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Review

An exploration of alternative proteins as a potential sustainable solution to meeting the nutritional needs of the ever-increasing global population

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Summary Proteins of animal origin have been used in various food formulations as they have been shown to possess excellent techno-functional properties such as gel formation, emulsification, foam ability and stability, among others. However, the production of proteins from animal sources does come at an environmental cost due to the greenhouse effect that is attributable to the rearing of animals. To mitigate against the environmental impact of the production of protein from animal origin, research has focused on alternative proteins such as those of plant, insect or mycoprotein origin. However, there are still several issues as well as mapping of alternative proteins in terms of their nutritional profile, sensorial attributes that include taste and texture as well and the challenges that exist in mimicking proteins of animal origin that need to be addressed which would broaden the window of application of alternative proteins. Therefore, this review explores some of the alternative protein sources that have been characterised in terms of their techno-functional characteristics and underpins the challenges such as allergenicity that exist in the use of alternative. Furthermore, this work aims to evaluate consumer perception and acceptance of alternative proteins which is pivotal in their success in food production and process as well as ensuring the safety of these proteins. As such, this work will contribute towards the existing knowledge on the possible applications of these proteins as sustainable, cheaper solutions.

Keywords Allergenicity, alternative proteins, animal proteins, consumer perception, food security, sustainability, techno-functional.

Introduction

Recent global events such as COVID-19 pandemic, conflicts and wars such as the Russian-Ukrainian war and the Israeli–Palestinian conflict, as well as climate change that have resulted in severe drought in places such as Sub-Saharan African have all shown the vulnerability of food systems and their inability to be resilient to crises if they are not sustainable (Sanderson Bellamy *et al.*, 2021; Caron *et al.*, 2023). Food systems are reported to be responsible for major environmental and climatic damage (Vermeulen *et al.*, 2012). Currently, (i) food systems account for nearly one-third of global greenhouse gas (GHG) emissions (Crippa *et al.*, 2021), (ii) are reported to consume a large amount of natural resources (Wunderlich & Martinez, 2018), (iii) contribute to the loss of biodiversity (Read *et al.*, 2022), (iv) could have negative health impacts which

could be due to both over- and under-nutrition (Neff *et al.*, 2009), and (v) do not always allow for fair livelihoods and economic returns for all actors, specifically for primary producers (Clodoveo, 2022). All these challenges continue to pose a challenge in meeting the nutritional needs of the growing world population. Thus, there is a need to redesign our food systems to make them more sustainable (Hendriks *et al.*, 2023; Thomsen *et al.*, 2023; Van Zanten *et al.*, 2023).

As the world population continues to grow, there is a concomitant increase in the demand for nutritious meals that include proteins. Proteins are a major nutritional component as they provide both non-essential and essential amino acids which cannot be synthesised by the human body and would therefore depend on nutrition for their provision (Langyan *et al.*, 2022). Depending on the source, protein can be either of animal or vegetal (plant storage) origin. Animal proteins have in the past been used for food production. However, given production cost, sustainability issues and availability, plant storage proteins are increasingly being used as a

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robust alternative to animal proteins (Khan *et al.*, 2023). In recent years, the use of novel protein sources (like insects, algae, duckweed, and rapeseed) has been explored as potential replacers for animal-derived proteins (Van der Spiegel *et al.*, 2013; Vauterin *et al.*, 2021). However, for proteins of plant origin to be successfully used as replacers for animal proteins, their techno-functional properties including gelation, and water-holding capacity which has been defined as ‘the ability of a matrix of molecules (network) to entrap water in such a manner that exudation is prevented’ (Fennema, 1996) solubility, foaming and emulsification among others (Aimutis, 2022) need to be evaluated and have to be at par with those of animal origin. Even though there has been an increasing study of proteins of plant origin which includes the characterisation of their techno-functional properties, most of the studies have concentrated on soy protein (Ma *et al.*, 2022a). Although soy is indubitably an established plant protein that provides a range of useful techno-functionalities, it does have some limitations. One main issue with the use of soy in food formulation is the allergenicity aspect. Secondly, research evidence has shown the potential adverse long-term health consequences associated with consuming soy infant formulas during developmentally sensitive windows (Suen *et al.*, 2022; Ma, Grossmann, Nolden, McClements, & Kinchla, 2022b) Additionally, the cultivation of soybean is shown to be a major contributor to the deforestation of the Amazon rainforest even though the cutdown for crops has drastically been reduced since the Brazil’s Soy Moratorium (Tyukavina *et al.*, 2017). Nonetheless, with the increase in demand for protein- and oil-rich crops, there is a possibility of this changing again in the future (Ma *et al.*, 2022). As such, it is important to explore other protein-rich ingredients to understand how they behave in food matrices, for instance in terms of gelation and liquid holding properties which are key in the formulation of meat alternatives as well as emulsification properties which are key in the formulation of dairy alternatives (Ma *et al.*, 2022). Therefore, this review aims to explore other alternative proteins (apart from soy) that can be used as potential replacers for animal proteins. The pros and cons and these proteins will be reviewed as well as the challenges and opportunities that exist in their use discussed.

Proteins in food formulation

Proteins can be classified into major broad categories based on far more general qualities: whether the protein is fibre-like and insoluble, globular and soluble or intermediate (Grover *et al.*, 2012). These two categories can further be divided into two categories (animal and alternative) depending on the source of protein (Fig. 1). Alternative proteins include those of plant,

algae, fungal (mycoprotein) or insect origins as illustrated in Fig. 1.

Major differences between proteins of plant or animal origin are mainly attributed to inherent differences such as differences in their quaternary and tertiary structures and this has a concomitant impact on the functionality of the proteins which includes their solubility, water-holding capacity, gelation, emulsification potential as well as the foaming ability and stability (Karabulut *et al.*, 2024).

Proteins from animal sources

Animal proteins have been and continue to be used in the food industry in the formulation of a range of food products. The use of proteins from animal sources is attributed to their techno-functional characteristics that range from gelation, and emulsification to foaming ability and stability. Animal proteins such as whey (Razi *et al.*, 2023; Yang *et al.*, 2023; Yigit *et al.*, 2023), egg (Munialo *et al.*, 2014), and gelatine (Ersch *et al.*, 2016) among others have been characterised in terms of their functional properties and their use in product development has been corroborated elsewhere (Cao & Li, 2013; Ismail *et al.*, 2020). The nutritional profile of proteins from animal sources has also been investigated and shown to have a higher biological value which includes a higher digestibility as well as bioavailability (Berrazaga *et al.*, 2019). However, the use of proteins from animal sources has come under a lot of scrutiny and criticism due to the environmental impact. This is mainly attributed to GHG emissions that contribute to global warming (Kozicka *et al.*, 2023; Pingali *et al.*, 2023). Consequently, the search for potential replacers that are cheaper and have a lesser impact on the environment has resulted in alternative proteins such as proteins from plant sources being explored (Aimutis, 2022; Ma *et al.*, 2022, 2022a) and in various new applications. Major sources of traditional and alternative protein foods and their new applications are shown in Table 1.

Plant-based proteins

In view of sustainability, alternative sources of proteins such as from plant origin have been characterised and are being used in the food industry in several formulations. Several plant proteins such as pea proteins (Munialo *et al.*, 2014, 2015), and soy proteins (Baiano *et al.*, 2011, Béné *et al.*, 2015, Bhatnagar *et al.*, 2017) have been characterised in terms of the functional properties. Different plants such as legumes, nuts, and seeds have been used as sources of protein as classified in Fig. 2 and their extraction, characterisation and functional modification are described in detail elsewhere (Nikbakht Nasrabadi *et al.*, 2021).

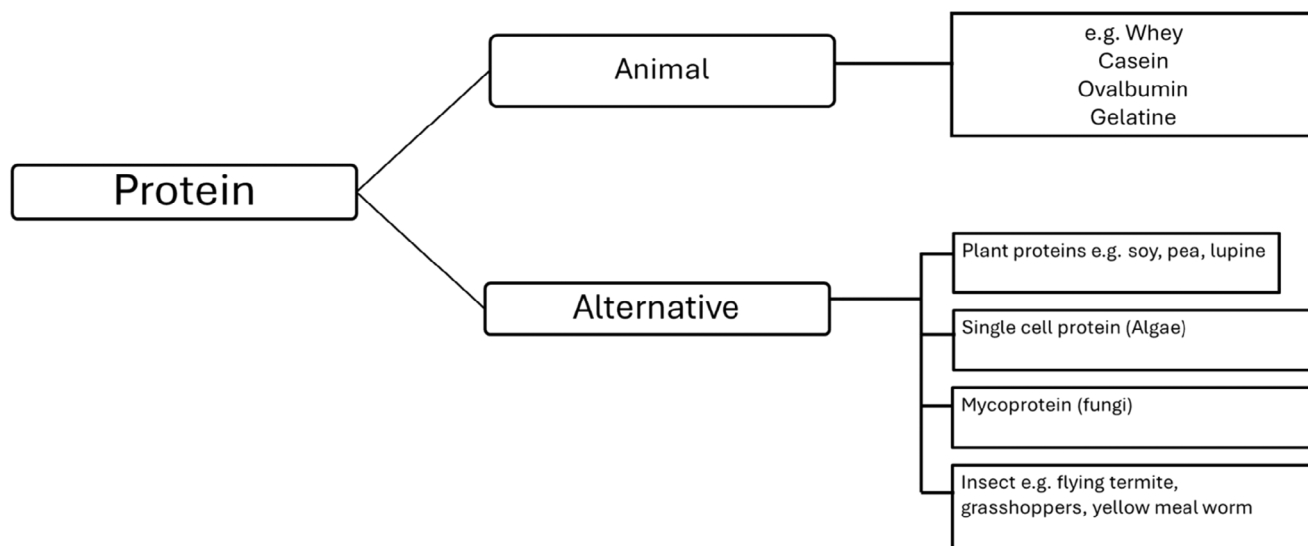


Figure 1 Broad classification of proteins based on their origin. Adapted from (Karabulut *et al.*, 2024).

Despite plant-based proteins being viewed as cheaper and more sustainable, several hurdles need to be overcome for them to be used as potential replacers for animal-based proteins. For instance, some researchers have shown the amino acid profiles of proteins from plant-based sources to be inferior to those of their animal counterparts. Proteins from plant-based sources have been shown to have a lower biological value and lower content of essential amino acids particularly lysine and other sulphur-containing amino acids (Munialo & Vriesekoop, 2023). Despite plant proteins having a lower biological, soy and peanuts are some of the proteins of plant origin that have a considerably higher biological value with soy (2.2) being close to casein (2.5) and milk (2.5) as shown in Table 2 which shows a comparison of the protein quality of proteins from animal and plant sources. The Essential Amino Acid (EAA) profiles of proteins from animal and plant sources (mg g^{-1} protein) are provided in Table 3. Some EAA of proteins from plant sources are comparable and, in some cases, higher than that of some proteins of animal origin (Table 3) with soybean having higher concentrations of these EAA. This makes soy a reasonable replacer for proteins of animal origin. However, there already exists an over-dependence on soy protein and this provides a challenge to the food industry to look for other alternative protein sources which open research opportunity that involves other under-utilised plant proteins such as buckwheat and hemp protein (Table 3). Furthermore, there are opportunities for formulating products using protein blends which could either be animal and plant protein blends or different plant

protein blends which not only would alleviate the over-dependence on soy proteins as aforementioned but also allow for the formulation of new and sustainable products.

The second limitation in the use of plant-based proteins is the presence of antinutrients such as phenolics, tannins, and phytates among others. Some antinutritional factors which are present in various plants including their concentrations ($/100$ g) are shown in Table 4.

Antinutritional factors possess the potential to limit the absorption and bioavailability of certain nutrients such as protease and trypsin inhibitors may result in maldigestion of proteins when they bind to some minerals and nutrients hindering their absorption (Munialo & Andrei, 2023). Other antinutrients such as alpha-amylase inhibitors can impair the digestion of carbohydrates, whereas others such as saponins and lectins are associated with autoimmune responses and leaky gut (Popova & Mihaylova, 2019; Langyan *et al.*, 2022). Oxalates, phytates, and tannins may result in the malabsorption of minerals, whereas goitrogens have the potential to cause inflammation and interfere with thyroid iodine uptake. These adverse effects of antinutrients have generally been seen in animals when they consume unprocessed proteins of plant origin (Popova & Mihaylova, 2019; Langyan *et al.*, 2022). However, antinutrients have also been reported to pose beneficial health effects. For example, a reduction in blood glucose levels, plasma cholesterol and triglycerides, has been documented at a lower level of lectins, phytates, enzyme inhibitors, saponins, and phenolic compounds (Popova & Mihaylova, 2019; Langyan

Table 1 Major sources of traditional and alternative protein foods and their new applications

Sources	Categories	Alternative proteins and applications	Reference(s)
Animal	Dairy products	<ul style="list-style-type: none"> Such as calcium caseinate & whey protein; being explored for meat analogues 	Khattab & Arntfield (2009)
	Insects	<ul style="list-style-type: none"> Such as: <i>Alphitobius diaperinus</i> protein concentrate; being explored for meat analogues <i>Tenebrio molitor</i> flour; being explored for snacks Cricket powder; being explored for pasta <i>Acheta domesticus</i> flour; being explored for bread 	da Silva Lucas <i>et al.</i> (2020)
Plant	Legumes and beans	<ul style="list-style-type: none"> E.g., hemp protein; being explored for meat analogues, plant-based milk, and bread 	Zahari <i>et al.</i> (2023)
	Cereals	<ul style="list-style-type: none"> E.g., gluten, being explored for meat analogues E.g., oats, being explored for plant-based milk 	Kyriakopoulou <i>et al.</i> (2019)
	Nuts and seeds	<ul style="list-style-type: none"> E.g., peanuts; being explored for use in edible coatings E.g., almonds, being explored for the production of plant-based milks 	Kazemian-Bazkiaee <i>et al.</i> (2020)
	Tuber	<ul style="list-style-type: none"> E.g., potato protein; being explored for use in meat analogues 	Kumar <i>et al.</i> (2017)
	Algae	<ul style="list-style-type: none"> E.g., <i>spirulina</i>; being explored for use in meat analogues 	Palanisamy <i>et al.</i> (2019)
	Leaf protein	<ul style="list-style-type: none"> E.g., grass protein (RuBisCO) being explored for various food applications 	Kaur <i>et al.</i> (2021)
Fungal	Microbial proteins	<ul style="list-style-type: none"> E.g., fermented fungus being explored for use in meat analogues 	Kim <i>et al.</i> (2011)
	Mushroom	<ul style="list-style-type: none"> E.g., <i>Colocvbeindica (dudhchatta)</i> mushrooms; being explored for use in analogue meat nuggets for the purpose of increasing the meaty flavour 	Kumar <i>et al.</i> (2017)

et al., 2022). Saponins are hypothesised to play a significant role in the functioning of the liver in addition to decreasing platelet agglutination. Some of the saponins as well as protease inhibitors, phytates, phytoestrogens, and lignans have the potential to reduce the risk of cancer. Additionally, tannins also have antimicrobial effects (Popova & Mihaylova, 2019; Langyan *et al.*, 2022). The presence of antinutritional factors is one of the limiting factors when it comes to the wide application of proteins from plant origin, hence the need to reduce their concentration in plant proteins and consequently, their adverse effects. Several strategies and approaches have been adopted to reduce the concentration of antinutrients. These range from thermal treatment of the product to the use of methods such as autoclaving, extrusion, hydro techniques, fermentation, soaking, gamma irradiation, sprouting (germination), genomic technologies enzymatic, and harvest treatments (Popova & Mihaylova, 2019). However, the use of some of these processes and techniques especially those that require heat treatment can pose other challenges such as the denaturation of proteins and this could result in conformational changes that can further impact their digestion and bioavailability. Undesirable Maillard reactions can

also occur which can result in the development of off-flavour, result in flavour loss, discolouration, degradation of amino acids and loss of protein nutritional value (Tessier & Birlouez-Aragon, 2012). Thus, there is a need for further research to evaluate how antinutrients can be minimised or eliminated from food products without impacting the nutritional profile of proteins.

The last but not least factor that impairs the use of plant-based proteins in food formulations is their allergenicity. A food allergy is fundamentally an adverse effect that results in the activation of an immune response when an individual is exposed to a food (Sampson *et al.*, 2014). Food allergens of plant origin are mainly categorised into four families, (i) the cupin superfamily, (ii) the profilins, (iii) the prolamin superfamily, and (iv) the Bet v 1 family. Over 50% of allergens of plant proteins are thought to fall into two categories, that is, the cupin and prolamin superfamilies (Shewry *et al.*, 2002). The most commonly found allergens are 2S albumins, cereal prolamins, α -amylase, lipid transfer proteins that are non-specific, and trypsin inhibitor, protein families (Langyan *et al.*, 2022). In most cases, allergic reactions in the body are due to small linear stretch of amino acids or a specific 3D

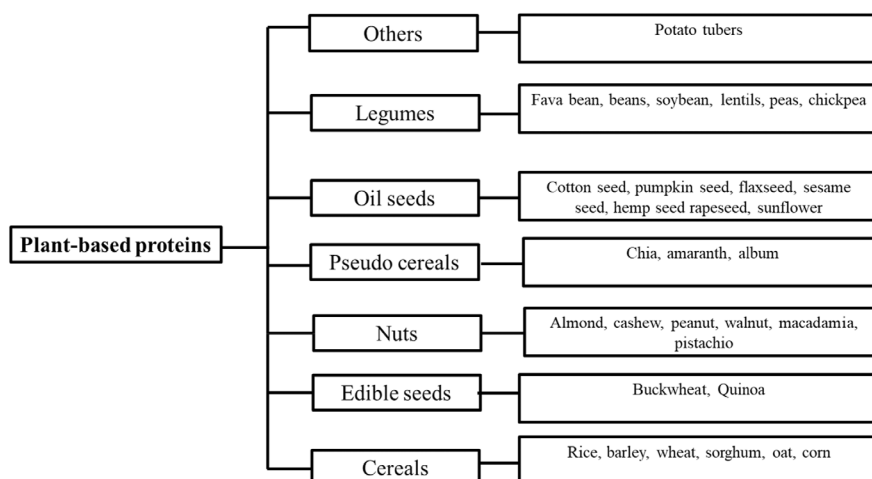


Figure 2 Major sources of plant-based proteins. Adapted from (Munialo, 2023).

Table 2 Protein quality rankings of some proteins of animal and plant sources

Type of protein	Biological value	Protein efficiency ratio	Digestibility of proteins corrected for amino acid score	Net protein utilisation
Casein	2.5	77	1.00	76
Whey protein	3.2	104	1.00	92
Milk	2.5	91	1.00	82
Egg	3.9	100	1.00	94
Beef	2.9	80	0.92	73
Wheat gluten	0.8	64	0.25	67
Soy protein	2.2	74	1.00	61
Black beans	-	-	0.75	-
Peanuts	1.8	-	0.75	-

Data adapted from (Hoffman & Falvo, 2004) and U.S Dairy Export Council, Reference Manual for U.S. Whey Products 2nd Edition, 1999 and Sarwar, 1997. “-” represents missing or unavailable data.

structure which is a part of a much larger protein, which are known as ‘epitopes’ (Vanga *et al.*, 2017). Subject to the method of food processing, these epitopes may either be destroyed or result in the formation of new ‘neoallergen’ epitopes which are thought to be the cause of allergic reactions in patients following the consumption of processed food (Vanga *et al.*, 2017). Both thermal processing which can either involve the use of moist (such as conventional thermal processing such as boiling, autoclaving and novel technologies such as microwave heating or dry heat) and non-thermal methods (such as conventional methods like proteolysis/hydrolysis, fermentation, ultrafiltration and novel technologies like gamma radiation, pulsed ultraviolet light, ultrasound and high hydrostatic

pressure (HHP)/high-pressure treatment) are used in food processing. These processing techniques have been shown to have varying impacts on food allergen epitopes when used individually or as a combination. Apart from the formation of neoallergens, some methods that are used in the processing of food may also result in an increase in the allergenicity of a certain protein or may cause no change in it and this has been discussed in detail elsewhere (Vanga *et al.*, 2017). As ways for processing the foods are huge and varied, the effect produced by each of them on the specific type of epitope (conformational and linear epitopes) is very important for analysing the effects on the allergenicity of a certain protein, hence, there is a need for continual research to be carried out to understand how allergic epitopes can be destroyed without impacting on the nutritional or functional properties of the proteins. This would provide more opportunities for proteins from plant sources to be used in food formulation.

Functional characterisation of plant-based proteins

For proteins from plant sources to be used as a potential replacer of proteins from animal sources for instance in product development, there is a need for these proteins to be extracted and characterised in terms of the techno-functional properties. The first stage of formulations including plant-based proteins is the extraction of proteins. In most cases, either wet or dry fractionation has been used as a strategy of protein extraction. The merits and demerits of both wet and dry fractionation have been reported elsewhere (Berghout *et al.*, 2015; Munialo *et al.*, 2022). The most common method for isolating proteins from plant materials is wet extraction which includes alkaline extraction; this method has, however, been often

Table 3 The profiles of Essential Amino Acid (EAA) of some common protein sources of animal and plant sources

Essential amino acid mg g ⁻¹ protein	Basic			Neutral			Aromatic			Neutral (branched chain)				Sulphur Methionine + cysteine	References
	Histidine	Lysine	Threonine	Phenylalanine + Tyrosine	Tryptophan	Isoleucine	Leucine	Valine	Isoleucine	Leucine	Valine	Valine			
<i>Animal proteins</i>															
Cow milk	28	84	47	95	14	53	99	65	30						Liu et al. (2019)
Sheep milk	29	90	49	99	17	54	105	69	33						Liu et al. (2019)
Goat milk	30	97	54	111	15	68	105	80	42						
Caseins	31	85	46	114	14	59	102	76	33						Liu et al. (2019)
Whey protein	16	109	88	75	17	74	121	69	52						Wu et al. (2016)
Beef	31	70	36	61	10	40	65	46	35						Hodgkinson et al. (2018)
Pork	37	76	40	68	11	39	71	47	30						Marinangeli & House (2017)
Chicken	34	100	46	40	n.d.	52	85	51	31						Day et al. (2022)
Marine fish	7	76	65	38	20	50	96	84	30						Day et al. (2022)
Fresh water fish	33	33	38	52	6	38	57	44	14						Day et al. (2022)
Whole egg	25	78	51	98	12	57	91	64	47						Day et al. (2022)
Egg white (albumin)	17	62	36	77	10	46	68	49	38						Day et al. (2022)
Egg yolk	33	104	71	114	18	65	115	72	50						Day et al. (2022)
House cricket (adult)	24	55	37	85	7	47	74	54	24						Poelaert et al. (2018)
<i>Plant proteins</i>															
Wheat flour	26	27	31	85	13	37	75	47	41						Cervantes-Pahm et al. (2014)
Duram flour	23	24	30	73	13	26	70	44	36						Cervantes-Pahm et al. (2014)
Corn	27	29	36	100	8	39	128	51	44						Cervantes-Pahm et al. (2014)
Corn meal	27	29	26	88	5	38	109	46	32						Day et al. (2022)
Oat	25	43	35	91	9	39	76	54	43						Day et al. (2022)
Rice	26	28	37	82	14	45	88	63	44						Kalman (2014)
Rice protein concentrate	17	21	28	104	11	34	62	45	39						Kalman (2014)
Rice protein isolate	17	23	29	86	12	34	64	44	40						Kalman (2014)
Sorghum	18	16	29	76	n.d.	34	127	43	7						Day et al. (2022)
Soybean	28	73	47	99	16	54	90	55	31						Day et al. (2022)
Isolated soy protein	25	56	39	94	13	49	56	51	26						Day et al. (2022)
Soy protein concentrate	25	62	36	86	12	45	76	47	28						Day et al. (2022)
Pea protein concentrate	24	67	38	97	9	44	76	49	19						Cervantes-Pahm et al. (2014)
Fava bean	31	63	39	77	8	35	85	48	27						Day et al. (2022)
Chickpea	23	55	32	82	10	38	65	37	22						Day et al. (2022)
Lentil	29	69	44	71	5	35	71	46	26						Day et al. (2022)
Lupin	39	45	40	80	10	40	69	41	13						Day et al. (2022)
Amaranth	27	43	31	55	n.d.	19	43	21	41						Day et al. (2022)
Quinoa	19	24	67	27	10	8	24	9	6						Ruales et al. (2002)
Buckwheat protein	25	52	35	80	17	38	68	52	42						Day et al. (2022)
Hemp protein isolate	32	27	34	83	10	36	65	47	32						Day et al. (2022)
Hemp seed protein meal	33	39	38	77	11	36	69	47	38						Day et al. (2022)
Peanuts	25	39	22	88	7	25	70	40	16						Day et al. (2022)
Microalgae	26	66	52	98	n.d.	45	93	61	27						Day et al. (2022)
Microalgae cultured	28	72	53	101	n.d.	44	92	62	26						Day et al. (2022)
Microalgae products	22	70	49	96	n.d.	51	91	62	29						Day et al. (2022)

n.d., not determined.

Table 4 Antinutrients that are available in different foods.

Source	Type	Amount
Grains such as barley, corn, millet, Kamut, oat, spelt, sorghum rye, and wheat	Phytic acid	50–74 mg g ⁻¹
	Oxalates	35–270 mg/100 g
Pseudo-grains such as amaranth, buckwheat, quinoa, Teff, and wheat	Goitrogens	0.04–2.14 ppm
	Lectins	0.5–7.3 g/100 g
	Phytic acid	
	Saponins	
Legumes such as chickpeas, lentils, peanuts, and soya beans	Cyanide	2–200 mg/100 g
	Saponins	106–170 mg/100 g
	Tannins	1.8–18 mg g ⁻¹
	Trypsin inhibitor	6.7 mg/100 g
	Oxalates	8 mg kg ⁻¹
	Phytic acid	386–714 mg/100 g
Seeds such as flaxseed, sesame, sunflower, poppy seed, and pumpkin	Alpha-amylase inhibitor	0.251 mg mL ⁻¹
	Cyanide	140–370 ppm
	Phytic acid	1–10.7 g/100 g
Nuts such as almonds, Brazil nuts, cashew, hazelnut, macadamia, pignola, pistachio, and walnuts	Phytic acid	150–9400 mg/100 g
	Lectins	37–144 µg g ⁻¹
	Oxalates	40–490 mg/100 g
Nightshades such as eggplant, pepper, potato, and tomato	Phytic acid	0.82–4.48 mg/100 g
	Tannins	0.19 mg/100 g
	Saponins	0.16–0.25 mg/100 g
	Cyanide	1.6–10.5 mg/100 g
	Oxalates	0.4–2.3 mg/100 g
Tubers such as carrot, manioc (or tapioca), Jerusalem artichoke, sweet potato and yam	Tannins	4.18–6.72 mg/100 g
	Phytates	0.06–0.08 mg/100 g

This data has been adapted from the work of Popoval and Mihaylova (Popova & Mihaylova, 2019)

shown not to be efficient and has also been suggested to have the potential of damaging some proteins (Munialo *et al.*, 2022). One disadvantage of wet extraction is the protein content that often varies depending on the type of protein. Consequently, a drying step (such as freeze drying, or spray drying among others) is often used to concentrate the proteins. The drying steps can however result in structural changes which could impact things like the solubility of the proteins (Munialo *et al.*, 2022) and this has consequences for the application of proteins of plant origin in food formulation and or production. Hence, strategies to enhance the solubility of the proteins need to be researched which will enhance their application in food processing and production.

The other issue that limits the application of proteins from plant origin is the protein yield. Most researchers report protein yield based on dry matter basis. However, there are several challenges with this as (i) the protein needs to be dried and this can result in issues such as a reduction in the solubility of the protein which is something that has been addressed above. This can impact the functionality of the protein, and (ii) if the solubility of the protein is reduced, there remain a couple of hurdles to overcome in terms of protein concentration as some of the formulations that require high protein concentrations would not be

possible when the yield and the concentration of the protein are low. Additionally, there arises the need to include other biopolymers such as polysaccharides which could act as thickeners or binders, and this has implications on the cost of production but also there arises the challenge of ensuring the consistency of the texture and flavour profiles before these proteins can be effectively used in product development. (iii) the need to concentrate the proteins before using them could concentrate antinutrients which are naturally present in plant extracts and the impact of this on protein digestion and bioavailability as well as human health needs to be investigated. Finally, (iv) if high concentrations of other biopolymers are required to formulate products, the question remains as to whether the new products would qualify to be referred to as protein-based products. A perfect example exists in the formulation of plant-based (vegan) cheese which in some cases has less than 1 wt./wt. % protein. When marketed as protein, the consumers would be purchasing inferior products that barely have a considerable amount of protein and this can compromise their nutritional value and as a result consumer health. As such, there remain barriers and hurdles that need to be overcome before plant-based proteins can become effective replacers of animal-based proteins. Even though some work has already been initiated on the

roadmap to replace animal with plant proteins, more work still needs to be done and this requires a concerted effort of various stakeholders to come up with effective strategies.

Insects as a potential source of alternative protein

In recent years, due to the drive for sustainability and the desire to diversify the existing protein sources to feed the increasing global population, attention has shifted towards insect proteins as part of a multifaceted strategy for achieving global food security using a more sustainable source of protein.

Entomophagy, which is the consumption of insect protein is reported to have been an integral part of the prehistoric diet in many areas worldwide (Kouřimská & Adámková, 2016). Edible insects have been documented to have the potential to contribute to increased human nutrition. This is mainly attributed to high amino acids (between 46% and 96% depending on the species), lipids, protein (which is around 77% in most species), lipids, and energy (Belluco *et al.*, 2013), in addition to the presence of various micronutrient content in edible insects (Melo *et al.*, 2011).

The consumption of insects can be in various forms. For instance, in some cultures, it is normal for whole insects to be consumed. Nonetheless, in other cultures and societies such as in the Western world, the consumption of whole insects has in most cases been met with resistance. This is mainly attributed to the fact that insects are associated with being bugs and transmitters of diseases. However, some studies and informal surveys (results not published) that have been carried out by the authors on various groups have suggested the incorporation of insect proteins in food products as either insect powders or protein extracts, or in product design such as in baked goods, and this has been shown to increase the general acceptance of these products (Del Valle *et al.*, 1982). The incorporation of edible insects to enrich cereal products has been suggested by some authors (Acosta-Estrada *et al.*, 2021). The replacement of 5%–40% of cereal flour with insect flour has been used in staple foods or snacks, with the ideal replacement being reported to be around 10% as shown in Table 5 (de Oliveira *et al.*, 2017). Bread that was enriched with 10% cockroach flour was shown to present the best nutritional characteristics with a 49% protein increase, without alterations in sensorial quality when compared with white and whole wheat bread (Acosta-Estrada *et al.*, 2021). The formulation of insect flours involves a shift towards extraction and characterisation of protein extracted from insects which is often followed by drying of the extracts to result in insect protein powders. However, one thing to note about the extraction and functional characterisation of protein from insects followed by subsequent drying does come

with an extra production cost. Furthermore, a knowledge gap remains in understanding the possible effects of technological processes (such as blanching, heat treatment and pasteurisation) which are often employed during product formulation and their impact on the protein extracts. Moreover, some authors have postulated that processes that are applied during the processing and production of insect protein-fortified products could have the potential to result in increasing allergic reactions which could raise regions (epitopes) of the proteins, and this could influence allergenicity and susceptibility to gastrointestinal digestion of proteins of insect origin (Munialo *et al.*, 2022). However, the impact of heat processing on allergenicity remains a controversial subject as some authors have reported the general lack of significant impact on allergenicity following the boiling of allergenic shrimp samples (Samson *et al.*, 2004) whereas an increased IgE-binding capacity based on crab and prawn studies has been reported by other authors (Abramovitch *et al.*, 2013) even though this was not shown to link with clinical allergic symptoms (De Marchi *et al.*, 2021). Several strategies such as enzymatic hydrolysis have also been researched as a potential way of reducing the allergenicity of insect proteins in different food matrices, which is based on the findings of Guadix and colleagues who used enzymatic hydrolysis to reduce allergenicity in milk proteins for commercial formulations (Guadix *et al.*, 2006). Enzymatic digestion has been carried out on insects with proteins in the 25–33 kDa range displaying a higher stability to digestion and heat treatments. Interestingly, protein fragments with different molecular weights were obtained following enzymatic digestion of insects with the proteins in the 25–33 kDa range and these protein fragments displayed greater stability to heat treatments and digestion (De Marchi *et al.*, 2021). However, there is still a need to investigate whether the resultant protein fractions retain the necessary functional properties for product development, given that a remarkable decrease in both heat stability and emulsifying ability with a significant increase in the degree of hydrolysis (from 27% to 35%) in whey protein has been reported (Euston *et al.*, 2001).

Mycoprotein

Various fractions have been extracted from mycoprotein and their techno-functional properties have been evaluated. For instance, Lonchamp and colleagues characterised the various fractions of mycoprotein in terms of their gel formation, foaming ability, and stability. The proteomic and metabolomic profiling of the various fractions of mycoproteins has also been carried out. For instance, metabolomic and proteomic analyses of the central fraction of mycoprotein showed the presence of an array of functional proteins and

Table 5 Benefits and challenges in the incorporation of insects in food products

Food products	Insect (replacement %)	Benefit	References
Fishmeal based fish feed	<i>Acheta domesticus</i> and <i>Hermetia illucens</i> (0%–75%)	Phosphorus and potassium levels increased with an increase in the level of fish meal substitution. A reduction on leaching of most minerals was observed by the diets than by the control diets.	Irungu <i>et al.</i> (2018)
Wheat bread	<i>Hermetia illucens</i> , <i>Acheta domesticus</i> and <i>Tenebrio molitor</i> (5%)	An average of 12.7%, 246%, and 120% increase in protein, lipids and fibre levels, respectively.	González <i>et al.</i> (2019)
Wheat bread	Cinereous cockroach (<i>Nauphoeta cinerea</i>) (5%–15%)	Large percentage of unsaturated fatty acids rich in ω -6 and ω -9 were found. There was an increase of protein to 49% by 10% wheat flour replacement.	de Oliveira <i>et al.</i> (2017)
Maize tortilla	<i>T. molitor</i> larva (6.5%)	Protein and fat content increased by 2% and 1%, respectively, as did essential amino acids (phenylalanine, tyrosine, and tryptophan) and polyunsaturated acids (linoleic acid).	Aguilar-Miranda <i>et al.</i> (2002)
Extruded cereals snacks	Grasshopper (<i>Sphenarium purpurascens</i> Ch.) (0%–40%)	An extruded snack that is consumer-friendly can be made from a combination of nixtamalised maize flour and grasshopper meal.	Cuj-Laines <i>et al.</i> (2018)
Extruded cereals snacks	Yellow mealworm larvae (<i>T. molitor</i>) (10% and 20%)	Digestibility of <i>T. molitor</i> proteins was improved by 33%. Additionally, the protein and fat content increased by 35% and 288%, respectively.	Azzollini <i>et al.</i> (2018) and Adeboye <i>et al.</i> (2016)
Wheat cookies	Palm weevil larvae (<i>Rhynchophorus phoenicis</i>) (10%–50%)	Cookies containing 10% insect larvae had higher protein (increased 86%), fibre (increased 642%) and fat (increased 30%) content.	Adeboye <i>et al.</i> (2016)
Pork emulsion sausages	Mealworm larvae (<i>T. molitor</i>) and silkworm pupae (<i>Bombyx mori</i>) (10%)	The protein content in emulsion sausages increased by 21%. Additionally, almost all minerals were increased (e.g., P, K, Ca, Mg, ZN, Mn) especially Zn that increased 89%, Ca and Mg which is double its amount. Cu increased 6 folds. Mealworm larvae flour contributed to Fe increases by 1.5 folds. Mealworm larvae flour contributed to Fe increases by 1.5 folds.	Kim <i>et al.</i> (2016)
Pork emulsion sausages	Cricket (<i>A. domesticus</i>) (5%–10%)	As replacement level increased, P, K, Mg, Zn, and Mn contents of meat emulsion were shown to increase. Insect treatments had higher protein 18%–48% compared to regular formulation (control emulsion).	Kim <i>et al.</i> (2017)
Soy meat analogue	<i>Alphitobus diaperinus</i> (15%–50%)	Meat analogues with 25%–31% of protein content were formulated.	Smetana <i>et al.</i> (2018)
Insect – soy like-fermented sauce	<i>T. molitor</i> larvae (60%–80%)	Essential and non-essential amino acids, as well as amino acid derivatives increased by 1.5 2 times during fermentation.	Cho <i>et al.</i> (2018)
Honey spread	Soldier termites (<i>Syntermes soldiers</i>) (8%)	Protein increased from 0.4% to 5.5% and Fe and Zn solubility increased to 42.8% and 27.1%, respectively, with contents of 3.80 mg/100 g and 1.75 mg g ⁻¹	Akullo <i>et al.</i> (2017)
Insect tea	Produced using insect faeces fed from tea leaves [<i>Aglossa dimidiata</i> Haworth, <i>Hydrillodes morose</i> Butler, and <i>Nodaria nippona</i> (Butler)].	Higher levels of human essential amino acids such as valine (3 folds), threonine (2.45 folds), and phenylalanine (2.35 folds) were observed.	Zhao <i>et al.</i> (2017)
Wheat based feed	<i>H. Illucens</i> (25%)	Extrusion process increased <i>in vitro</i> organic matter digestibility by 16.8% compared to unextruded control.	Ottoboni <i>et al.</i> (2018)

metabolites that include cell wall components (including chitosan and chitin), cerato-platanin protein guanine and guanine-based nucleosides as well as

nucleotides (Lonchamp *et al.*, 2022). Several surface-active protein families of filamentous fungal origin have been reported, including cerato-platanins

(Frischmann *et al.*, 2013). Cerato-platanin EPL1 of the *Trichoderma atroviride* origin has been shown to that the ability to self-assemble into films at air/water interfaces, resulting in the formation of excellent foaming properties (Frischmann *et al.*, 2013). Furthermore, cerato-platanins that were isolated from the marine fungi *Aspergillus terreus* MUT 271 and *Trichoderma harzianum* MUT 290 have been reported to display high surface-active properties, while emulsions that were prepared with these isolates were also shown to exhibit high stability (Pitocchi *et al.*, 2020).

Mycoprotein is shown to also contain some carbohydrates with known functional properties. These include the fungal cell wall components N-acetylglucosamine polymers chitin and chitosan (Denny *et al.*, 2008). Both chitin and chitosan and their derivatives (including those of fungal origin) (Quintela *et al.*, 2012) are reported to display high emulsifying, thickening and gelling properties (Lapasin *et al.*, 1996). One important thing to note is that instead of a hydroxyl group (OH), the glucose molecules in chitin have an amyl group attached that consists of carbon and nitrogen. The presence of nitrogen does have implications on the determination of protein content as the use of any method that is dependent on nitrogen such as Kjeldahl can result in an over-estimation of the protein content as it can be difficult for one to be able to decouple the nitrogen that is related to the protein and the chitin which is present in the cell wall of fungal proteins. Additionally, relatively high levels of nucleotides are reported to be present in the concentrate which is the result of the breakdown of RNA during the heat-shock RNA-reduction step (Ward, 1996). The ability of guanine in the guanine-based nucleotides to self-associate has been hypothesised to be responsible for the high thickening and gelling properties (Peters & Davis, 2016). Given the fact that guanine is important to the structure of DNA and RNA, and is reported to play a significant role in the determination of how the genes in the human body will be expressed, one would wonder whether its self-association could in any way result in complexes that become difficult for the human body to be able to breakdown during the process of digestion and this can impact on its bioavailability. Hence, there could be a potential to further study and investigate the impact of the self-association on nutritional quality of various components of mycoprotein including guanine.

There are nutritional challenges that are related to mycoprotein. One major issue is allergenicity. A study by Jacobson and coworkers (Jacobson & DePorter, 2018) reported Quorn® products to have the potential to cause allergic reactions, including urticaria and anaphylaxis, which occurred within 4 h of Quorn consumption, hence, a reason why some people would not consume mycoprotein. However, it is still possible for biotechnological and bioengineering ways to be

researched that can result in a reduction in allergenicity. The other issue is the RNA content. High levels of RNA have been linked to gout hence the reason why the RNA reduction step is added to the production of mycoprotein (Lonchamp *et al.*, 2022).

Issues, challenges, and future prospects of plant-based proteins and their utilisation in food products

Consumer, knowledge, perception, and acceptance of alternative proteins

Without a shadow of a doubt, consumers are becoming increasingly aware of the impact of food production and in particular animal production on global warming. Some are open, flexible, and adaptable when it comes to the incorporation of alternative protein sources in their diets. However, in some cultures and societies where animal production is the main source of livelihood, the consumption of alternative proteins has always been met with resistance as this is seen as a threat as they depend on animal husbandry either for commercial viability or for their livelihood (Safdar *et al.*, 2022), even though it is not yet clear whether the rise of the plant-based industry does pose a threat to the sustainability of the animal meat sector (Van Loo *et al.*, 2020). The other issue arises when it comes to the preparation of these proteins as some consumers would feel that they do not have the knowledge and skills that are needed, for example, in cooking alternative meat products (Santo *et al.*, 2020). The cost of these alternatives also remains a barrier to their introduction into local cuisines. Products such as meat analogues which are made using alternative proteins have also been classified as being ultra-processed foods and this can impact their uptake, especially for health-conscious consumers. The taste of alternative proteins also remains a barrier to their introduction into the food system. The presence of off-notes has been reported when proteins from plant sources have been incorporated into product design and this does not often have appealing sensory profiles (Ismail *et al.*, 2020). Thus, factors such as willingness to purchase need to be taken in to account when decisions are being made on the processing and production of alternative proteins. Consumer education can play a significant role when it comes to the adaption of alternative proteins in the human diet.

Environmental impact of alternative proteins and food Laws and regulations

There is no doubt that alternative proteins are reported to have a lesser GHG emission and hence have a lower carbon footprint, and this does look great in terms of the environmental impact of GHG.

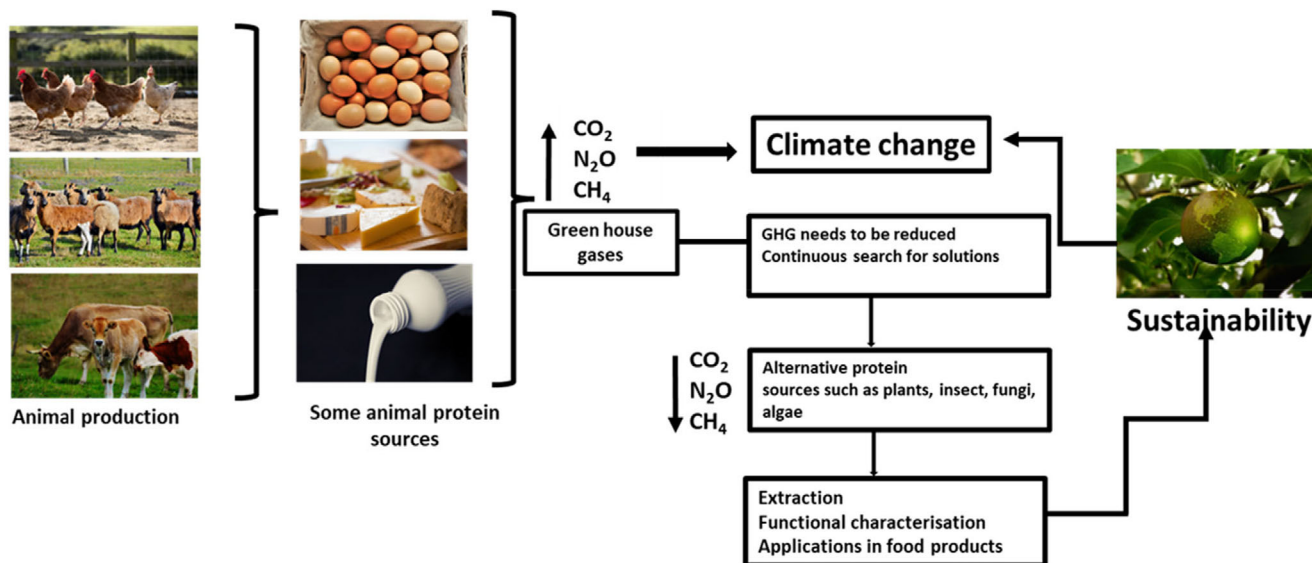


Figure 3 Authors perception of environmental impact of protein of animal versus plant origin.

Fig. 3 shows the correlation between proteins of animal versus plant origin and their perceived impact on the environment.

However, one often neglected part is the water footprint of these proteins. For instance, the preparation of protein extracts from alternative sources such as plants is often highly water-consumptive. To put it in context, some studies have shown the water footprint of one California almond to be averaged at 12 L (3.2 gallons) (Fulton *et al.*, 2019). The work of (Berardy *et al.*, 2015) shows the production of 1 kg of isolated soy protein requires 38 950 L of water which is not a sustainable solution. One key factor to consider is the final destination of the wastewater following the processing of these alternatives. If the wastewater is treated or recycled, it means extra costs of production in terms of the resources that are required for this is to be carried out successfully. If the wastewater makes its way to the water bodies, there can be an increased risk of eutrophication, and this could have dire consequences for ecological biodiversity. Hence, there arises a need for both the carbon and water footprint to be looked at hand in hand. If researchers and governments of the nations of the world focus mainly on the carbon footprint and forget about the water footprint of these alternatives, we could end up with a situation where less carbon is emitted to the environment with the reduction of the rearing and consumption of animal proteins but a massive issue with a water management.

In some countries and contexts, alternative proteins can be classified as ‘novel’ foods. Consequently, several EU food laws govern the introduction and use of these foods in various food processing. There have also been several protests by farmers in various

countries in the EU which culminated in the ban of “meaty” names/terms of some plant-based meat analogues, for example, in France and Italy (Mancini & Antonioli, 2022) (Lähteenmäki-Uutela *et al.*, 2021). Some other issues related to the ban are the safety of alternative proteins which can be attributed to the ingredients and additives that are used in their formulations. Thus, there still exists several barriers and hurdles that need to be overcome to see the continual booming market of these protein alternatives, and this provides more opportunities for more research to fill the knowledge gap that would enhance the expansion of these proteins in the food industry.

Conclusion

There is a myriad of alternative proteins that have been researched as potential animal protein replacers. This is partly driven by consumers becoming aware of the environmental impact of animal husbandry as well as lifestyle changes that include a reduction in meat consumption. However, before alternative proteins can be fully adopted in food production, there are several hurdles such as protein yield, allergenicity, as well as the cost of production that need to be overcome. There is also a need to consider the water footprint of alternative proteins which needs to be included in the lifecycle analysis of these proteins. Additionally, there are legal issues surrounding these alternatives that call for more research to find answers that would clear the doubts about the use of these ingredients in food production and processing. This provides an opportunity for various stakeholders to work together to achieve common sustainability goals whilst ensuring the

provision of a balanced diet to feed the world population which continues to increase.

Author contribution

Claire Darizu Munialo: Conceptualization; investigation; writing – original draft; methodology; formal analysis; writing – review and editing; supervision; data curation; project administration; validation.

Data availability statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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