protein, high concentration of protein return for each
	unit of food input, can be small scale, can be local, can be cheap, can utilize waste products from agriculture as feedstock, does not
	need vast areas of land, insects can be reared with minimal
	experience or training.
255	To my knowledge, this product would provide a conveniently high
256	nutritional value compared to the energetic need for its production I am a Dietetics student and in our Global Food security module we
200	learned that incorporating more insects into our diet can reduce
	carbon footprint and still supply us with protein. It was described as
	a good alternative to meat and other animal products. It can also be
257	used to feed animals instead of using grains or other type of feed. High protein content as well as low fat content.
201	righ protein content as well as low fat content.
	Requires a fraction of the water, energy and space to produce than
	other commercially produced meat products.
258	Cheap source of protein that can be raised on waste products
259	Mass production, range of amino acids, cheap to produce
260	Effective way of consuming protein. Lower environmental impact during production. Less space required to produce more protein?
261	Better for the environment as take up less space. More
	concentrated protein. Less carbon footprint
262	More efficient than mammals. Could become more efficient than
263	poultry. Potential health benefits from chitin
200	
	protein content
264	High protein, Low production costs, high yields
265	They are nutritious and widely available. They can also be used in
266	food products (such as sweets) as a replacement for other animals. energy dense food, good source of protein, more environmentally
200	friendly
267	More sustainable protein source - can rear many insects in a small
	space so more efficient than raising vertebrates for food. Potentially
	less carbon footprint, land use and habitat loss. Able to feed more people using less resources so benefits food security

268	Environmentally efficient. Less use of water, less production of co2 per G protein produced.
269	Nutritious food source
270	insect food production will have less of an impact on the environment, it may be a less expensive allowing more people to have access to protein for their diet
271	High in protein, low in fats
272	An additional food source not currently being used and we need to think about these as the world population grows. I think on the programme I saw they said that insects were nutritious.
273	High protein low fat food
	A more sustainable protein source compared to current protein from livestock
274	Not only protein source but high in other micronutrients, very easy to cultivate, benefit to developing countries, accessibility, taste like chicken!
275	Insects can provide a good source of high quality protein.
276	low fat content
	high in protein, many containing essential amino acids
	possibly containing antioxidents
	much higher edible proportion to inedible/undesirable parts (then for example a cow or pig)
277	Insect based products have more high quality protein content than animal protein.
278	They are high in protein and easy to eat as a last resort if living in the desert.
279	A different type of protein so benefits for allergies/intolerances to regular proteins? (Just thinking in terms of atopic dogs etc).
280	The nutritional benefit is primarily to do with diversity of foods as
	part of healthy diet. From environmental perspective, I don't have enough knowledge to assess how efficient and environmentally friendly insect production will be. The main concern are allergies that can prevent widespread utilisation of insect-based products in other types of food, i.e. beyond those cases where history of use have been well documented.
281	More sustainable source of protein
282	-they can be farmed on waste
	-good source of protein for humans and animals
	-take up less space when farming
283	I imagine they are high in protein, including essential amino acids and low in fat
284	lower environmental impact than mammal or poultry meat, as greater feed conversion and shorter rearing times.

	Doesn't require the same amount of land as traditional food animals, so less pressure on land use.
285	-They require very little input for the amount of useable energy they produce
	- small area of land needed for farming
	- little water needed
	- grow very fast
286	High in protein, good source vitamins and minerals. Also, they are much cheaper to produce.
287	Good source of protein and add flavour to food
288	They're abundant and are high in protein.
289	Less reliance on animals
290	More sustainable
291	reduces need for animal breeding for food purposes
292	probably similar to other protein sources we consume
293	Less impact on the environment (land use of farming cattle and feed, water usage, methane etc) cleaner source of protein, more nutrient dense.
294	High level of protein. Less energy needed for insect production so greater yield.
295	High protein and there are lots of them!
296	Good source of proteins, able to reproduce quickly
297	High protein
298	They are non-sentient, easily reared, and very good animo acid profiles
299	Insects are very efficient at converting feed to product
	Insects use less water and energy than conventional animal protein sources
300	High in protein, (but not always bioavailable). Convert feed to protein more efficiently than traditional protein sources. Contain unsaturated fats. Require less space for production.
301	Insects are a more sustainable protein source than meat and is more complete than plant proteins
302	Strong protein source with little energy production
303	A wild guess here: less CO2 produced; less concern re. ethics of production; fewer areas of land used in production; greater sustainability.
304	much more protein can be produced within the same amount of land with much lower environmental and monetary costs.
305	Good sources of protein and a source of micronutrients (though I could not name any specifically)
306	In general more environmentally friendly:
	 fewer resources required (to make same content of protein) than traditional meats

	and food off contain woods products
	- can feed off certain waste products
	- short life-cycle
307	Alternative source of protein or feed for animals.
308	High protein content
000	
	Less green house gases from farming
	5 5 5
	Reduces other meet consumption
	Cheaper?
309	Different plants edible for protein, high protein concentration
310	Relatively low intensity production methods?
311	Less requirement of resources to farm animals, take up less space,
	less negative impact on environment. A visual summary of this on
	a documentary showed the requirements of beef cattle, versus a
312	large tank of insects providing the same amount of protein. High protein, low in fat
012	righ protoin, iow in ruc
	They take very little space, energy etc to farm so can produced very
	economically
313	A way to increase protein intake for free
314	I would say that as opposed to traditional animal protein, insects
	are much cheaper to produce as a protein source.
315	Smaller- less space taken up to grow / mass produce them and you
	don't need massive amounts of land etc like with farm animals
	also less cruel on larger animals that feel more pain
316	Sustainable, highly nutritious, insect breed faster than other
	mammals that we're consuming
317	Provide a good source of protein.
	Aren't expensive to produce as they eat waste materials.
	We won't waste edible resources raising them like we do with other
	protein sources at the moment.
	Very abundant.
318	Very high protein ratio, relatively simple feedstock and growing
	conditions, low fat.
319	To provide an alternative protein source that is more sustainably
	farmedd and enable a reduction in processed red meat consumption.
320	Low environmental impact of production of the insects, for example
520	less water required and fewer fgeenhouse emissions produced
321	Hight protein content. sustainable/low polution
322	Low intensity 'farming'
022	
	high yield of protein compared to animal based protein for a low
	outlay of resources

202	protoin, alternative source
323	protein, alternative source
324	High protein and readily available/easy to cultivate
325	eating insects mean that you can produce more protein using less resources and there isn't the issue of animal welfare like there is
	when farming cows etc
326	Alternative source of protein.
327	Not as damaging to the environment as traditional protein sources. More sustainable way of getting protein
328	They are high in protein and low in fat. They require a lot less water
020	to produce than traditional meat per kg. Less farm land is needed as well.
329	It is sustainable and has protein
330	they can be 'vertical farmed', meaning less space is need to grow
331	them. as well a good source and protein, high in protein too. The production of insects uses less resources including space and
331	water compared to meat. Insects can also be fed leftover food,
	reducing waste. Insects are generally higher in protein and can
	contain less fat than meat and contain nutrients, depending on the
	insect species and stage of life. being a healthy nutritious food,
	insects could help reduce obesity related with eating too much
	meat. Given that insects are found all over the world, they can be a
	nutritious food source that can be found anywhere, particularly in
	developing countries. They are easily and quickly produced, so
	there is unlikely to be a deficit. Insects are more environmentally
	friendly, producing less greenhouse gases compared to meat and
222	are more sustainable.
332	High in energy/protein
333	Easy to mass produce a cheap source of protein
334	Good sustainable source of protein
335	Purer protein with less fat and carbohydrate (causes less obesity).
	Abundance of some species.
	Insects require little space.
	More humane than killing mammals or birds.
	Insects convert a larger portion of their food into protein.
336	High protein and easy to produce, can be dried or mashed to create different textures for different uses (like crisps)
337	High in protein, and are much more environmentally friendly than
	other animal alternatives. They produce less emissions.
338	They are a good source of protein and micronutrients, and provide
	an under-utilised and sustainable resource. I would assume they
	have a smaller carbon footprint than livestock.
339	High levels of protein and low saturated fats. Similar functionalities
	to some protein with further investigation in to other properties that
	wold be beneficial in manufacturing e.g. gelling and foaming (my
	dissertation).
340	Protein source, takes less time to produce than eg a cow? less
0.0	resources, greater numbers to supply demand

341	cheap
342	Good level of protein and fat, close to animal protein sources
343	Good source of protein, cheap, wide range of product uses
344	They are apparently a good source of proteins
345	High protein content. Famous Bear Grylls quote " pound for pound,
010	more protein than beef"
346	High protein content and lower cost than meat.
347	Protein based foods
348	they contain a lot of protein
349	Add taste to the food, source of protein
	Easy to breed and house
	Cheap
	Readily available in lots of different forms
350	less inputs
351	Sustainable, highly productive and low cost animal protein
	production. More environmentally friendly.
352	Less land used for greater amount of protein
252	Easy to farm/harvest
353	High in protein, low cost to production, readily avaiable
354	high protein.
	good food conversion rates
	geod lood controloin lates
	easy to produce
355	High protein/weight ratio. Less waste than many animal based
	meats eg no bones etc.
	I think they have good nutritional value
356	Insects are plentiful
	They can be a nuisance so people don't have such a strong
357	emotional bond with them Less environmental impact compared to animal protein, less of a
307	welfare issue than consuming 'higher' animals.
358	Protein source
359	Protein rich. Can be used to fortify other products.
360	less meat derived protein so good for the environment??? maybe
	better protein?
361	reduce costs, reduce water and energy use, high protein content,
	sustainable
362	Better for the environment
	Less emissions
363	insects are known with high protein. in my country people can
200	accept to eat locust as is eat plants but not as a main food source
364	another resource of protein
	•

365	They are a novel protein so less allergies, and they are widely available due to their vast numbers.
366	High protein source. Production is cheaper. Less impact to the
007	environment. Sustainable
367	They are cheap to farm, so a cheap way to get protein. They are low in fat and probably high in fibre.
368	Cheap and readily available
369	less methane will be produced
	less cost is needed to produce insects
370	Greater availability
371	Less fat? Lean protein. More minerals?
372	Insects are high in protein and are cheaper and much more environmentally friendly to farm than traditional livestock.
373	High sources of good quality protein, plentiful supply.
374	Low water consumption in production, low fat content and high levels of protein. Cheap as well
375	High protein and an alternative source to meat.
	Very sustainable
376	No idea
377	Better for environment as need less land to farm
378	high protein source, low fat. High yield.
379	High protein content with little input of useful biomass. Easy to store and maintain populations
380	much more available
	high source of protein
381	Much easier and less cost intensive to produce insect-based
	protein and much more accessible to the wider world.
382	They have a high protein content, but need a smaller land area to
	farm compared to other animals bred for consumption. They require
	less water input. They can be farmed in vertical farms with various different levels rather than lots of land as needed for other animals,
	and particularly free-range produce.
383	Low production cost, low environmental impact, fewer animal rights
	violations.
384	Cheaper, better for the environment, lean protein
385	They have a high protein content and are easy to produce in large
	quantities with far less environmental cost than other animal based
	protein sources.
386	Less animal welfare concerns
387	In my culture, when people where poor the eat insects, and now the
	new generation they like to do the same in the same perion where
	the insects become available for just traditional food, however, its
	impossible for me to eat now but if there is no food I may do to
000	survive only
388	Very high protein content for their ratio
	Little area needed to breed them

389	high protein low fat
390	There are lots of them, they are small and I imagine easier to farm than like cows etc., they have quite a lot of protein for their size (I think)
391	Alternative to animal meat sources. Less methane production
392	possibly. Lean protein, manufacture takes up less space and poses less of a
393	threat to the environment There are benefits in production of insect protein over animal
394	protein but the consumption bit is unclear. More sustainable and possibly healthier
395	Insects are common, easy to farm and have high protein content.
396	A substitute protein option
397	1. improve protein diversification, mainly because the population is growing up and the need for new food resources is necessary
398	Very high in protein- essential amino acids? Quite cheap, sustainable to produce, low in saturated fat and sugars
399	It could be an alternative source of nutrients in case of food shortage
400	High yielding, quick reproducing, minimum carbon imprint. A quick and environmentally friendly alternative to animal protein. High in protein, minimal waste, and when it comes to meal worms, will literally eat anything.
401	very short life cycle so breed rapidly therefore very cheap to produce. Do not require a lot of space. cheap food
402	They are rich in proteins and amino acids and fatty acids which are extremely beneficial to human health. They are easy to farm on a large scale to help meet the growing deman for protein in a increasing population, using far less resources to cultivate such as land.
403	Contain protein, lots of them available if know the right ways to catch them, no ethical issues related to them unlike e.g. chicken
404	high level of protein
	potential to be cheap to produce
405	High protein and fat content. It takes less space and feed to raise insects than other animals for human consumption.
406	A new source of food, lots of insects are available (species and numbers), less space required compared to animals, less religious problems (most likely)
407	easily generated protein rich food source
408	Greater economy, less landfill, less carbon footprint emission, less water necessary to produce kg of protein.
409	A more sustainable way of producing animal protein. Less inputs per unit.
410	Cheaper
411	Additional Amino Acids
412	Aids in sustainability and a good source of protein
413	More energy per gram

414	High quality, relatively cheap, protein without the environmental impact
415	The protein content of insects can be manipulated lending itself to add protein content to food efficiently. Furthermore it means reduction in animal products. However while some may feel this could be environmental friendly there are some consequences of insect farming. Nonetheless the need for large areas for cattle etc to graze.
416	High in protein and micronutrients but lower energy density
417	good protein source
418	consuming insects can be a substitute of meat product
419	High protein, little fat (I think)
420	More environmental friendly to harvest protein from vast amount of insects than having to get protein from livestock
421	Higher energy conversion efficiency compare to conventional methods
422	Sustainable farming; high protein
423	More efficient use of resources (including recycling), reduced C footprint, welfare
424	source of protein that is more sustainable than animal sources esp beef
425	sustainable protein
426	good protein source amenable to farming
	lower GHG emissions
427	?Relatively high protein content per gram compared to plants
428	cheap, renewable
429	Less land and resources needed to produce high level of protein when compared to Cattle and other livestock
430	- we do it anyway (as a contaminant of jams etc)
	 less impact on environment (energy/land use/water use) and hence more sustainable
	-better yield
431	Sustainable - much lower greenhouse gases compared to animal protein
432	Effective food ingredients Insects are good at transforming waste material into protein and have a high protein content themselves. Insect farming is thus relatively straightforward on a large scale.
433	High protein content
	More sustainable compared to other livestock as farming insects requires less water and release less green house gas
	Satisfying for curious people

	Might be accepted by some vagetarian
434	Easy to produce and have a high level of protein/nutrients for their size
435	A sustainable source of protein
436	High protein. Sustainable.
	Less waste.

Question 13: Have you previously tasted edible insects in any format? If no, would you be willing to try insects?

Respondents	If no, would you be willing to try insects?
1	no
2	Yes, as long as it didn't look like an insect
3	no because I'm vegeterian
4	Yes
5	yes
6	Not at all
7	Yes
8	No
9	im vegetarian
10	yes
11	yes
12	Yes
13	yes
14	I think so
15	no, I'm vegan
16	NO
17	I would not be keen
18	No
19	If they don't move in my mouth then yes
20	maybe
21	yes
22	maybe
23	yes
24	yes
25	Yes
26	No
27	no
28	definitely
29	not sure
30	Yes
31	yes.

00	Vaa
32	Yes
33	Yes
34	Yes
35	No.
36	Maybe
37	Yes
38	No
39	Perhaps
40	Yes
41	yes
42	Yes
43	Yes
44	bbq roasted meal worms - at a science museum (I was 12)
45	Yes
46	Yes, particularly ground into meal in flours etc.
47	Yes as long as it doesn't look like an insect!
48	yes
49	Yes
50	yes
51	Yes
52	Yes
53	yes
54	No
55	Maybe
56	Yes
57	yes
58	Yes
59	yes
	Probably not
61	Yes
62	yes
63	Yes
64	Yes
65	If they were processed is some form
66	Maybe
67	yes
68	Yes
69	Yes
70	Maybe
70	yes
71	Yes
72	Yes
73	Yes!
74	Depends on so many factors
75	Depends on so many racions

76	I'm vegetarian, so no
70	Yes
78	unsure
78	Might not
80	Yes
81	I do not think so.
82	yes love to
83	Yes I would try
84	not at this point
85	yes,
86	vegan
87	Maybe?
88	Yes
89	Possibly
90	no
91	Not sure
92	Yes, why not
93	yes, I would like to try
94	Maybe
95	probaby not
96	Yes
97	l'm vegetarian so probably not
98	Yes
99	Definitely
100	Yes
101	No
102	No
103	As long as it didn't look like an insect, I'd be happy to try it.
104	Possibly
105	Definitely
106	No I am vegan
107	Yes
108	Unsure
109	Potentially
110	no
111	Insect-based foods, yes.
112	No
113	I think so, may need some convincing but may depend on my mood at the time
114	Maybe, depending on what it was. Not live ones!
115	Not knowingly
116	Yes, particularly if it was part of a product and not as a whole insect
117	No
118	Yes
119	Probably not, I am currently vegan.
113	r robusty flot, i all outfolity vogali.

120	I would yes
120	No
121	Maybe
123	No.
124	yes however I doubt it would become common within the UK so don't expect to
125	Yes
126	yes
127	yes
128	Maybe
129	Possibly
130	yes
131	yes
132	Not particularly
133	maybe
134	No
135	yes
136	Yes
137	no
138	Yes
139	No
140	yes
141	No
142	Yes
143	I think I would rather eat them in a recognisable form rather than ground up and used as an ingredient in other foods.
144	Depends on how they are cooked / prepared
145	maybe
146	Me personally no, because I am vegan however I had previously read into insect protein and understood the premise but when there are alternatives available such as plant proteins (pea etc) I'll opt for those
147	No
148	yes
149	No
150	I'd give it a go.
151	Yes
152	Yes
153	Possibly if as part of a dish, the texture is too offputting alone.
154	Yes
155	No
156	never
157	Yes
158	Yes
159	yes
160	yes
	·

4.04	Voo
161	Yes
162 163	Yes
	If they are in a product then yes but i wont eat them straight up
164	Yes
165	unsure
166	no
167	yes Definitely
168	Definitely
169	Yes, would be happier if they were ground up into something rather than whole
170	Depends what it looks like
171	yes
172	Yes
173	No
174	Yes
175	Perhaps
176	yes in powder
177	no
178	yes
179	Yes if they were incorporated into products, for example in the form
	of insect flour.
180	Not really
181	Yes!
182	Yes
183	Absolutely
184	I would, but I think I would have an issue with the appearance
185	Yes
186	maybe
187	Yes
188	No, I'm allergic to crustaceans so may be allergic to these.
189	yes
190	Maybe
191	Depends on how they are presented (whole or ground etc)
192	No
193	Yes
194	No, I am vegetarian.
195	Potentially
196	Yes, but in a form when I can not see its an insect, e.g. powder, a bar.
197	No
198	Don't know
199	Possibly
200	Yes
201	No, the whole idea repulses me
202	Yes
203	No

204	No
205	no
206	Yes
207	Depends not as an insect but as flour etc would be fine.
208	Yes possibly in baked form using flour
209	yes
210	Possibly
211	Yes
212	yes. But I would be happier with insect derived protein added to food as I am not too keen on the look and do not think I would like the texture.
213	yes - as long as crunchy consistency, I do not fancy soft maggots/larval stages; woudl dfeintly find raosted locusts quite appealign to try - some of it might be quite similar to prawns etc
214	Yes
215	I have an allergy to chitin. This is often overlooked.
216	Yes
217	Not sure, I don't eat meat right now and I'm not sure how this would fit in
218	Yes

Question 15: Does your home country eat insects as part of their regular diet? If Yes, where are you from?

Respondents	If Yes, where are you from?
1	Mexico
2	Mexico
3	China
4	The Provence of South China
5	Thailand
6	Mexico
7	Parents are Indian
8	Indonesia
9	Nigeria
10	China, in some provinces, insects diet is very prevailing
11	I have no idea whether people eat insects as part of their diet although I don't believe it to be common
12	New Zealand
13	Mexico
14	Mexico
15	Thailand
16	mexico
17	Oman. people eat locust only but not as regular food
18	México
19	

Question 22: Would you be willing to purchase insect ingredients if they cost more than

similar products? Maybe - under which circumstances?

Respondents	Maybe - under which circumstances? Maybe - under which circumstances?
. 1	If instead of wheat flour, if it is a particularly cheap cut of meat, I haven't really made up my mind yet on how much I would be willing
	to pay
2	It would depend on how much preparation would need to be done on the ingredient
3	flavour and perception
4	increased nutritonal value
5	If we were aware of the environmental impact
6	If I got my head round using them
7	If they didn't cost much more, and tasted good
8	If they were healthier alternatives
9	Sustainability credentials
10	If the alternative was very damaging to the environment or of poor quality
11	Depends on the information regarding welfare and ethics in production
12	As a staple, maybe not, depending on the nutritional content.
13	If they were very sustainable
14	Better for the environment
15	If they didn't cost much more, and tasted good
16	My current view is that they're expensive but this is because I live in the UK and they aren't widely available here. I would purchase more out of curiosity as a one off - not for common usage
17	If tastier
18	if they were going to be more sustainable as a protein source.
19	If they tasted better or had a better texture to similar products may pay more
20	If they are proven to be healthier or more sustainable
21	If they were better for the environment
22	if they were moderately more and provided benefits as above
23	If they taste nice
24	reduced carbon footprint
25	Taste better than alternatives
26	if they provided a premium effect
27	for giving a try
28	If they were substantially better for the environment, I'd prefer to pay less though
29	Out of curiosity and maybe if it turns out to be a good way to replace animal protein
30	If they were better nutritionally and tasted better
31	Improved ethics
32	if I felt motivated to encourage the success of the product, as I am motivated to encourage and use my own market force to grow the market for edible insects
33	Flavour, environmental benefits, how intriguing
34	if the product was better or more sustaible and proved to be

35	if they tasted good, and provided benefits to the environment and the body
36	If they were needed for a specific dish
37	If i found that they tasted well in dishes I already like
38	it is the overall value nutritionally or sensorily that needs considering
39	they are demonstrably better for the environment and taste good
40	If they were more sustainably produced
41	If there were many benefits and if these were explained to me
42	it depends their value
43	to be environmently friendly
44	If they add a more desirable characteristic, or if they were more locally sourced
45	If they are more nutritionally beneficial for me AND I can tell little difference between the two products
46	If I had tried insects and liked them
47	if they are better quality than other similar products
48	Dependent on my liking of the insect ingredient and the nutritional value of it
49	Nutritional quality and ease of foodstuff use
50	Depends on how much I want to try it
51	If it was something I enjoyed consuming then I would consider it.
52	If there was strong proof of its sustainability
53	If the overall environmental impact is lower
54	if i enjoyed cooking and eating them
55	In times of food shortage
56	If they didn't cost much more, and tasted good
57	for balanced diet, add flavour
58	If there was any reason why I would not want to buy the cheaper option (ethical/environmental)
59	To try it out, especially powdered. Not if textured as normal.
60	Depends on the product and the taste
61	if I tried them and though they were better/tastier than other ingredients
62	If I had tried insects and liked them
63	As long as the price is not really high
64	Dependant on nutritional values and economical contribution
65	if there were recipies more available and people has less of an adversion to seeing them
66	never willing to purchase
67	If I liked the taste of the product
68	If i found i liked it more than the similar products then maybe
69	superior taste/cooking potential
70	If i knew i liked them
71	Depends on other factors such as food mileage, weight for weight value etc.
72	tasted better/ better for environment/ ethically produced
73	to get protein when avoiding meat

74	If they tasted better or knew they were better for me
75	If they were in a powdered form rather than whole insects
76	If I enjoyed them more and if they had superior health benefits.
77	High quality, easily metabolized protein
78	Flavour of products
79	if I wasn't a student with very little money
80	I'm currently a vegetarian so it is difficult to answer this question. Some meat alternative products are more costly than meat so perhaps I would still be happy to pay more for insect ingredients. I am more concerned about environmental impact and sustainability of the products I purchase rather than what they cost.
81	If the production of them was proven to be more environmentally sound. If they tasted ok.
82	If organically produced
83	If the benefits of eating insects was clearly explained
84	particular dishes. See no difference from crustaceans.
85	depends on the product
86	If I knew that it was substantially better than other products

Question 25: What type of products would you expect to contain cricket powder? Select all that apply. Other (please specify)

Respondents	Other (please specify)
1	pet food
2	Burger, sausage style foods - like quorn type products, but made with insect protein instead
3	World foods
4	Т
5	8
6	I have no idea - also your previous question assumes I have an idea of what it tastes like, and I have no idea so it's asking a blind person what they thing red is like.
7	Protein powder - health supplements
8	takeaways
9	Supplements
10	I have no point of reference for Q23-27 as I don't know what cricket powder tastes like or it's traditional uses
11	bread/other carbohydrates
12	Packed ingredients for cooking, baking or adding to salads
13	if I had to pick one, health products but I wouldn't expect any
14	Protein shakes
15	'Meat' Substitutes - like quorn products, but with insects

Question 26: what occasions would you associate with the potential of eating products

containing cricket powder? Select all that apply. Other (please specify)

containing cheket pe	Swder's Select an that apply. Other (please specify)
Respondents	Other (please specify)
1	Н
2	it doesn't matter
3	i think the flavour repeats on you a bit (for this question and next question). Don't know what you mean by supper.
4	When protein requirements are higher e.g. unintentional weight loss/wound healing.
5	Supplements
6	I have no point of reference for Q23-27 as I don't know what cricket powder tastes like or it's traditional uses
7	any meal
8	post-workout

Question 31: Do you have any dietary restrictions or lifestyle choices which may prevent you from eating insects? (edible insects are an animal product which have been linked with having similar allergen restrictions to shellfish) If yes, please give details.

Respondents	If yes, please give details
1	Vegetarian
2	vegetarian
3	Vegetarian - but would support eating insects in lieu of traditional animal products to promote it's normality and change society's perception that people need meat.
4	Histamine intolerance Pescatarian
5	vegan
6	vegan
7	I am pescatarain, but happy to eat insects
8	Vegetarian
9	I a vegetarian but for sustainability and lifestyle reasons.
10	I have a shellfish allergy
11	Vegan
12	Vegan
13	I am vegetarian, but would most likely eat insects if they were ethical in my view and cheap
14	I eat plant based. But I would try insects
15	Shellfish fish nuts egg
16	trying to be vegan but would eat insects as I struggle to get protein as it is so good alternative as wouldn't bother me about welfare and if it's good for the planet
17	The idea of eating insects is repellent and would stop me eating the food

18	Vegetarian
19	Vegan, but still curious about insects as a protein source
20	Vegetarian
21	vegan
22	Vegetarian for ethical reasons
23	Vegan
24	Vegan
25	I would not eat them as I am vegan
26	Shellfish allergy
27	vegan
28	vegetarian
29	I have eaten insects in the past, as I lived in East Africa. They're nothing very exciting really.
	Now I am vegan, I have no desire to eat animal products, and wish we would concentrate on plant proteins.
30	Vegan
31	Vegetarian
32	allergic to shellfish
33	Re question 30 - I wouldn't know how to rank this.
34	Allergy to seafood
35	I don't eat a lot of meat, I prefer to eat plant-based products on the whole.
36	vegetarian
37	Celiacs
38	Vegetarian
39	Pescatarian
40	Following vegetarian/vegan diet
41	vegetarian anyway, if forced to choose though I would eat meat / fish over insects easily
42	No but I just don't fancy it
43	Pregnancy currently so would need to know possible risks.
44	I am a vegetarian
45	Vegan
46	Vegetarian for over 40 years
47	Pescatarian
48	Vegetarian

Question 38: If your answer was bad or indifferent - what is your reason? Select all that apply. Other (please specify)

Respondents	Other (please specify)
1	Don't like the packaging and looks sweaty inside
2	photo does not appeal

3	The packaging is quite dark not giving an exciting emotion about it.
4	never tried it before so don't have any idea of how it tastes, but looks a little slimy
5	Repelling package
6	I've eaten products containing insect protein before

Question 40: Would the potential ethical / sustainability benefits of meat reduction using insects be enough for you to consider trying the new product? Maybe - please specify additional requirements.

Respondents	Maybe - please specify additional requirements
1	Novelty
2	yes, so long as it is similar price or not excessively expensive.
3	Depends what the ingredients of new product is and its visual presentation
4	Depends on the product
5	&
6	Main emphasis on try
7	it would take a bit more convincing to try eating products containing insects
8	As a protein replacement but putting in pasta is not doing that is it. We eat enought protein. Burger is a better proposition
9	still a bit squeemish
10	it would be something i would consider, but i would not consider it to be a good enough reason on its own
11	If benefits were significant and had sound evidence behind them. If we needed a drastic change to our meat consumption in the world for some reason.
12	upon trying the product if i liked it i would continue to eat it however if i did not like it i would go back to meat regardless
13	depends on whether the sustainability of the insect production was good enough to exceed the potential flaws of meat production, some insect systems require a lot of energy for growth
14	Never tastedbut the thought puts me offmaybe I need educating!
15	Flavour masking would need to be very effective
16	depending on the price - I would be willing to pay a little more but if it is too expensive I couldn't afford it
17	Not concerned about sustainability - more concerned with nutrition aspects
18	if tried and tested products
19	Nutrition
20	if it doesn't have any difference in texture