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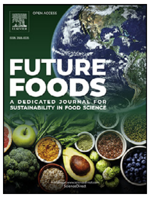

# Extraction, characterisation and functional applications of sustainable alternative protein sources for future foods: A review

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ABSTRACT

For many centuries, animal proteins have been used as the conventional food proteins in the food industry to produce a variety of food products due to their functional properties that range from gelation, water holding and binding capacity, foaming ability and stability, solubility, and emulsification. However, the production of proteins from animal sources does come at a cost due to its impact on the environment. Thus, the search for potential replacers for animal proteins that are sustainable, cheaper, and environmentally friendly has resulted in the exploration and the incorporation of alternative proteins in different food product formulations. Even though studies have been carried out to investigate the functional properties of several alternative protein sources, there are technological, sensorial, and nutritional challenges that still need to be overcome in order to make alternative protein sources a more feasible exchange for animal-based proteins. Additionally, the impact of processing on the functional and structural characteristics of alternative proteins in addition to the health impact still need to be understood. Therefore, this review discusses alternative protein sources that have been researched and documented as potential substitutes for animal-based protein sources. The extraction, characterisation, functional properties, and the nutritional quality of alternative proteins in comparison to the conventional animal-based protein sources will also be discussed. Additionally, this review aims to highlight opportunities and challenges of incorporating alternative proteins in food processing and manufacturing.

## 1. Introduction

The increase in global population has a concomitant impact of the demand of food production for adequate nutrition. As such, there is a progressive use of land for animal and plant production which could potentially be devoted to agriculture and this compromises the provision of adequate human nutrition (Tessari et al., 2016; Willett et al., 2019). Additionally, modern food production has been reported to play an important role in the “environmental footprint” (Khan et al., 2009) with the Inter-Governmental Panel on Climate Change (Mbow et al., 2017; Shukla et al., 2019) reporting that 21 – 37% of total greenhouse gas (GHG) emissions are attributable to the food system. Recently, GHG life cycle assessment indicated that livestock production contributes approximately 18% to the global anthropogenic GHG emissions which accounts for 37% of anthropogenic methane and 65% of anthropogenic nitrous oxide and dairy cattle sector 4% of total anthropogenic GHG emissions (Patra, 2014). The FAO, in their Global Livestock Environmental Assessment Model (GLEAM) (Anon, 2017)) identified that “Livestock supply chains emitted an estimated total of 8.1 gigatonnes carbon dioxide ($CO_2$)-eq in 2010 (using 298 and 34 as global warming potential for $N_2O$ and $CH_4$ respectively). Methane ($CH_4$) accounted for about 50% of the total emissions. Nitrous oxide ($N_2O$) and ($CO_2$) represent almost equal shares with 24 and 26%, respectively.” Furthermore commodity analysis identified meat production from beef cattle, buffalo and small ruminants as the largest emitters.

Animal proteins have been used as conventional food proteins in the past and presently still constitutes a large part of food production. Animal-based proteins, predominantly milk and egg proteins, have been highly studied for many decades. However, some authors have shown the production of 1 kg animal protein to require ~ 6 kg plant protein (Aiking, 2014; Pimentel et al., 1975; Pimentel and Pimentel, 2003). Consequently, the large-scale production of animal proteins by means of factory farming is reported to be a major driver of the loss of biodiversity, climate change, and the depletion of freshwater (Aiking, 2014). Conversely, in view of production cost, sustainability and availability, plant storage proteins have increasingly been used as a robust alter-

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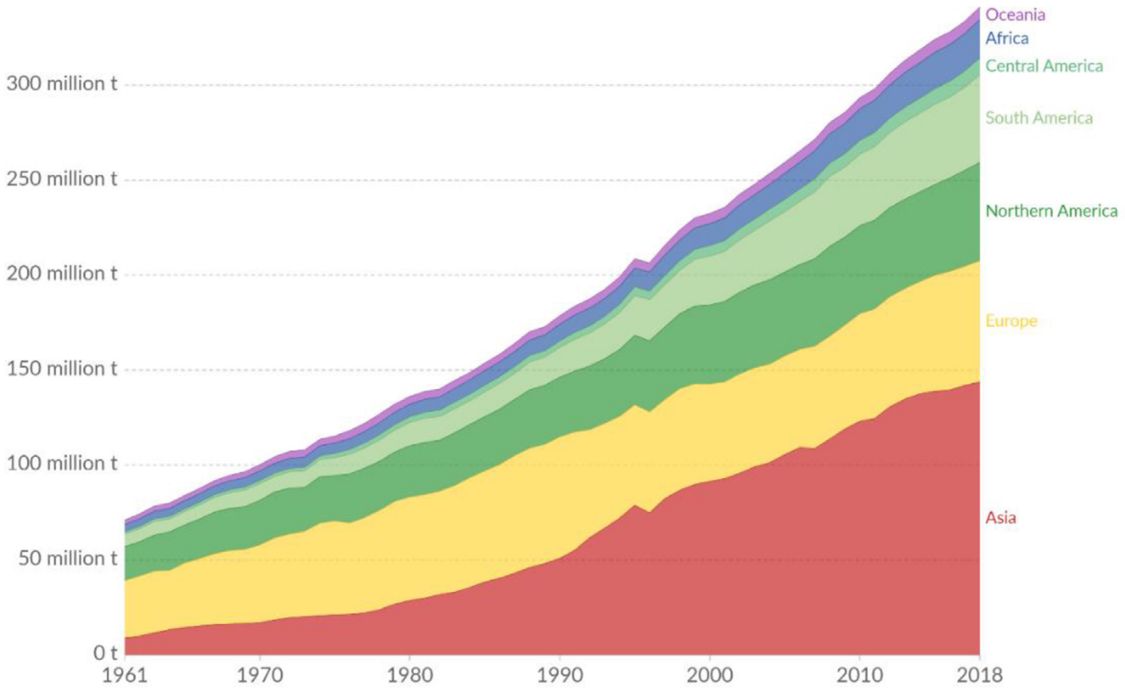

**Fig. 1.** Global meat production from 1961 – 2018. Source: UN Food and Agricultural organisation (FAO). The use of this figure is allowed under Creative Commons license: https://creativecommons.org/licenses/by/4.0/.

native to animal proteins (Munialo et al., 2018, 2015, 2014). In recent years, the use of alternative protein sources (such as plant-based proteins like quinoa, insects, and fungi) has been explored as potential replacers for animal-derived proteins (Maurya and Kushwaha, 2019; Onwezen et al., 2021) and the national strategies being developed and adopted to realise this diversification of dietary protein elegantly reviewed by Clark (2021). Notwithstanding the fact that plant-based proteins are preferable from a land use and GHG emission point of view than animal-based proteins, there are also environmental concerns over plant proteins. Such criticisms mainly relate to the use of plant-based protein for animal feed and the concomitant increased environmental pressures that is mainly associated with the development of industrialised farming systems to meet this demand (Henchion et al., 2017).

There has been a growing awareness of the environmental impact of animal production and the claimed health and nutritional benefits of plant-based diets. As such, one would imagine that there should be a global decline in animal production. Contrastingly, Food and Agricultural Organisation (FAO) has shown an increasing trend in the production of meat (Fig. 1) with the developed countries consuming more meat per capita than the developing countries (Figs. 2 and 3). Proteins from both animal and non-animal origins are a major nutritional component, providing both non-essential and essential amino acids (those that cannot be synthesized by the human body and would therefore depend on nutrition for their provision) (Tessari et al., 2016). The increasing demand for proteins arises from population growth and is driven globally by socio-economic changes such as increased urbanisation, an increase in income, and an increased recognition of the contribution of protein to healthy aging, and recognition of the role of protein in a healthy diet (Henchion et al., 2017). Studies in, for example, Africa identified that increasing urbanisation was associated with increased consumption of meat, dairy and fish at home and in the form of processed foods (Cockx et al., 2019). At least part of this is down to animal products being viewed as superior in nutritional quality than plant-based sources (Berrazaga et al., 2019).

To be able to use alternative proteins, there is need for these proteins to be characterised in terms of their functional and structural properties. The enhancement of texture, gelling, emulsifying or foaming ability are some of the specific functional properties that are important within formulations. The functional properties of proteins extracted from pseudocereals such as quinoa, buckwheat and amaranth have been reported in literature (Alonso-Miravalles and O'Mahony, 2018). Research has been conducted on future foods that include insect proteins, cell-cultured meats, and pseudo cereals among others with the aim of establishing their functionality, processing, and industrial applications (Colgrave et al., 2021; Sexton et al., 2019; Tan et al., 2021). Additionally, there has been a growing interest in the valorisation of by-products or waste streams obtained from other processes as a way of contributing to food security and sustainable food production (Platt et al., 2021). However, knowledge gaps still exist and as such a need for further research on the impacts of alternative protein sources on human health (e.g., food allergies) as well as understanding their bioavailability in relation to other characteristics such as structure, taste and flavour. Even though there is presumption that a shift to alternative proteins should lead to healthier and overall, more sustainable diets, this depends on the nature of the shift, e.g., shifting from processed meat to another nutrient-poor, highly processed protein source might not provide the desired health benefits. Therefore, in this work we aim to review the various methods that are used in the extraction of proteins from alternative sources and discuss how this impacts the structure and functional characteristics of alternative protein sources. Additionally, we will review the nutritional quality of alternative protein sources and shed some light on the opportunities and challenges of incorporating alternative proteins in food processing and manufacturing.

## 2. Alternative protein sources

Given that many protein sources have been investigated as potential and sustainable alternatives to animal protein, this review focuses on those with the most potential, namely insect, fungal, and a selection of plant-proteins.

### 2.1. Insects

Edible insects contribute to increased human nutrition in many parts of the world due to their high protein, lipids, energy, and various mi-

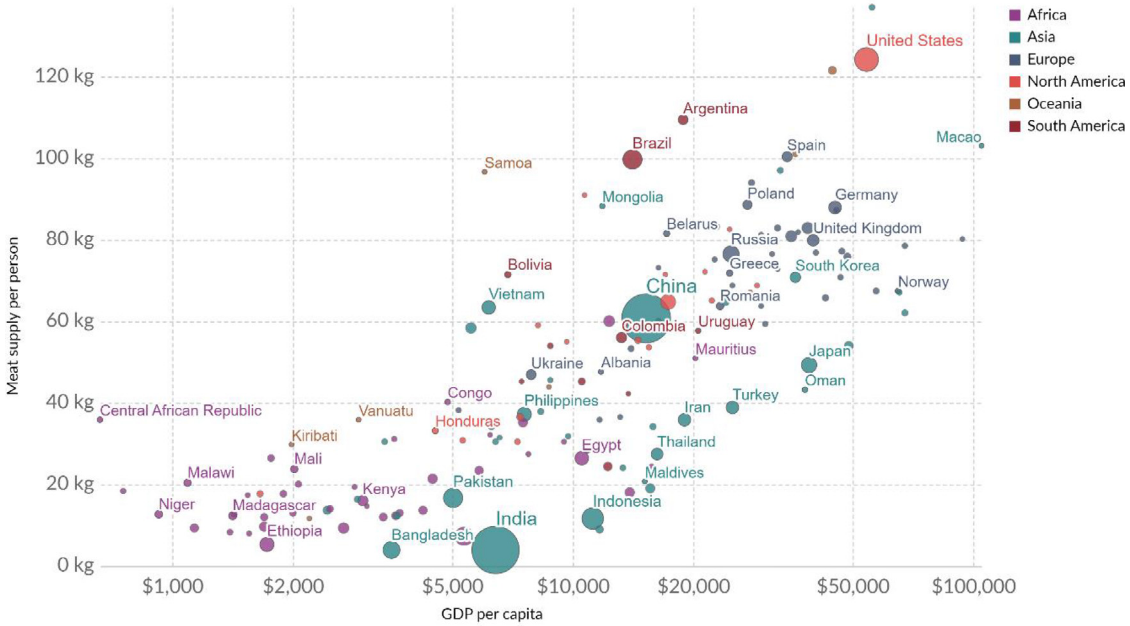

**Fig. 2.** Meat consumption versus gross domestic product (GDP) per capita in 2017. The average meat consumption per capita is measured in kg/year versus GDP measured in 2011 international unit - $. Source: UN Food and Agricultural organisation (FAO). The use of this figure is allowed under Creative Commons license: https://creativecommons.org/licenses/by/4.0/.

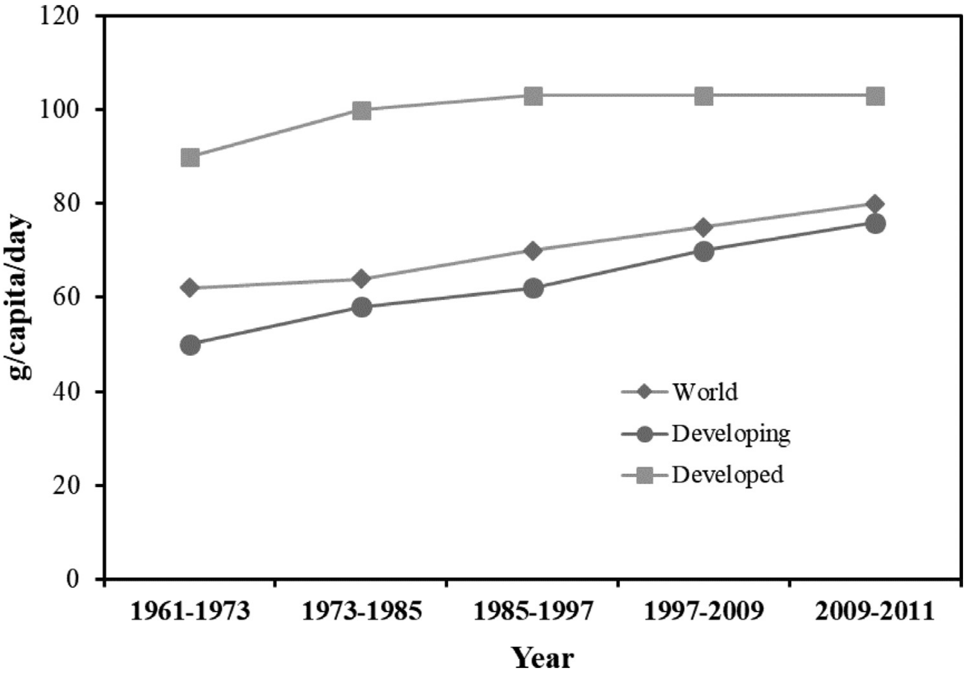

**Fig. 3.** Evolution in protein consumption per capita (g/capita/day). Authors' analysis based on food balance and population data obtained from http://faostat3.fao.org.

cronutrients content (Kouřimská and Adámková, 2016). According to Akhtar and Isman (2018), there are approximately 1500 – 2000 edible insects species recorded worldwide with the most common being those that are readily available. The protein content of different edible insect species is presented in Table 1 and the comparison among either raw or cooked edible insects, reptiles, fish, mammals, or a selection of proteins from plant sources is presented in Table 2. Some raw edible insects such *Sphenarium purpurascens* have a considerably higher protein content than fish, beef, chicken, lamb, pork and several plant-based proteins.

### *2.2. Fungi*

Continuous fermentation of the filamentous fungus *Fusarium venenatum* (PTA 2684) produces a high-protein, food ingredient known as mycoprotein. This fungus was found in the wild and was later developed specifically for the production of food-grade mycoprotein that has been in production since 1985 (Finnigan et al., 2019). During the production of mycoprotein, first the organism is grown in an aerobic, axenic, continuous air-lift fermentation system in a broth composed of food-grade carbohydrate substrates and other components that are needed for growth.

**Table 1**
Insect order, their percentage availability and their protein content (% dry matter basis).

| Order and % availability | Insect examples | Stage | Protein content range (%) |
|---|---|---|---|
| Coleoptera (31%) | Beetles,<br>Weevils | Adults and larvae | 23 - 66 |
| Diptera (2%) | Flies | Larvae | 37 - 63 |
| Isoptera (3%) | Termites | Larvae and Nymph | 38 - 64 |
| Lepidoptera (18%) | Butterflies,<br>Moth | Pupae and larvae | 14 - 68 |
| Hemiptera (10%) | True bugs | Adults and larvae | 42 -74 |
| Homoptera | Mealybugs | Adults, larvae and eggs | 45 - 57 |
| Hymenoptera (14%) | Bees, | Adults, pupae, larvae and eggs | 13 – 77 |
| Odonata (3%) | Dragonflies, Damselflies | Adults and naiad | 46 – 65 |
| Orthoptera (13%) | Grasshoppers,<br>Locust,<br>Crickets | Adults and nymph | 23 – 65 |
| Others (5%) | - | - | - |

Source: Modified from Akhtar and Isman (2018). Insects as an Alternative Protein Source in Proteins in Food Processing (Second Edition) pp. 263-288.

**Table 2**
A comparison of the average protein content (g/100 g fresh weight) of raw or cooked edible insects, reptiles, fish, and mammals.

| **Animal group** | **Species and common name** | **Edible product** | **Protein content (g/100 g fresh weight)** |
|---|---|---|---|
| **Insects (raw)** | Locusts and grasshoppers: *Locusta migratoria, Acridium melanorhodon, Ruspolia differens* | Adult | 13 - 28 |
| | Locusts and grasshoppers: *Locusta migratoria, Acridium melanorhodon, Ruspolia differens* | Larvae | 14 - 18 |
| | *Sphenarium purpurascens* (chapulines – Mexico) | Adult | 35 – 48 |
| | Silkworm (*Bombyx mori*) | Caterpillar | 10 – 17 |
| | Palmworm beetles: *Rhynchophorus palmarum, R. phoenicis, Callipogon barbatus* | Larva | 7 – 36 |
| | Yellow mealworm (*Tenebrio molitor*) | Larva | 14 – 25 |
| | Crickets | Adult | 8 – 25 |
| | Termites | Adult | 13 – 28 |
| **Reptiles (cooked)** | Turtles: *Chelodina rugosa, Chelonia depressa* | Intestine | 18 |
| | | Liver | 11 |
| | | Heart | 17 - 23 |
| | | Flesh | 25 - 27 |
| **Fish (raw)** | Crustaceans | Lobster | 17 - 19 |
| | | Malaysian prawn | 16 - 19 |
| | | Shrimp | 13 - 27 |
| | Finfish | Tilapia | 16 – 19 |
| | | Mackerel | 16 - 28 |
| | | Catfish | 17 - 28 |
| | Mollusc | Cuttlefish, squid | 15 - 18 |
| **Cattle** | | Raw beef | 19 - 26 |
| **Chicken** | | Raw | 26 |
| **Lamb** | | Raw | 25 |
| **Pork** | | Raw | 27 |
| **Mycoprotein** | | Wet weight | 11.5 |
| **Some Plants** | Soy | Soybeans, green, raw | 12.95 |
| | Peas | Green raw | 7.9 |
| | Lupins | Uncooked | 36 |
| | Quinoa | Uncooked | 24 |

Source: Modified from Van Huis et al. (2013).

Once the fermentation is in the steady stage growth phase, batches of the mycelium of the fungus are harvested, subsequently heat-treated to reduce the ribonucleic acid (RNA) content since the nucleic acids of RNA are a risk factor in gout (Hafez et al., 2017). Once the RNA level is reduced, the mycelia are recovered by centrifugation to give a paste with 75% water content. This paste is the mycoprotein. In the case of *F. venenatum* (PTA 2684), the hyphae are of a length and diameter that make them suitable for creating meat-like texture in products. Binders (typically egg white), flavourings, and other ingredients are added, and the mixture processed further to achieve the desired organoleptic and physical properties. Mycoprotein is typically 12% water, 3% fat, 3% available carbohydrates, 6% fibre, 2% ash, and 56% total protein (N × 6.25) (Gilani and Lee, 2003).

### *2.3. Plant proteins*

Plant-based proteins (particularly legumes) have gained popularity amongst food scientists, mainly as a response to consumers' growing interest in a perceived more economical and environment-friendly diet, in addition to the rise of veganism and vegetarianism in western cultures (Gravel and Doyen, 2020). The most common example is soy protein, which has been linked to good gelation properties that are essential in the formulation of simulated meats and tofu (Singh et al., 2008). Other plant-based proteins that have been researched include pea proteins (Munialo et al., 2014, 2015, 2014), lentil (Jarpa-Parra, 2018), almond (Maykish et al., 2021) quinoa (Alrosan et al., 2022), lupins (Pelgrom et al., 2014), and amaranth protein (Bressani and Garcia-Vela, 1990) among others.

## 3. Extraction of alternative protein sources

Protein from any given source is a heterogeneous mixture of different types of proteins. Extraction and purification of the protein using different methods may result in a different protein profile, quality, and functionality (Ismail et al., 2020). Protein isolates of animal-, plant- or even edible insect-based flours can be obtained using a variety of methods and techniques which mainly depend on the raw material and its proximate composition (Boye and Barbana, 2012). However, it is still possible to describe a general 5-step food protein processing procedure which includes: (i) Pre-treatment, (ii) Defatting, (iii) Protein solubilisation and recovery, (iv) Protein purification, and (v) Drying.

For the extraction of a protein isolate from insects, the first step usually involves the insects being dried by either a freeze-drying process or by being cooked in the oven, followed by the insects being ground and sieved to produce a fine powder. The fine powder increases surface area, promoting better protein-solvent contact in the subsequent extraction steps. Some authors have used a defatting step to improve insect protein yield at the laboratory scale (Choi et al., 2017). An increase in the protein content of both *Tenebrio molitor* and *Hermetia illucens* from 58 ± 1.2% to 65 ± 0.3% and 35 ± 0.2% to 45 ± 1.4, respectively, was observed when insect flour was defatted in hexane (Bußler et al., 2016). Others have observed a protein yield increase of 28% for mealworm and 34% for cricket following defatting and subsequent sonication. These results as well as the documented high fat content of most comestible insects (Tzompa-Sosa et al., 2014) emphasise the importance of the defatting step in producing insect-based protein-enriched ingredients. Defatting has also been used during the processing of soy flour and was shown to result in enrichment of protein from 37 g/100 g to 45 g/100 g dry basis (Xing et al., 2018).

Various methods have been used in the extraction of insect protein isolates. For instance, an aqueous solubilisation method was used by Yi et al. (2013) to obtain protein isolates from the supernatants of 5 different insect species, achieving protein yields and purity ranging from 17 to 23% and 50 to 61%, respectively, whereas Chatsuwan and colleagues obtained protein isolates from *Patanga succincta* and *Chondracris rosea* with extraction efficiencies of 49% and 43%, respectively (Chatsuwan et al., 2018). Furthermore, a 51.7% protein yield and 82.3% purity was obtained by Purschke et al. (2018) who produced a protein concentrate from *Locusta migratoria* where 1 M or 4 M NaOH was used to enhance the solubility of the protein. These findings show the potential of both aqueous and alkaline solubilisation being used for insect protein isolate production.

The processes that are commonly used for production of plant-based protein concentrates (50-80% protein) and isolates (> 80% protein) can be broadly categorised into aqueous and dry fractionation. Raw material containing a high oil content, such as pulses and oilseeds, first undergo oil extraction. Taking sunflower seeds as an example, the process consists of milling, solvent extraction of oil (using organic solvents such as hexane or ethanol), dispersion in extraction solvent (NaOH or water), centrifugation, membrane filtration and drying. The membrane filtration usually entails ultrafiltration to concentrate the protein to either a concentrate or an isolate depending on protein content. Concentrate and isolate production can also include an additional step of acid precipitation of protein at their iso-electric pH, to facilitate the concentration process (Berghout et al., 2014; Geerts et al., 2018).

Dry fractionation involves milling of the seeds and solvent extraction of oil followed by air classification where the protein particles are separated from starch granules and husks in a cyclone-type separator (Pelgrom et al., 2013; Schutyser and Van der Goot, 2011). The aqueous extraction process gives a higher purity than dry fractionation (45–55% protein) although it is more expensive than dry fractionation due to a larger number of processing steps, the yield of protein is also lower and the use of NaOH can lead to safety issues. Preparation of concentrates by aqueous extraction is cheaper than isolate preparation (but still more expensive than dry fractionation). Dry fractionation of de-oiled sunflower cake is very cheap to produce after the initial capital purchase cost of the separation equipment. It gives the highest protein yield but lowest purity (protein concentrate/flour) (Ismail et al., 2020).

Ultrafiltration has been used in the extraction of proteins from various sources. Protein fractionation using tangential ultrafiltration was applied using a membrane molecular weight cut-off of ≤300 kDa on algae proteins solubilised at pH 7 and 12 (Ursu et al., 2014). However, most of the proteins remained in the retentate, which was attributed to the molecular size of the complex macromolecular aggregates of algae proteins being above the molecular weight cut-off. However, both permeate and retentate solutions showed excellent emulsifying properties (Ursu et al., 2014). Emulsification and foaming properties of rapeseed protein fractions that were prepared via ultrafiltration with a membrane of molecular weight cut-off of ≤8 kDa were higher compared to extracts that were prepared using the aqueous extraction method comprising of alkaline solubilisation and acidic precipitation methods (Dong et al., 2011). Lonchamp and colleagues obtained a retentate via a ≤100 kDa ultrafiltration from mycoprotein (Quorn) fermentation co-product (centrate) which displayed good foaming stability, emulsifying, and rheological properties (viscosity, viscoelasticity, and gelation) (Lonchamp et al., 2020a). Membrane filtration has yet to be explored with insect proteins given that a knowledge of the protein size and properties is required, and little information on insect proteins is currently available. It is also worth noting that even though membrane processes are considered to be more efficient, environmentally friendly and economical, their main shortcoming is membrane fouling, thus a trade-off between productivity and selectivity emerges, especially when pure isolates are an ultimate goal.

The final step in protein extraction is the drying of the extract. Drying of protein extracts, in particular insect extract can be done by oven-drying and microwave drying (Melgar-Lalanne et al., 2019). Although less commonly used, fluidized bed drying was also reported as an alternative drying method for insect flour production (Kröncke et al., 2018; Purschke et al., 2018) and this could have the potential of being used to dry insect protein extracts. Spray drying is also another common approach for drying liquid foods to powders (Jayasundera et al., 2009) but only recently has this been applied to insect protein (cricket) preparations (Bassett et al., 2021). Finally, freeze-drying can also be used for both wet solid and liquid samples (Assegehegn et al., 2019) and is the most commonly used laboratory scale technique in insect protein processing, even though the parameters used are not always well documented. The various drying methods and their effects on nutritional, functional and microbial properties have been compared by several authors (Huang et al., 2019; Kamau et al., 2018; Purschke et al., 2018; Vandeweyer et al., 2017; Womeni et al., 2012). As such, more studies are needed to establish which drying methods are suitable for example in the case of insect protein isolate where mass production is required.

The recovery of protein from waste or co-product streams to capture value and reduce environmental footprints is the focus of significant research interest (Platt et al., 2021). Processes such as chromatography have been developed to recover the protein from the liquid pot ale, a co-product of the distilling process by Horizon Proteins using ion-exchange chromatography (Traub Modinger, 2015). Although the protein content of pot ale is low (less than 1%), the volumes are very high and ion-exchange chromatography can be a very cost-effective method for the extraction of proteins from dilute streams as long as robust and inexpensive ion-exchange resins can be found. It turns out that zeolites or other calcium carbonate-based resins (which are very cheap) are suitable for the extraction and very high-volume throughput chromatography can be achieved, resulting in a high purity protein suitable for aquaculture uses.

## 4. Functional characterisation of some alternative proteins

Given that proteins from different sources will have different structural characteristics that contribute to differences in their solubility and reactivity under various extraction conditions, there is need for dry and wet extraction protocols to be developed which would enhance protein yield and purity while maintaining structural integrity and functionality (Ismail et al., 2020).

The functional properties of *Chlorella vulgaris* species of microalgae were determined following the use of alkaline solution at pH 12 to enhance the yield of proteins solubilisation The final protein matrix exhibited lower emulsifying capacity and emulsion stability, while solubilisation under pH 7 induces lower yield but proteins with higher emulsifying capacity and emulsion stability (Ursu et al., 2014). The water and oil absorption capacity and foaming properties of *Chlorella pyrenoidosa, Arthrospira platensis,* and *Nannochloropsis oceanica* species of microalgae protein have also been reported. *Nannochloropsis oceanica* exhibited the highest water absorption capacity per g of protein compared to *Chlorella pyrenoidosa* and *Arthrospira platensis* (Fradinho et al., 2020). The variation in the water binding capacity of the protein isolate was attributed to the protein conformation, hydrophilic-hydrophobic balance of amino acids and other intrinsic characteristics of the protein (Hyrslova et al., 2021).

Yi et al. (2013) extracted proteins from five insect species. In terms of the foaming ability, insect protein supernatant fractions showed negligible foam ability at a concentration of 3% w/v, at pH 3, 5, 7, and 10. The poor foaming ability of the insect protein supernatants was attributed to the protein concentration in the supernatant fraction solution (around 1.7% w/v) which were too low to generate stable foam. In terms of gelation, insect protein supernatants at pH 7 and a concentration of 3% w/v formed a weak gel, whereas at 30% w/v a strong gel was formed. In a recent study, Kim et al. (2020) extracted proteins from various edible insect species namely *Tenebrio molitor* L., *Protaetia brevitarsis* larvae, and *Allomyrina dichotoma* larvae. *P. brevitarsis* and *A. dichotoma* proteins exhibited excellent ability to form heat-induced gels. Higher apparent viscosity was observed for gels prepared with *P. brevitarsis* whereas, *T. molitor* had the lowest apparent viscosity.

Some authors have shown the nutritional composition of insect protein extract to impact the rheological properties of gels. Proximate fat content of *P. brevitarsis*, *T. molitor*, and *A. dichotoma* was 12.92%, 31.30%, and 17.04%, respectively (Ghosh et al., 2017). The gels prepared from *T. molitor* had lower gel strength that the others which was attributed to its higher fat content compared to the others. The fat in the gel matrix was hypothesised to act as a lubricant when it is subjected to external forces (Kim et al., 2020). This finding suggests the possibility of incorporating defatting as an initial step during protein extraction and this could yield protein fractions that have improved functional properties and mitigate against the impact of fat lowering the strength of gels prepared from insect proteins.

Lonchamp and colleagues carried out the functional characterisation of mycoprotein as a partial replacer of egg whites as foaming and/or gelling ingredient (Lonchamp et al., 2020b). In one of their studies, mycoprotein was sonicated and the foaming properties determined. The sonication process was reported to result in an increase in foaming ability, which was higher than that of a sonicated commercial whey protein concentrate. The mycoprotein also displayed good emulsifying ability in oil/water emulsions. Their study showed large hyphal aggregates to be present in solutions and gels, which correlated with their high viscosity and viscoelasticity as well as the foam stability (Lonchamp et al., 2020b).

Plant proteins tend to have inferior functionality compared to animal-based proteins as they tend to be more insoluble and often have associated off taste properties. The functional properties of these proteins are influenced by a plethora of factors that range from the sample conditions such as pH, extraction method, the nutritional composition among others. Functional properties of pea, soy, lentil, and lupine, among others have been reported as aforementioned. However, to diversify the existing protein sources with the aim of meeting the increasing demand for protein, it could be interesting to investigate the potential of using mixtures of these proteins in product development and investigate how this influences the attributes of the products as well consumer perception and acceptance of the final products.

## 5. Challenges in the application of alternative proteins in the food industry

### 5.1. Flavour and taste

One of the challenges of using plant-based proteins, such as legume proteins, in food is the persistent off-flavours that can be perceived by consumers (Ismail et al., 2020). Sensory descriptors that have been given to the off-flavours present in soy proteins include “beany,” “grassy” “green,” and “painty” (Rackis et al., 1979). These off-notes are attributed to lipoxygenase-initiated peroxidation of unsaturated fatty acids (MacLeod et al., 1988) which is commonly related to the source of the raw material, processing, and/or storage (Ismail et al., 2020). Flavour compounds such as aldehydes, saturated and unsaturated alcohols, alcohols, ketones, and their ester derivatives, and methoxypyrazines were reported in raw, stored, and cooked peas (Malcolmson et al., 2014). Many different strategies to mitigate against off-flavours and aromas have been attempted in pea and other plant protein sources (Mittermeier-Kleßinger et al., 2021; Trindler et al., 2021). These approaches can encompass the selection of germplasm with absent/diminished odd notes, better purification processes, added fermentation steps, the use of bitter inhibitors, post processing marinating/seasoning, matrix adaption for flavour (Mittermeier-Kleßinger et al., 2021) through to the latest developments such as the application of supercritical fluid extraction, using $CO_2$ and ethanol to remove the problematic compounds from pea protein isolate (Vatansever et al., 2022). However, this has been met with several technological challenges given that aroma is the sum of a pattern of the responses of numerous receptor types. This leaves a challenge to the food researchers and industry to carry out accurate flavour profiling which will lead to identifying approaches that eliminate the problematic off-flavours rather than attempting to mask them.

### 5.2. Allergenicity

All foods that contain proteins have the potential of being allergenic. A major concern for all emerging proteins for future foods, is their potential to cause immune-mediated adverse reactions, which are commonly known as food allergy. Case reports of allergic reactions to protein sources include the edible algae (Le et al., 2014) and the fungal protein source Quorn® (Hoff et al., 2003). Allergic reactions to different insects also have been described in countries where insect consumption is common (De Marchi et al., 2021). However, in the western countries where insect proteins are mainly used as ingredients to enrich fortified products, there remains a knowledge gap in understanding the possible effects that the different technological processes (such as blanching, pasteurization, and sterilization) in addition to heat processing as well as the complex food matrices that include starch, proteins, etc. may have on allergic reaction raising regions (epitopes) of the proteins, and how this could influence their allergenicity and susceptibility to gastrointestinal digestion. Some studies have reported a lack of significant impact on allergenicity following the boiling of allergenic shrimp samples (Samson et al., 2004) while others authors have reported an increased IgE-binding capacity based on crab and prawn studies (Abramovitch et al., 2013) even though this did not necessarily correlate with clinical symptoms (De Marchi et al., 2021). Several technological processes such as enzymatic hydrolysis have been investigated as a tool to reduce the allergenicity of insect proteins in different food matrices. Similarly, enzymatic hydrolysis is used to reduce allergenicity in milk proteins for commercial formulations (Guadix et al., 2006). Protein fragments with different molecular weights were obtained following enzymatic digestion of insects with the proteins in the 25 - 33 kDa range displaying greater stability to heat treatments and digestion (De Marchi et al., 2021). Even though this could improve allergenicity, the question still remains as to whether the resultant insect protein fractions retain the necessary functional properties for product development, as some authors have reported a remarkable decrease in both emulsifying ability and heat stability with a significant increase in degree of hydrolysis (from 27 to 35%) in whey protein (Euston et al., 2001).

### 5.3. *Protein solubility*

Solubility of proteins has been defined as “the maximum amount of protein that remains in a visibly clear solution (i.e., does not show protein precipitates, crystals, gels, or hazy soluble aggregates), or does not sediment at 30,000 g centrifugation for 30 min”(Ries-Kautt and Ducruix, 1989). It is, however, important to note that the aqueous solubility of proteins is a challenge to assess given that proteins at higher concentrations may form aggregates upon concentration or form gels (Jung et al., 2008). Additionally, some protein solutions at higher concentrations do separate spontaneously into two phases or more distinctive aqueous phases and this can hamper the use of conventional methods to determine protein solubility (Hofmann et al., 2018). Protein solubility that is often determined is apparent solubility given that the determination of true solubility of a protein as a hydrocolloid is difficult to define (Kramer et al., 2012). During protein extraction, the concentration and subsequent drying steps such as lyophilising protein and in the absence of an excipient can promote protein aggregation and a reduction in solubility. A good example of where an excipient is important is the drying of milk protein powders (Rupp et al., 2018). When milk protein concentrates were first developed using a membrane filtration process it was found that as protein content increased, solubility decreased. Subsequently it was realised that lactose acts as an excipient during drying and protects the protein against heat damage. Thus, the higher the protein content the lower lactose and consequently, lower solubility.

The solubility of proteins can be influenced by several factors, which can either be extrinsic and intrinsic. Extrinsic factors that influence the solubility of proteins include ionic strength, pH, temperature, and the presence of various solvent additives and the polarity of these solvents (Ries-Kautt and Ducruix, 1997). When these extrinsic factors are varied, the solubility of protein solutions can be increased (Bagby et al., 2001; Schein, 1993). It is however important to note that altering the conditions of protein solution conditions will not always be a sufficient or appropriate measure to increase the solubility of the proteins to the required extent (Ries-Kautt and Ducruix, 1997). Intrinsic factors that influence the solubility of proteins are on the other hand mainly defined by the number and type of amino acids that are present on the surface of the protein (Schein, 1993), the folded structure and for glycol- and lipoproteins, the structure, and the composition of the glycans and lipids (Kramer et al., 2012). In general, proteins that contain a large proportion of hydrophobic amino acids that have aliphatic side chains (such as tryptophan, phenylalanine, and tyrosine) tend to be insoluble or to have a lower solubility in water (Lodish et al., 2000b). Molecules that are hydrophobic in nature do avoid water by coalescing into an oily or waxy droplet and this enables these molecules to pack in the interior of proteins, away from the aqueous environment (Lodish et al., 2000a). It has been reported that the addition of glycans to proteins that are made up of large quantities of hydrocarbons increases the protein solubility in water. The properties of solvents such as pH, salt concentration and the presence of specific ligands can affect protein solubility as aforementioned under the extrinsic factors that affect protein solubility.

## 6. Nutritional properties of alternative proteins

Insects are a good source of essential amino acids, and are high in vitamins $B_1$, $B_2$ and $B_3$ and the minerals iron and zinc (Belluco et al., 2013). However, many insects have been reported to be deficient in certain amino acids, that include tryptophan and lysine, and as well as they contain chitin exoskeletons which have lower levels of digestibility (Henchion et al., 2017). In the cases where insect proteins are included in product design especially in cultures where the consumption of whole insects is considered unpalatable or not accepted, there is still need for research to be carried out to determine the impact of the type of preparation and processing on the amount of bioaccessible nutrients (Ojha et al., 2021). Additionally, there is a paucity of research regarding the influence of various innovative techniques and process parameters on digestibility of insects-based food systems (Acosta-Estrada et al., 2021; Elhassan et al., 2019).

Mycoprotein is high in protein and fibre and low in fat (Finnigan et al., 2019). However, its high RNA content of 10% of dry weight compared to beef liver and heart ofals which contain approximately 2 and 0.6% of RNA has raised some health concerns (Souza Filho et al., 2019). The consumption of excessive quantities of RNA can lead to an increased amount of uric acid in the body, which is a risk factor for gout (Denny et al., 2008). During the production of mycoprotein, the biomass of *Fusarium venenatum* undergoes a heat treatment where the fungal biomass (still in the broth) is rapidly heated to temperatures above 68°C, maintained at this 20 - 45 min, resulting in a reported reduction in RNA content to $\leq$2%. The thermal treatment degrades the RNA into monomers which then diffuse out of the cells (Raats, 2007).

Even though plant-based protein sources have been shown to often lack one or more amino acids in sufficient quantity to meet human nutritional needs, the intake of a combination of different proteins, and supplementation, can be used as a strategy to help to overcome deficiencies especially in individuals who consume strict vegan or vegetarian diets (Henchion et al., 2017). Some plant-based protein sources can also offer significant health benefit as a rich source of bioactive peptides which have gained increased interest as agents in the control of chronic diseases (Gobbetti et al., 2004). The nutritive value of plant storage proteins is reported to be lower than that of animal ones. Plant storage proteins have less of an anabolic effect than animal proteins because of their lower essential amino acid content (especially leucine), deficiency in other essential amino acids, such as the sulphur-containing lysine and methionine, as well as lower digestibility (Berrazaga et al., 2019). Furthermore, the plant-based protein foods (such as the so called “vegan cheese”) are nutritionally inferior to products that are made using animal-based proteins. To enhance the nutritional composition, sensorial and techno-functional properties of plant-based proteins, some authors have suggested the use of protein modification via physical, chemical and biological means (Nikbakht et al., 2021). However, the energy and cost efficiency of these techniques still need to be evaluated in order to deliver a product desirable to the consumer all whilst fully aligning with sustainable development goals.

## 7. The opportunities and challenges in exchangeability of animal proteins with alternative proteins

There are opportunities that exist in the exchangeability and replacement of animal proteins with alternative sustainable proteins. For instance, the consumption of proteins from edible insects and plant-based proteins is shown to be more sustainable compared to animal (livestock) protein sources due to the comparatively elevated GHG footprint of the latter as mentioned earlier. As such, alternative sources of protein have the potential of contributing towards food security. Alternative protein sources are also perceived to be cheaper than animal-based protein sources (Possidónio et al., 2021). Additionally, due to changes in lifestyle, social demographics and the emergence of data supporting a reduction in mortality and morbidity (English et al., 2021; Kim et al., 2019), it is becoming increasingly easier/acceptable for alternative protein sources to be incorporated in human diets especially for people who would like to make changes in their health or contribute towards a reduction in the impact of animal husbandry on the environment. However, in terms of the digestibility and bioavailability of some of the alternative protein sources, such as the plant-based proteins, there have been reports that have shown these proteins to be lower compared to their animal counterparts (Berrazaga et al., 2019). Additionally in terms of their functional properties some researchers have shown that higher concentration of the plant-based proteins is required to achieve close to or similar functionality as animal proteins. Munialo et al. (2014) compared the gelling propensity of pea proteins in comparison to whey proteins. In their work, they showed that up to 6 × higher concentration was needed to form gels from pea proteins fibrils that had the same gel strength as fibrils made from whey proteins. As such, it may be that as much as alternative protein sources are perceived to be cheaper than animal-based protein sources, more of the same will be needed to end up with similar functional properties as animal proteins, and this could still have potential cost implications for the food industry.

Other challenges include the cost of production. For instance, the application of microalgae in food products has been hampered by their high production costs, technical difficulties in extraction and refining, and sensory and palatability issues, even though nutritionally, they are comparable to other plant proteins (Henchion et al., 2017). Based on such challenges and the fact that higher concentrations of alternative proteins are needed to achieve functional properties that are as good or superior to animal-based protein, then it may be that one needs to look at the cost-benefit analysis and the production costs before making a decision to replace animal proteins with alternative sources of proteins. There are also technological challenges that the food industry will face in terms of replacement and or exchangeability of animal proteins with plant proteins. This is not straightforward, mainly due to the inherent differences (such as in their tertiary and quaternary structures) that exist between animal and other protein sources. Thus, one needs to map the functional properties of the animal proteins to be able to mimic these with alternative sources, whilst ensuring that the textural as well as sensorial properties of the end protein (or processed food matrix) remain similar or at least close to the protein that is being exchanged. There also remains a challenge in examining the environmental impact of the alternative proteins sources when incorporated in the formulation of food products. For instance, some researchers have suggested that producing 1 kg of animal protein requires about 100 times more water than 1 kg of grain protein (Smil, 2001). However, other authors have reported on the water footprint of meat analogues with there being suggestions that the production of soy protein isolate consumes almost x3 more water than texturized soy protein and x7 more than soybean oil, even though all three products are derived from the same soybeans. Other reports have also identified isolated soy protein as highly water consumptive (Fresán et al., 2020). For example, Berardy et al. (2015) report that producing 1 kg of isolated soy protein requires 38,950 L of water and this is clearly not a sustainable solution.

The exploitation of existing plant processing co-product streams is identified as a sustainable source for some protein replacements, thereby allowing for diversification of feedstock and increased sustainability (Platt et al., 2021). An example is shown by the emerging plethora of plant-based dairy milk alternatives from sources such as potatoes, nuts, and grains among others (Bridges, 2018). However, even if the existing protein sources were diversified and alternative sources characterised with the aim of providing sustainable and nutritious food for the growing world population, food waste remains a blight that affects the food industry. Some of the food waste occurs after harvesting. Post-harvest losses have been reported to be partly a function of the technology that is available in a given country, in addition to the extent to which markets have developed to market the food produce (Parfitt et al., 2010). Mirabella and colleagues have shown the proportion of losses post-production to include up to 42% at household level, 39% in food manufacturing, 14% in the foodservice sector, and that 5% of losses occur during distribution (Mirabella et al., 2014). Thus, there remains a global challenge in minimising food (and hence protein) waste as this would contribute to food security and other emerging technologies that can be employed in food production, and the continual search for protein alternatives would help in diversifying the protein sources rather than this being the main driver and push towards sustainable food production that has a lower impact on the environment.

## 8. Conclusion

Alternative protein sources exhibit significant potential in the food industry as a substitute to animal-based protein sources. The protein content of alternative protein sources has been evaluated and, in most cases, found to be similar close or in some cases higher than animal-based protein sources. Several studies have been carried out that investigated the functional properties of alternative protein sources such as insect, microalgae, and mycoprotein among others. The profile of alternative protein sources was found to be in some cases lower than that of animal-based protein sources. This was mainly attributed to the inherent differences between these two protein sources. The solubility of proteins such as those obtained from plant-based sources was generally lower than that of the animal-based protein sources. The nutritional quality of alternative protein sources has also been evaluated in comparison to animal-based proteins sources and animal-sourced proteins were shown to have superior digestibility and bioavailability compared to the plant-based protein sources. A clear understanding of the mechanisms, principles and differences between these two broad types of proteins will help enhance the formulation and exchangeability window for alternative protein sources. Increasing global meat intake remains an issue, which is predicted to rise by 14% by 2030 (OECD/FAO, 2021) and the factors contributing to this, and the implications of the associated environmental food print compared to plant-based foods and proteins need to be evaluated. Additionally, the issue of food loss and waste from farm to fork need to be addressed and this does call for a unified and combined effort of all the stakeholders that are involved in food production. Tackling the issue of food waste does contribute to responsible consumption and production which is part of the UN sustainable development goals.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Ethical guidelines

Ethics approval was not required for this research.

## Data availability statement

Research data are not shared.

## Ethical statement - studies in humans and animals

The authors declare that the research presented does not involve any animal or human study.

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