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Alternative proteins for meat and dairy replacers: Food safety and future trends

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ABSTRACT

Traditionally, meat and dairy products have been important protein sources in the human diet. Consumers are eating more plant-based proteins, which is reflected in current market trends. Assessing how alternative proteins are processed and their impact on food safety helps realize market opportunities while ensuring food safety. In this review, an analysis of the food safety hazards, along with current industry trends and processing methods associated with alternative proteins for meat and dairy products for the European Union market is described. Understanding the effects of processing and safety alternative proteins is paramount to ensuring food safety and understanding the risks to consumers. However, the data here is limited. With the expected further increase in protein alternatives in consumers' diets, the risk of food allergens is apparent. The occurrence of processing contaminants in plant-based alternatives may occur, along with anti-nutritional compounds, which interfere with the absorption of nutrients. Further, typical food safety hazards related to the plant, the product itself, or processing are relevant. Although hazards in insects and seaweed are being addressed, other protein alternatives like cultured meat and SCPs warrant attention. Our findings can aid industry and governmental authorities in understanding current trends and prioritizing hazards for future monitoring.

KEYWORDS

Allergy; hazards; plant-based; processing; protein

Introduction

Traditionally, meat and dairy products have been important protein sources in the human diet. Yet, current market developments generate increasing interest in alternative protein sources (Aschemann-Witzel et al. 2021). The European Union (EU) has explored EU protein plant production opportunities that provide economic and environmental advantages (European Commission 2018). In the Farm to Fork Strategy, the EU Commission seeks to reduce dependency on crops like soy grown on deforested land and instead desires alternative EU-grown plant proteins (European Commission 2020).

Branding and claims for protein alternatives must be transparent to the consumer for the market to succeed (FMCG Gurus for Fi Global 2020). Consumer perception can influence the successful introduction of less traditional protein sources like insects, cultured meat, or single-cell proteins (SCPs). The impact of consumer trends of reducing meat consumption when given a flexitarian diet on human health has been debated. However, the shift toward a flexitarian diet is seen as a first step toward reducing the over-consumption of meat in the Western world, a noteworthy trademark of the protein transition (Dagevos 2021). Consequently, there appears to be a trend toward plant-based

proteins to be used in the near future given the fewer barriers to trade, legal authorization, and consumer perception and understanding compared to other alternative proteins like insects, cultured meat, seaweed, and SCPs (van der Spiegel, Noordam, and van der Fels-Klerx 2013).

Drivers like human health benefits, environmental impact, and animal welfare influence the development of plant-based (meat) alternatives (He et al. 2020). In the European food industry, barriers influencing the future growth of the plant-based sector are bridging the price gap between animal and plant-based products, improving user experiences regarding taste and nutritional profiles, and increasing the distribution and availability of plant-based products (Geijer and Gammoudy 2020). According to Innova Market Insights, a global market intelligence company specializing in trends for food and beverages, plant-based production has been a continuing trend. It has diversified in 2021 into a range of plant-based alternatives for meat, fish, cheese, and eggs, along with drinks, snacks, and ready-to-eat meals (Innova Database 2021). The global market for plant-based proteins is forecasted to grow, with an estimated turnover of 27 billion dollars by 2030 (P&S Intelligence 2021).

This study reviews food safety hazards (chemical and microbiological) related to the current transition toward alternative proteins for meat and dairy products. Given

expected short-term trends, the analysis preliminarily focuses on plant-based proteins for EU consumers. The effect that processing has on the safety of plant-based proteins is also described. Beyond this, we touch upon other alternative proteins like insects, cultured meat, seaweed, and SCPs in our analyses. Results were based on the available literature concerning food safety hazards, expert input, and upcoming trends as a protein replacer for meat and dairy products.

Material and methods

Demarcation of the study

The study focused on alternative proteins used to replace meat or dairy products intended for human consumption. Animal feed was not considered in this study. The EU market and consumer were considered for the trend analysis. Plant-based proteins like cereals, legumes, seeds, nuts, potatoes, mushrooms, and green leaves were foremost examined. Other alternative proteins included were insects, cultured meat, seaweed, and SCP products. The list of alternative proteins is non-exhaustive, yet it depicts proteins currently used or where consumer use in the EU is expected to grow in the coming five years.

Trends

Several sources were consulted to elucidate current developments of alternative proteins. First, an Innova search was performed on product introductions for meat and dairy replacers between July 2016 and July 2020 in the EU, with grains, insect-based products, nuts & seeds, plant proteins, pulse & pulse products, and soy & soy products as ingredients. Moreover, reports from food-focused news sites, like Food Navigator, trade journals like *Vakblad voor de voedingsmiddelenindustrie* (VMT), sector forecasts from the financial sector (ING, ABN AMRO), and the Center for the Promotion of Imports from developing countries (CBI), among other scientific literature reporting on trends were used. Also, internal Wageningen University & Research (WUR) reports that included trends on proteins were assessed. The focus was on information from the last five years.

Literature review

Google Scholar was consulted for literature on alternative proteins. General terms like “plant-based,” “protein alternative,” and “trends” were preliminarily used. When required, protein-specific (pea, insect, etc.) and hazard-specific (allergen, mycotoxin, etc.) terms were applied. We also used literature reviews performed by Wageningen University & Research (WUR) for several plant-based ingredients. Given the novelty of plant-based proteins as meat and dairy replacers, additional literature searches were performed for legumes and green leaves to elucidate chemical and microbiological food safety hazards from about the last 10 years. Appendix A is the search strings used for these specific searches. The

bibliographic databases Scopus and CAB abstracts were used. After applying the search strings, the relevance of the references retrieved was foremost processed based on the title and keywords. Then, the selected references were further evaluated based on the abstracts. Relevant papers were read in full to analyze for potential hazards. In addition, we used the snowball method to focus our search and find specific information on trends and hazards per protein type. Moreover, we consulted scientific reports from governmental organizations like the European Food Safety Authority (EFSA), the World Health Organization (WHO), and the Food and Agriculture Organization of the United Nations (FAO). Finally, during the expert elicitation (interviews), reports and scientific literature noted by the expert were also consulted.

Expert elicitation

To better understand the current trends, knowledge gaps, and food safety hazards of plant-based and other alternative proteins, 15 experts were interviewed. Interviews were performed with scientific researchers with knowledge on topics like protein trends ($n=7$), processing techniques ($n=5$), and/or food safety hazards ($n=15$). Each interview followed a qualitative interview guide (Appendix B). Questions were tailored to the interviewees and further shaped during the interview depending on the answers provided.

Results and discussion

The results found on trends and food safety hazards related to plant-based proteins are described in “Plant-based proteins,” focusing on the short-term alternative proteins. The section “Other alternative proteins” describes trends and food safety hazards related to proteins from insects, cultured meat, seaweed, and single cells as examples of proteins expected to be used in the longer term. Images of these protein sources and some potential food safety hazards are shown in [Figure 1](#).

Plant-based proteins

Globally, the market for plant-based meat and dairy proteins is rapidly developing (Aschemann-Witzel et al. 2021, Sha and Xiong 2020). These developments are adapted to meet consumer demands, with legumes like soy and pea being used to develop meat analogs (Sha and Xiong 2020). When considering plant-based milk alternatives, the nutritional composition, appearance, and taste can vary. However, pulse protein concentrates or isolates may be used to achieve nutritional needs (Vogelsang-O’Dwyer, Zannini, and Arendt 2021). Market trends indicate increased potential for business opportunities and innovation of plant-based alternatives (Sha and Xiong 2020, Aschemann-Witzel et al. 2021). The following sections examine trends, processing effects, and review food safety hazards (chemical and microbiological) for several plant-based protein alternatives.

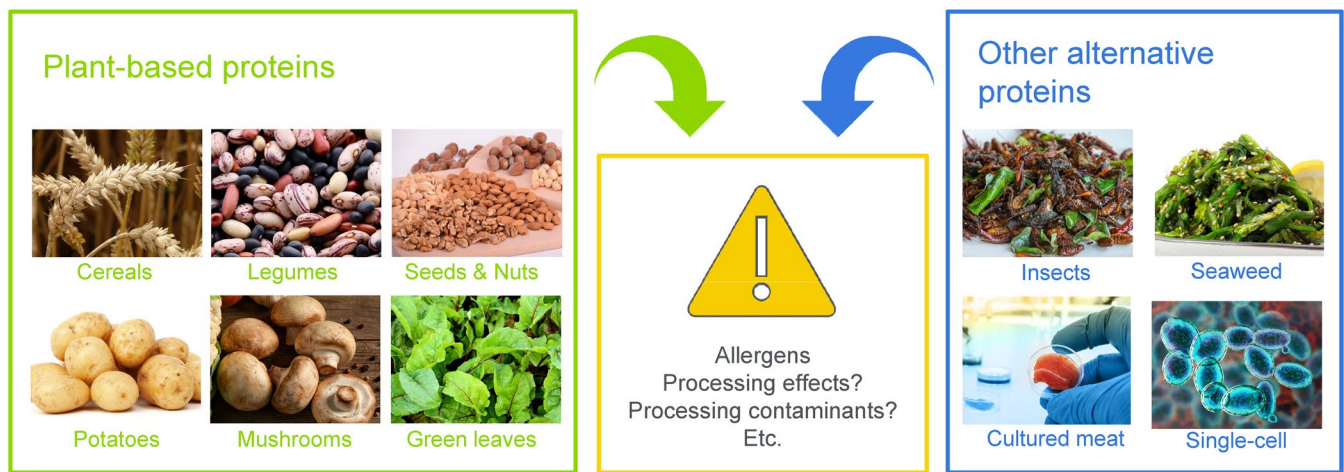


Figure 1. Images of plant-based and other alternative proteins along with some potential food safety hazards.

Trends for plant-based proteins

Although soy is already widely used, there is a shift toward using other plant-based products as a protein source. For instance, cereals like wheat and oats, other legumes like peas, chickpeas, and (fava) beans, seeds, nuts, potatoes, mushrooms, and green leaves, like beet leaves, are used as alternatives. Technological hurdles for plant-based alternatives, along with the effects on product quality, cost, and safety, are current challenges (Sha and Xiong 2020). The following sections further elaborate on trends for the various plant-based proteins.

Cereals. Wheat is readily available and often used with soy in meat substitutes to achieve the right texture. However, there are increasing demands for “allergen-free” products, including gluten, dairy, soy, or nut-free options (VMT 2020). According to data from Innova, there is no clear trend for ancient grain introductions, and the hype for quinoa appears finished. However, there has been a clear increase in oat product introductions in the EU over the last five years (Innova Database 2021). According to a 2019 report from the company Givaudan, oats were the leading cereal candidate for future protein ingredients with favorable factors like supply, nutritional value, taste, and color, as well as regulatory approval (Givaudan 2019). It is especially used as an alternative for dairy products (Geijer and Gammoudy 2020, Menkveld 2021). Besides its use as a milk alternative, the use of oats in other dairy substitutes like yogurt, crème fraiche, and desserts is seen in EU supermarkets. In general, though, the amount of protein in oat-based dairy alternatives is lower when compared to dairy-based products.

Legumes. Soy is a common plant-based ingredient and alternative to animal proteins. Although a shift toward other alternative proteins is seen, soy remains an important alternative to meat and dairy in the coming

years (Geijer and Gammoudy 2020). According to the European Vegetable Protein Association (EUVEPRO), in 2018, textured soy flours and concentrates, hydrolyzed protein, and peptide/peptones of soy were seeing market growth (Agrosynergie et al. 2018). Possible explanations for replacing soy may be attributed to its adverse health effects as a phytoestrogen (Rietjens, Louisse, and Beekmann 2017), which experts have also noted. Also, the increased availability of other plant-based alternatives, concerns about soy’s sustainability, and its classification as an allergen may prompt alternatives. According to Innova data (2016-2020), the use of soy is relatively constant in the EU (Innova Database 2021). Therefore, soybeans are still included in the list of 50 future foods, as established by Knorr and WWF (2019).

Pea protein, like green and yellow pea protein, is an upcoming alternative protein. According to the EUVEPRO, textured pea protein and functionalized isolates were introduced to the market, and pea isolates, concentrates, and functional pea flours were seeing market growth (Agrosynergie et al. 2018). Companies like Cargill and Roquette are investing in pea protein as it is a low allergenic alternative to soy and wheat. In the EU, retailer Albert Heijn has consciously chosen non-soy vegetable burgers, like the pea vegetable burger from Beyond Meat (VMT 2020). Innova data (2016-2020) parallels this trend and shows an increase in pea protein product introductions in the EU (Innova Database 2021). Despite the traction gained from pea protein as an alternative for other plant-based options, the supply is still limited since large investments in facilities for protein extraction, breeding, and quality is needed (Geijer and Gammoudy 2020).

Chickpeas, also known as garbanzo beans, have been ranked in the top 15% of plant-based proteins (Givaudan 2019). Also, the Knorr brand and the WWF specified sprouted chickpeas on the list of 50 future foods (Knorr and WWF 2019). According to the CBI, chickpeas are increasingly being used for new products and alternatives due to the plant-based movement for foods and beverages (CBI

2020a). Falafel and hummus are familiar sources of chickpeas for consumers. Given consumer familiarity, versatility, and low prices, EHL Ingredients expects the demands for chickpeas will increase in 2021 and have indicated it as one of four food trends for 2021. However, there has been no clear trend for chickpea product introductions in the EU over the last five years (Innova Database 2021). In general, convenience is a crucial driver for the EU consumer. Therefore, providing new chickpea products in easily accessible forms opens opportunities for future growth (CBI 2020a).

Broad beans, also known as faba or fava beans, are a promising future vegetable protein (Knorr and WWF 2019). The Innova database also shows a clear increase in bean product introductions over the last 5 years, especially for fava beans (Innova Database 2021).

Seeds. Based on a study evaluating forty-two plant proteins, seeds like sunflower seed and flaxseed have been ranked in the top 6 plant-based proteins. The supply, nutritional value, and regulatory application of sunflower seeds are very favorable. Advantages for flaxseeds include the environmental impact, taste, and color (Givaudan 2019). Seeds were predicted to be a popular superfood for 2020 (Sloan 2020), while flaxseeds, hemp seed, and sesame seeds have been listed as being part of the 50 future foods by Knorr and WWF (2019). Despite their popularity as a superfood and potential as plant-based proteins, data from Innova (2016 to 2020) shows no clear trend for seed product introduction in foods in the EU (Innova Database 2021).

Nuts. Given a healthy image, the consumption of nuts has previously reported growth, with almonds reported as particularly popular (Klüche, Hoek-van den Hil, and van Asselt unpublished). Almonds are also an alternative to soy in plant-based beverages, like dairy drinks (VMT 2020). The introduction of alternative plant-based drinks from nuts is advancing in the market (Geijer and Gammoudy 2020). Data from Innova (2016 to 2020) shows no clear trend for nut product introductions in the EU. However, there is some increase in the nut category as a whole and in product introductions containing almonds and hazelnuts (Innova Database 2021).

Potato. Potato protein is already used on the European market as a food ingredient. It is a low allergenic alternative to soy and wheat, and it can be used as a vegetable-based emulsifier (VMT 2020). According to EUVEPRO, the market for potato protein is growing (Agrosynergie et al. 2018).

Mushrooms. Mushrooms have a health-promoting appeal and are considered a promising ingredient (Sloan 2020). According to Knorr and WWF (2019), enoki mushrooms, maitake mushrooms, and saffron milk cap mushrooms

all make the list of 50 future foods. According to the CBI, the European market for dried mushrooms shows stable growth, most likely attributed to the rising interest in animal protein alternatives and vegan diets. Also, the sustainable and ethical production of mushrooms appears important to EU trade and consumers (CBI 2020b). The increased interest in mushroom products coincides with Innova's (2016-2020) data, which shows an increase in product introductions containing mushrooms in the EU (Innova Database 2021).

Green leaves. A review on the potential use of plant-based proteins originating from green leaves identified various crop plants that are considered potential protein sources for use in food. For example, studies reported the use of cowpea leaves (Badar and Kulkarni 2011), lucerne (Badar and Kulkarni 2011), berseem (Badar and Kulkarni 2011), and cassava and kapok leaves (Stuetz et al. 2019), as attractive nutritional sources of proteins and other micronutrients. Potentially a wide range of green leaves can be used as sources of plant-based proteins for the food sector. For example, the leaves of nopales, amaranth, kale, moringa, bok choy (Chinese cabbage), spinach, watercress, and parsley root were mentioned by Knorr and WWF (2019).

No trends were found for the introduction of proteins from green leaf products in the last five years (Innova Database 2021). However, commercial-scale production of ribulose-1,5-bisphosphate carboxylase-oxygenase (rubisco) extracted from sugar beet leaves, pending its approval as a novel food, is expected (VMT 2020); experts identified this as an upcoming protein source.

Processing toward a plant protein ingredient. Plant-based products, like cereals, legumes, or seeds, can be used as a whole and as protein concentrate ingredients in meat and dairy replacers. Experts have indicated that when processing plant-based protein substitutes, the industry may assume it follows the same way of processing as meat and dairy. Thus, we may be unaware of the effect of processing on potential food safety hazards in the final product, like allergens, anti-nutritional factors, processing contaminants, and microbiological quality.

Protein from plants can be produced using dry fractionation or wet fractionation. The choice of technology depends on the crop composition (protein, oil, and starch content). Dry fractionation using air classification, a method to separate materials by air velocity and particle size, is mainly applied for starch-containing crops such as pea, faba bean, or mung bean to make protein concentrates from flour. Protein content depends on the protein and starch content of the beans or seeds, the differences in size between the protein body and the starch granules, and the milling efficiency (the presence of fat has a negative impact). Air classification leads to an enrichment in anti-nutritional factors

(Arntfield and Maskus 2011), e.g., plant phenolics (tannins) (Mohan, Tresina, and Daffodil 2016), phytic acid (Kumar et al. 2010), and protease inhibitors. Ways to mitigate this include, for example, toasting (the conditions will depend on the crop, and if dry or wet toasting is applied), which is commonly applied in soy processing to inactivate enzyme and protease inhibitors and create positive off-flavors (Bühler et al. 2020). Wet processing is a standard processing technique to make protein isolates. It is used mainly in oilseed and pulse processing toward a protein isolate. In the case of oilseeds (e.g., soybean or rapeseed/canola), a defatting step is required (by pressing or hexane extraction). In a simplified way, it includes a hydration step, a decanting step to remove the starch and insoluble fiber, isoelectric precipitation to extract the globulin fractions of the proteins, and a spray-drying step. A high protein purity of the ingredients offers more flexibility in the formulation and the removal of the anti-nutritional factors (mainly during the isoelectric precipitation step). Proteins from cereals (e.g., wheat gluten) are extracted using a simple washing step using water (to remove the starch). In most cases, potato proteins are recovered from the potato fruit juice (after starch and fiber extraction) by an acidic-heat treatment (90-105 °C), resulting in low-added value proteins for feed application. Due to their high functionality, potato proteins are also extracted and purified using a combination of expanded adsorption and simulating bed technology leading to highly functional ingredients (Alting et al. 2011).

Experts suggest that dry fractionation with air classification is a processing technique that will grow in the coming years; however, it will not completely replace the need and functionality of protein isolates. A combined system of air classification and wet processing to increase efficiency can be expected in the next 3 to 5 years. At this moment, another technique for protein extraction known as electrostatic separation needs further efficiency improvements (yield) before company investment is expected. A trend toward fewer refined ingredients influences the digestibility of the ingredients (anti-nutritional factors). There is the need, which is also recognized by experts, to better understand which processing steps are needed to inactivate anti-nutritional factors and partially remove them. Apart from research on the aforementioned compounds, little is known on the effect of processing on other contaminants such as pesticides or heavy metals.

Once proteins are extracted from plants, further processing is needed to produce meat or dairy replacers from these proteins. The main technology involved in producing meat analogs is extrusion either to produce texturized vegetable proteins used in, e.g., burger patties or to produce high moisture meat analogs like chicken pieces. A critical step toward soy protein meat alternatives is applying high moisture extrusion to achieve fibrous structures similar to meat. Alongside this application, allergenicity, odor, and development of off-flavors during processing influence its potential as a meat alternative (Zhang et al. 2021). To process plant proteins into dairy replacers, such as plant-based beverages, starch or other hydrocolloids are added since plant proteins have lower solubility and functionality (especially gelling

properties) than milk-based products. Experts anticipate food safety concerns during the processing of new protein sources and the use of side-streams as a source of proteins.

Food safety hazards related to plant-based proteins

Several chemical and microbiological food safety hazards are related to using plant-based proteins as alternatives to meat and dairy products (Table 1). Literature on the hazards was mainly related to the ingredients used to produce proteins, meaning further processing into the plant-based product may influence the presence of hazards. For example, processing may introduce microbiological hazards like *Staphylococcus aureus*, mainly via food handling (contact with skin) or *Listeria monocytogenes* during processing, as it can be in the factory environment and is especially of concern in (chilled) ready-to-eat products. Also, viruses may be introduced via contaminated food handlers. On the other hand, microbiological hazards like bacteria and viruses may also decrease due to the heating steps while producing alternative proteins and their subsequent processing into meat and dairy replacement products. Experts have also pointed to mycotoxins or other natural toxins, like certain lectins, the latter of which can be a concern if not heated well enough. Similar questions arise when looking at processing contaminants and the emergence of, e.g., advanced glycation end-products (AGEs). Previously, we were less aware of these kinds of contaminants. More recently, we are looking at the effect of processing (Maillard reaction - acrylamide, AGEs, furans, 3-monochloropropane diol (MCPDs), 2-MCPDs, and glycidyl esters). Again, the effects depend on the processing (e.g., temperature-timing) and how the products are treated, as well as the protein source used. For instance, protein denaturation, aggregation, or glycation may reduce or aggravate the allergenicity of certain allergens (Vissers et al. 2011, Deng et al. 2019). Experts have also noted that some mycotoxins can be of concern in plant-based products since heating cannot remove them.

In the following section, hazards are identified per type of plant protein. A separate section is added on allergens since they are relevant human health hazards related to several of the below-mentioned plant-based proteins.

Cereals. A comprehensive review described chemical hazards in cereals such as wheat and oats based on scientific literature and Dutch monitoring data. The following were relevant for cereals in general (type unspecified): polycyclic aromatic hydrocarbons (PAHs), fumonisins, and ochratoxin A (OTA). Pesticides were relevant for both wheat and oats. Deoxynivalenol (DON) and zearalenone (ZEA) were relevant in wheat, while the T2/HT2-toxin was relevant for oats. Experts have also noted the presence of mycotoxins and pesticides residues in plant-based products, including cereals, as potential food safety hazards. Finally, a few hazards had knowledge gaps: tenuazonic acid (TA), enniatins, methylmercury, and nickel, as limited information was available to evaluate its relevance for cereals (Klütche, Hoek-van den Hil, and van Asselt unpublished).

Table 1. Overview of potential hazards in plant-based proteins, based on literature and expert opinion.

Hazards	Cereals	Legumes	Seeds	Nuts	Potatoes	Mushrooms	Green leaves
Allergens	x	x	x	x	x ³	x ²	x ²
Microplastics and nanoplastics	–	–	–	–	–	–	–
Chemical hazards							
Anti-nutritional factors	x ¹	x	x ¹	x ¹	x ¹	x ¹	x ¹
Brominated flame retardants	–	–	–	–	–	–	–
Dioxins and polychlorinated biphenyls (PCBs)	–	–	–	–	–	–	–
Elements	x ²	x	x ²	x ²	–	x	–
Heavy metals	x ²	x	–	–	x	x	x
Marine biotoxins	–	–	–	–	–	–	–
Mycotoxins	x ²	x	x ²	x ²	x ²	–	–
Plant protection products and biocides	x	x	x ²	x	x	x	x
Plant toxins or compounds	–	x	x	x	x ²	–	x
Polycyclic aromatic hydrocarbons (PAHs)	x	–	x ²	–	–	–	x
Processing contaminants	x ¹	x ¹	x ¹	x ¹	x	x ¹	x
Veterinary drug residues	–	–	–	–	–	–	–
Microbiological hazards							
Bacterial toxins	–	x ¹	x ¹	x ²	–	–	x
Genetic engineering/genetically modified organisms	–	–	–	–	–	–	–
Parasites	x ³	x	–	–	–	–	x
Prions	–	–	–	–	–	–	–
Spore-forming bacteria	x	x	–	–	x	x	x
Vegetative bacteria	x	x	x	x	x	x	x
Viruses	x ³	–	–	–	x	–	x

Dash: no indications were found that this would be a potential hazard.

¹Mentioned as a general, potential hazard in plant-based proteins.

²Knowledge gaps of some hazards.

³Rarely occurs.

Salmonella spp., *Bacillus cereus*, *Clostridium perfringens*, and *Escherichia coli* O157:H7 were detected in cereals and grains and reported as a causative agent in outbreaks with these foods (Young et al. 2015). More recently, *Salmonella* spp., *B. cereus*, *C. perfringens*, and *St. aureus* have been reported in cereal products and legumes (European Food Safety Authority (EFSA) and European Center for Disease Prevention and Control (ECDC) 2021). Virus and parasite contamination of cereals and grains is considered low. The EFSA developed a model to identify and rank specific food/pathogen combinations that are most often linked to human illness cases originating from food of non-animal origin in the EU. In this ranking, the pathogen/food combination pathogenic *E. coli* and fresh pods, legumes or grain ranked second (EFSA Panel on Biological Hazards (BIOHAZ)) 2013). Furthermore, strong associations were reported for *B. cereus* and buckwheat and bulgur wheat and cereals and grains in general (EFSA Panel on Biological Hazards (BIOHAZ)) 2013, FAO and WHO 2014). The proportion of Shiga-toxin producing *E. coli* (STEC) cases attributed to foods in the EU is for grains and beans 1.15% and nuts 0.00% (WHO and FAO 2018). This is low compared to the relative contributions for beef and produce, 11.83% and 10.77%, respectively (WHO and FAO 2018). Overall, several foodborne pathogens are linked to cereals, but processing into alternative proteins will largely reduce the levels present.

Legumes. A chemical hazard associated with soy are the so-called phytoestrogens (which are plant derived compounds), including the isoflavone compounds daidzein, genistein, and glycitein. These bear structural resemblance with human estrogenic hormones such as 17 β -estradiol and display in-vitro estrogenic activity. Whereas possible positive impacts of these phytoestrogens have been considered in literature, such as reduction of osteoporosis in postmenopausal women, there may also be risks of endocrine disruption involved, particularly in young and adolescent children. The Nordic Council of Ministers (2020) recently reviewed the available evidence from epidemiological human and laboratory animal studies on a possible correlation between soy phytoestrogen intake and risks of, for example, premature onset of puberty, breast cancer, hypospadias, and thyroid dysfunction. Although some case correlations were established, this was inconsistent across studies or the number of studies on subjects representative for Scandinavian consumers were too few to conclude confidently. Based on food intake data for the Danish population, hypothetical scenarios of substitution of animal products by soy were shown to give rise to slight exceedances to health-based guidance values in young (4-10 year-old) boys and girls would occur (Nordic Council of Ministers 2020).

Other food safety hazards have been reported in legumes. Kleter et al. (unpublished) evaluated West-African soy, cowpea, and Bambara groundnut. Besides allergens in soy, food safety concerns included the potential for mycotoxins, pesticide residues in cowpea and Bambara groundnut, bacterial contamination/presence of enteric pathogens in soy, and anti-nutrients in cowpea. Anti-nutritional factors have also been flagged in soy (De Angelis et al. 2017). Experts mentioned the presence of protease inhibitors and lectins in legumes as anti-nutrients, some of which can bind to the walls of the human digestive system and affect the gastrointestinal tract. Lectins can usually be degraded after prolonged heating, as then they are less toxic. However, it is currently unknown whether the heating involved in the production of dairy and meat replacers is sufficient to degrade lectins to safe levels. Pesticide residues are another concern that has been noted in chickpea grains as being associated with its storage, even though processing can decrease these (Kaushik, Satya, and Naik 2016). Also, heavy metals may be a food safety concern. Wei and Cen (2020) attributed concerns for residents of Beijing, China, with Cr, Mn, and Cu through dietary consumption of cereals, legumes, and their products. Experts have also emphasized concerns with other legumes like fava beans, as part of the population cannot degrade the glycoalkaloid (vicine) in fava beans due to a missing enzyme (G6PD-deficient). This leads to favism.

Outbreaks of *B. cereus* are reported for navy beans in GBR (Nicholls et al. 2016) and refried beans in the USA (Carroll et al. 2019). *Clostridium botulinum* caused outbreaks after consuming peas and green beans (Bergeron et al. 2019, Hellmich et al. 2018). As aforementioned, *Salmonella* spp., *B. cereus*, *C. perfringens*, and *St. aureus* have been reported in cereal products and legumes (European Food Safety Authority (EFSA) and European Center for Disease Prevention and Control (ECDC) 2021). Food vehicles related to outbreaks in the USA with legumes were green peas, peas, green beans, and refried beans and involved *C. jejuni*, *C. botulinum*, *C. perfringens*, *B. cereus*, STEC, *Salmonella* spp., norovirus, and the parasite *Cyclospora cayetanensis* (Centers for Disease Control and Prevention and National Center for Emerging and Zoonotic Infectious Diseases (NCEZID)) 2018). The parasite *C. cayetanensis* contaminated fresh sugar snap peas imported from Guatemala to Canada. It resulted in an outbreak of 35 confirmed and 10 probable cases (Whitfield et al. 2017). As indicated earlier, EFSA ranked pathogenic *E. coli* and fresh pods, legumes or grain second concerning human illnesses (EFSA Panel on Biological Hazards (BIOHAZ)) 2013). Several cases can be linked to STEC and grains and beans (WHO and FAO 2018). It is plausible that processing can eliminate or reduce these risks.

Seeds. Several mycotoxins have frequently been found in seeds, namely aflatoxins, DON, OTA, T-2 and HT-2 toxin, and ZEA. Also, pesticides and plant toxins were relevant hazards. The formation of hydrocyanic acid (HCN) from cyanogenic glycoside in linseed (i.e.,

flaxseed) was described as a concern. A few hazards have knowledge gaps in seeds. These included nickel, PAHs, *Alternaria* toxins [alternariol (AOH), alternariol monomethyl ether (AME), TA, and tentoxin (TEN)], and fumigants, like 1,2-dichloroethane (DCE) (Klüche, Hoek-van den Hil, and van Asselt unpublished). Until recently, the seeds of canola (i.e., low-erucic-acid oilseed rape varieties) used to be crushed for extraction of the oil mainly for human consumption, while the residual protein-rich meal would get diverted into animal feed. New extraction and processing methods have enabled the manufacture of protein isolates with lower, acceptable levels of antinutrients, such that they have become fit for human consumption. The safety assessments of such protein isolates by the EFSA NDA Panel point to the possibility of allergenicity of these isolates in mustard-allergic patients, such as through cross-reaction of the napins, major storage proteins from both crop species, with allergic patients' IgE antibodies (EFSA Panel on Dietetic Products and Nutrition and Allergies (NDA)) 2013, EFSA Panel on Nutrition Novel Foods and Food Allergens 2020).

Seeds for consumption have been linked to 8 *Salmonella* spp. outbreaks with 376 cases, 4 hospitalizations, and 1 death (Young et al. 2015, FAO and WHO 2014). The WHO/FAO attributed a level 3 priority for risk ranking to sesame seeds with *Salmonella* spp. and *Bacillus* spp. (FAO and WHO 2008). There have been many reports of *Salmonella* related to sesame seeds in the Rapid Alert System for Food and Feed (RASFF), thereby providing evidence that *Salmonella* is a relevant hazard related to sesame seeds and products thereof (Somorin, Odeyemi, and Ateba 2021). In general, limited information is available for food pathogens associated with edible seeds in scientific literature.

Nuts. Three chemical hazard groups are relevant for nuts: aflatoxins, pesticides, and natural plant toxins. The formation of HCN from cyanogenic glycosides is important to consider in almonds (Klüche, Hoek-van den Hil, and van Asselt unpublished). Almond milk is typically prepared with sweet almonds, which have lower amounts of HCN than bitter almonds (300-4000 mg HCN/kg); however, HCN in almond milk has been reported at 3.0 mg HCN/kg (RIVM and RIKILT - Wageningen University & Research 2018). There may be a greater risk if consumers prepare their own almond milk using the wrong type of almonds. Also, the concern with cyanogenic glycosides in foods like almonds has been raised by EFSA, although data to determine the chronic risks are limited (Schrenk et al. 2019). Besides these hazards, Klüche, Hoek-van den Hil, and van Asselt (unpublished) identified *Alternaria* toxins – AOH, AME, and TA – acrylamide, and nickel as knowledge gaps.

Salmonella spp. is considered a microbiological hazard in almonds. In 2008, the WHO and FAO attributed a level 3 priority for risk ranking to almonds (FAO and WHO 2008). There is a reported outbreak in the USA for *Salmonella* Bredeney in, among others, almond butter (Centers for Disease Control and Prevention 2013). Overall, few microbiological hazards are described for almonds. The presence of rhizoxins, toxins produced by endofungal bacteria, has been reported in plant-based products like ground nuts (Lackner, Hertweck, and Madhani 2011, Birol and Gunyar 2021).

Potato. A review based on scientific literature, Dutch monitoring data, RASFF reports, and expert opinion has described chemical hazards for potatoes. In total, 16 chemical hazards were prioritized as relevant to human health: cadmium, solanine, acrylamide, and 13 plant protection products (chlorpropham, chlorpyrifos, diquat, ethoprophos, fluazifop-p-butyl, fluopicolide, fosthiazate, glufosinate-ammonium, lambda-cyhalothrin, linuron, lufenuron, metribuzin, and thiacloprid) (Nijkamp et al. 2017). The risk of glycoalkaloids (GAs), like solanines, from potatoes, has been evaluated for potato and potato-derived products. Health concerns for younger age groups were found when considering the highest mean and P95 exposures. Data on the occurrence of GAs and their aglycones should be collected to improve the assessment and decrease uncertainty in these calculations (Schrenk et al. 2020). Besides the 16 hazards indicated by Nijkamp et al. (2017), the following data gaps were mentioned for potato products: the mycotoxins DON and diacetoxyscirpenol (DAS), the plant toxin calystegin, perfluorinated compounds (PFCs), and the process contaminants furan and AGEs. Given potato protein production, as described earlier, the formation of process contaminants may be an issue if higher temperatures, like those applied during deep-frying, baking, or toasting, are used during processing to make plant-based alternative products (Mesias, Delgado-Andrade, and Morales 2019).

In fresh, unprocessed potatoes, *B. cereus*, *C. botulinum*, *E. coli* O157:H7, and *L. monocytogenes* were detected (Doan and Davidson 2000). However, since potato processing involves heating steps, the major hazards are spore-formers, specifically, *B. cereus* (Hayrapetyan et al. 2018). According to an EFSA data analysis concerning outbreaks, *Salmonella* spp., *B. cereus*, and norovirus are linked to potato products (EFSA Panel on Biological Hazards (BIOHAZ)) 2013). This can be caused by the addition of contaminated ingredients to the potatoes (like herbs and spices containing *Salmonella* spp.) or infected food handlers (norovirus) (Lehmacher, Bockemühl, and Aleksic 1995).

Mushrooms. A review on chemical hazards in cultivated mushrooms identified the following 26 chemical hazards as relevant: heavy metals and elements (cadmium, lead, mercury, arsenic, platinum group elements,

and aluminum), pesticides (bifenthrin, carbendazim, carbofuran, chlorpyrifos, deltamethrin, fenpropathrin, fipronil, permethrin, procymidone, propoxur, tetramethrin, lambda-cyhalothrin, beta-cyfluthrin, and beta-cypermethrin), and disinfectants, including by-products thereof (like formaldehyde, trihalomethanes, haloacetic acids, and chlorate) (Hoek-van den Hil and van Asselt 2019).

A literature-based study on microbiological hazards in the mushroom chain found that *L. monocytogenes* were detected in the substrate and during cultivation, processing, and storage. Other pathogenic bacteria of concern reported in the mushroom chain included *Bacillus* spp. (including *B. cereus*), *Campylobacter* spp. (*C. jejuni* and *C. coli*), *Clostridium* spp. (*Cl. botulinum* and *Cl. perfringens*), pathogenic *E. coli*, *Salmonella* spp., *St. aureus*, and *Yersinia enterocolitica* (Nieuwland et al. unpublished). Overall, several chemical and microbiological hazards can occur in the mushroom chain.

Green leaves. Chemical hazards associated with the processing of green leaves were reported, such as corynetoxins on ryegrass (Penno et al. 2012), pesticide residues on leafy vegetables (Farha et al. 2018), heavy metal accumulation in alfalfa (Rezaeian et al. 2020) and pea plants (Slima and Ahmed 2020). Results from a WUR literature study on chemical hazards in leafy vegetables (lettuce, spinach, cabbage, chicory, etc.) showed that aluminum, cadmium, copper, and lead, along with nitrate, PAHs, several plant production products (>40), processing contaminants acrylamide and 5-hydroxymethylfurfural, disinfection by-products chlorate and perchlorate, and the quaternary ammonium compound benzalkonium chloride were reported hazards (Banach et al. unpublished). Experts have also noted that the leaves of some vegetables, like tomato and cassava, are toxic, meaning these would need to be detoxified, e.g., through an additional processing step, before use.

Results from a WUR literature study on the microbiological hazards in leafy vegetables (lettuce, spinach, cabbage, fresh herbs, etc.) showed that pathogenic *E. coli* are the group of microorganisms most frequently reported. Other bacterial human pathogens related to the leafy vegetable chain are *Salmonella* spp., *L. monocytogenes*, *Campylobacter* spp., *Shigella* spp., *Yersinia* spp., and other pathogenic bacteria like *Clostridium* spp., *Vibrio* spp., *B. cereus*, *St. aureus*, *Cronobacter* spp., and *Arcobacter* spp. Additional microbiological hazards that may be encountered include parasites (*C. cayetanensis*, *Cryptosporidium* spp. among many others like *Toxoplasma* spp., *Giardia* spp., *Entamoeba* spp., *Ascaris* spp., *Fasciola* spp., *Taenia/Echinococcus* and *Toxocara* spp.) and viruses (mainly human norovirus and hepatitis A virus). However, the processing of leafy greens into proteins requires some heating steps, so this will affect the presence of vegetative bacteria, parasites, and viruses (Van Bokhorst-van de Veen et al. unpublished). Pathogenic bacteria like *Salmonella* and pathogenic *E. coli* may internalize in green

leaves during food processing procedures, such as during washing when leaf damage may occur (Mulaosmanovic et al. 2021). Overall, several chemical and microbiological hazards can occur when green leaves are utilized as an alternative protein source.

Allergens. In recent decades, introductions of new and advanced food products have contributed to the general increase in the prevalence of food allergies (Loh and Tang 2018). This development will likely continue throughout the global protein transition. Since more meat and dairy replacers are expected, exposure to soy in one's diet can also be expected to increase (Zhang et al. 2021). Analyses on the allergenic potential of soybean protein showed gastrointestinal resistance to fragments of allergenic proteins, beta-conglycinin and glycinin, meaning their structure could trigger an immunoreaction (De Angelis et al. 2017). The allergenic potential of other plant-based proteins was assessed by Mishra and Kumar (2021). Chickpea and mung bean lipid transfer proteins (LTPs) were closely related to other reported allergenic LTPs (Mishra and Kumar 2021). Also, sensitization and allergy to legumes were shown to play a minor role compared to peanut and soybean consumption. Rather intrinsic factors like the "different proteins, processing, matrix, frequency, timing and route of exposure, and patient factors might play a more important role" regarding peanut sensitization (Smits et al. 2018).

The incorporation of new protein sources into food products, or increased intake of existing protein sources using purified protein concentrates, may entail the following:

- (1) A rise in existing food allergy prevalence may occur by taking proteins out of their natural matrix and incorporating these in products at a much higher quantity. For instance, soy is often the main ingredient of plant-based alternatives and can trigger allergic reactions. Its popular substitutes are wheat, rapeseed, and almond, which will hardly reduce the need for allergen labeling on meat-replacement products.
- (2) Development of novel food allergies. By introducing proteins from plant parts that we currently do not or hardly consume, primary sensitization to these novel proteins can occur, or they may show immunoglobulin E (IgE) cross-reactivity to existing allergens. Concentrated protein alternatives to soy, which are currently considered rarely allergenic when consumed as the whole vegetable, could raise allergy prevalence if such purified protein concentrates increase in our daily diet. For instance, the increased use of concentrated pea protein as a replacer has increased the incidence of pea-related allergic reactions. The same is expected for other legumes, such as fava beans.

- (3) Sensitization to novel proteins may cause cross-reactive events toward foods currently considered not or rarely allergenic, increasing the prevalence of both types of foods.

Experts have noted the challenges in estimating the severity of allergic reactions and the knowledge gaps in novel proteins, e.g., if they are a primary sensitizer and the threshold doses of novel proteins.

A high-quality protein increasingly used as a soy alternative is potato protein. Reportedly, of all the plant-based and animal protein sources, consuming potatoes has the lowest incidence of allergenicity (Alting et al. 2011). Although food allergy to potatoes is rare, elevated potato protein concentrations in our diet may boost these low prevalence rates. Industrial processing of potato protein might limit these risks, as documented allergic reactions involved mostly raw potatoes and lesser boiled potatoes (Seppälä et al. 1999); however, glycation through the Maillard reaction with galactose and galacto-oligosaccharides actually increased the total immunoreactivity of patatin in vitro (Seo, L'Hocine, and Karboune 2014).

The use of canola/rapeseed protein-rich meal as a rest-stream for human consumption is already regarded as a risk for primary sensitization, as well as of IgE cross-reactivity reactions for individuals allergic to yellow mustard. As such, appropriate labeling has been recommended (EFSA Panel on Dietetic Products and Nutrition and Allergies (NDA)) 2013).

Beetroot and other vegetable leaves are also considered sustainable sources of alternative proteins. However, there is very little literature available about their allergenicity, other than allergens present in pollen. Case reports published about the vegetable, Swiss chard (*Beta vulgaris L. cicla*) showed it is rarely allergenic, with only a few cases of asthmatic symptoms caused by inhalation of cooking vapor described (González-Mancebo et al. 2000, Parra et al. 1993). Therefore, the risks of allergic reactions to beetroot leaf protein, which is botanically related to swiss Chard, seem nihil when consumed as a processed, ready-made product.

Other alternative proteins

Besides plant-based proteins, other protein alternatives like insects, cultured meat, seaweed, and SCPs, can be used for meat and dairy replacers. However, in most cases, these are relatively new ingredients for which a novel food dossier is needed in the EU. Furthermore, other aspects may influence placing these proteins on the market, such as consumer perception. These factors ensure that they will not be readily available on the EU market to consumers in the next 1-3 years. However, future forecasts beyond 3 years do foresee introducing these proteins. Some of them are already on the market in other parts of the world. The following sections summarize future trends and food safety hazards for insects, cultured meat, seaweed, and SCPs as upcoming alternative proteins. Potential chemical and microbiological

food safety hazards related to using other alternative proteins in meat and dairy replacers are summarized in Table 2.

Insects

Although 25% of the world population regularly eats insects, in Europe, this is only about 0.1% (9 million) (International Platform of Insects for Food and Feed 2020). Despite Europeans' psychological, social, and ethical hesitation to consume insects, the positive ecological footprint, high nutritional value, and the need to tap into alternative protein sources appear to drive the forecasted growth of the European insect sector. The International Platform of Insects for Food and Feed (2020) estimated an increased production to 260,000 tonnes and 50% of Europeans consuming insects by 2030. Swift approval to market insects as food through the Novel Food Regulation (258/97 EC) seems a prerequisite for this growth. Currently, whole insects are consumed, but in the next five years, trends are toward specialty/functional foods, bakery products, and meat analogs. Since 2020, the yellow mealworm (*Tenebrio molitor* larva) has been the first species considered safe to eat as a novel food in the EU.

The available literature regarding food safety hazards in insects for human consumption has increased in the last decade. Factors influencing food safety are the production method (wild harvested or reared) (Murefu et al. 2019) as well as the transfer, accumulation, and source of contaminants, insect type and life stage, and the substrate conditions (Meyer, Hoek-van den Hil, and van der Fels-Klerx 2022). Experts have also noted a potential concern with the accumulation of different hazards and the use of side-streams to feed insects. Several papers indicate that heavy metals

like cadmium, arsenic, mercury, and lead can accumulate in insects like black soldier fly and yellow mealworm larvae (Meyer, Hoek-van den Hil, and van der Fels-Klerx 2022, van der Fels-Klerx et al. 2018). Other chemical hazards like pesticide residues, veterinary drugs and hormones, and dioxins and polychlorinated biphenyls (PCBs) were found in insects (van der Fels-Klerx et al. 2018). Mycotoxins do not accumulate in insects (Meyer, Hoek-van den Hil, and van der Fels-Klerx 2022, van der Fels-Klerx et al. 2018) and could even be degraded by the insects, albeit research on these metabolic pathways is needed (Meyer, Hoek-van den Hil, and van der Fels-Klerx 2022). PAHs were also found not to accumulate in insects (Meyer, Hoek-van den Hil, and van der Fels-Klerx 2022).

The presence of pathogenic bacteria in insects cannot be disregarded. Although data on the presence of pathogenic viruses and prions are limited, these could act as vectors (van der Fels-Klerx et al. 2018). In addition, Mézes and Erdélyi (2020) and Skotnicka et al. (2021) indicated the potential for pathogenic bacteria, parasites, and prions in insects. However, authors advocated that with proper insect farm management, insects could remain pathogen-free.

Finally, the allergenic potential of insects, especially in mealworm species, was also specified (Mézes and Erdélyi 2020, Murefu et al. 2019, van der Fels-Klerx et al. 2018). The EFSA did indeed flag the risk of allergic reactions to yellow mealworm proteins, especially in subjects with a preexisting allergy to crustaceans and dust mites (Turck et al. 2021). This concern may also apply to other edible insect species. This remains a data gap, along with other concerns like anti-nutritional factors (Murefu et al. 2019).

Table 2. Overview of potential hazards in insects, cultured meat, seaweed, and single-cell proteins, based on literature and expert opinion.

Hazards	Insects	Cultured meat	Seaweed	Single-cell proteins
Allergens	x	x	x ¹	x ¹
Microplastics and nanoplastics	–	–	x ¹	–
Chemical hazards				
Anti-nutritional factors	x ¹	–	–	–
Brominated flame retardants	–	–	x ¹	–
Dioxins and polychlorinated biphenyls (PCBs)	x	–	x ¹	–
Elements	–	–	x	–
Heavy metals	x	–	x	x
Marine biotoxins	–	–	x ¹	–
Mycotoxins	x ¹	–	–	x ¹
Plant protection products and biocides	x	x ¹	x ¹	–
Plant toxins or compounds	–	–	–	–
Polycyclic aromatic hydrocarbons (PAHs)	–	–	x ¹	–
Process contaminants	x ¹	x	–	x ¹
Veterinary drug residues	x	x	x ¹	–
Microbiological hazards				
Bacterial toxins	–	–	–	x ¹
Genetic engineering/genetically modified organisms	–	x	–	–
Parasites	x	–	–	–
Prions	x	x	x	–
Spore-forming bacteria	x	x	x	x ¹
Vegetative bacteria	x	x	x ¹	–
Viruses	x	x	x ¹	–

Dash: no indications were found that this would be a potential hazard.

¹Knowledge gaps of some hazards.

Cultured meat

December 2020 hallmarked the first commercial sales of a cell-cultured animal food product. A Singaporean restaurant started selling dishes containing cultured chicken meat produced by the Eat Just company. According to the Good Food Institute (GFI), Commercialization of cell-cultured meat and seafood is expected within the next few years (GFI 2020). As for European and UK markets, the South Californian BlueNalu company and the European frozen food provider Nomad Foods announced their collaboration to commercialize cell-based seafood in Europe (Davey 2021). Also, the Good Food Institute (GFI) foresees that from 2022 onwards, production in Europe will move up from pilot to a demonstration-scale (GFI 2020). Like with insects, introduction on the EU market requires prior approval through the Novel Food Regulation (258/97 EC).

Cell-cultured product production consists of the following five stages, as outlined by Ong et al. (2021): (i) Procurement of cells where biopsies are taken from live or slaughtered animals. (ii) Cell preparation production: The preferred cell type is isolated, further cultivated, and multiplied in cell culture (e.g., in flasks, Petri dishes). At this stage, antibiotics/antimycotics may be used to ward off cell culture infections. (iii) Biomass production: The prepared cells are transferred to bioreactors, where they are further grown to differentiate into the desired cell type, to amass into desired shapes and format, and to manufacture the actual product. For this purpose, cells may be grown into 3-dimensional “scaffolds” from natural biomaterials. Culture media can be added containing nutrients and growth factors, like hormones. In some cases, recombinant proteins produced by genetically engineered microorganisms can be used to replace animal-derived culture additives, like fetal calf serum. (iv) Harvesting of the product: This may be done with or without removing the scaffold. In the first case, specific physical or biochemical methods (e.g., enzymes) may be used to dissociate cells from the matrix. Removal may not be needed if the scaffold is made from edible materials and if its presence in the final product may also impart meat-like physical properties. (v) Processing of the food product: The harvested material will be processed into (semi-)final commercial products.

For each step, food safety hazards have been identified by Ong et al. (2021) based on consultations with cell-based product manufacturers. Production of cell-based products will likely be done according to Good Manufacturing Practices (GMP) and with a Hazard Analysis Critical Control Points (HACCP) approach. Nevertheless, some additives added to the intermediate products during production may adventitiously end up in the final product and introduce new hazards. Microbiological hazards (bacterial pathogens, viruses, and prions), along with allergens, antibiotics/antimycotics, contaminants formed during freezing, chemicals leached from plastics, genetic engineering, growth factors (e.g., hormones), dissociation agents used during harvest, processing contaminants formed during processing, and the potential accumulation of contaminants when recycled media is used were identified (Ong et al. 2021). The findings of Ketelings, Kremers, and de Boer (2021) aligned with a

number of these. For example, isolated cells may become genetically engineered or transformed into starting cell lines, with the latter triggering genetic alterations that need to be checked for. Concerns linked to possible zoonotic viruses and prions have also been suggested by others (Hadi and Brightwell 2021). Also, any alternative used instead of bovine serum that could end up in the final product would need regulatory approval. The same consideration applies to materials used to build scaffolding structures where the cells can grow on or microcarriers for added substance (Ketelings, Kremers, and de Boer 2021).

Seaweed

Seaweed is promising biomass with applications in food, beverages, feed, nutraceuticals, and pharmaceuticals. In the Western world, we have seen an increase in the production of food contributions from seaweed (Banach et al. unpublished). It has also been suggested as an alternative solution for the food industry to replace meat as a source of gelatin and protein (Lloyd's Register Foundation 2020). Seaweed consists of several macroscopic species of algae that can be classified based on their pigmentation into brown (Phaeophyta), green (Chlorophyta), and red (Rhodophyta) algae. It provides various amounts of protein, ranging from $3 \pm 15\%$ dry weight for brown alga, $9 \pm 26\%$ dry weight for green alga, and up to 47% dry weight for red alga (Fleurence, Morançais, and Dumay 2018). Currently, in Europe, upscaling production volumes alongside technological and market developments are key drivers to boost growth in the sector (Araújo et al. 2021). Algae is included in the top 50 future food (Knorr and WWF 2019), owing to its potential as a rich protein and its meat-like umami flavors, meaning it can make a potential meat replacer. Laver seaweed (*Porphyra umbilicalis*, a red alga also known as nori) and wakame seaweed (*Undaria pinnatifida*; a brown alga) are on this list (Knorr and WWF 2019).

Factors like the seaweed type, cultivation and harvest conditions, and further processing, among others, were found to influence the presence of hazards in seaweed (Banach, Hoek-van den Hil, and van der Fels-Klerx 2020). Recent literature shows that (inorganic) arsenic, cadmium, lead, mercury, iodine, pathogenic bacteria like *Salmonella* spp., *Bacillus* spp., *Vibrio* spp., and norovirus have been reported as relevant hazards in seaweed (Banach, Hoek-van den Hil, and van der Fels-Klerx 2020, Banach et al. unpublished). These results concur with an EFSA analysis on the risk assessment of seaweed, finding that seaweed can be a source of increased exposure to inorganic arsenic, cadmium, lead, mercury, and iodine (Sá Monteiro et al. 2019). Despite the wealth of research on these hazards, data gaps on the presence of other hazards exist. Banach, Hoek-van den Hil, and van der Fels-Klerx (2020) indicated data gaps for pesticide residues, dioxins and polychlorinated biphenyls, brominated flame retardants, PAHs, pharmaceuticals, marine biotoxins, allergens, micro- and nanoplastics, and pathogenic microorganisms *E. coli*, *Listeria* spp., *St. aureus*, norovirus, and hepatitis E virus. Moreover, although research describing the effects of processing like washing

seaweed to reduce microplastics or marine biotoxins (Banach et al. unpublished) as well as soaking or washing and cooking to reduce inorganic arsenic and iodine in seaweed have been reported (Banach, Hoek-van den Hil, and van der Fels-Klerx 2020, Banach et al. unpublished), there remain research gaps on the effects of processing. Also, additional monitoring of seaweed and data collection on the health effects, e.g., dietary exposure, to further characterize hazards is needed (Banach, Hoek-van den Hil, and van der Fels-Klerx 2020).

Single-cell proteins

Single-cell proteins (SCPs) are derived from fungi, microalgae, yeast, or bacteria. Examples of microbial biomass foods mycoproteins from *Fusarium venenatum*, e.g., Quorn™ and Abunda™, among other ingredients like AlgaVia's whole algae. A long history of yeast SCPs facilitates its market acceptance (Ritala et al. 2017). At present, commercial applications of bacterial proteins are unknown (Boukid et al. 2021). Experts expect potential for microbial biomass proteins, with major developments in the next 5-10 years. Current research on fungal biomass production is trying to demonstrate improvements in the nutritional value of substrates. Experts have indicated that research is warranted to understand pathogen behavior in these newer environments and the effects of fermentation (30°C, high pH) followed by pasteurization or sterilization before packaging.

There is limited research on the potential allergic and toxic effects of SCPs, and consequently, the safety of some SCPs is largely unknown (Hadi and Brightwell 2021, Hashempour-Baltork et al. 2020). The highest risk for processing some SCPs is if the fermentation is performed incorrectly. *Bacillus* spp. (aerobic spore formers) may form if the surface to volume ratio is too low. Also, the mold is creating anaerobic conditions, so there is a risk for the proliferation of anaerobic spore formers (expert opinion). Previous research has suggested that SCPs from filamentous fungi are likely to be restricted to solid-state fermentation and the mycoprotein from Quorn™, given the potential risks for mycotoxins and lengthy regulatory acceptance for new products (Ritala et al. 2017). More generally, food safety hazards related to SCPs include the presence of toxins, allergens, heavy metals, and high ribonucleic acid (RNA) content (Ritala et al. 2017, Hadi and Brightwell 2021). Food safety hazards like mycotoxins become relevant as more molds (*Rhizopus*, *Aspergillus*) are being used for alternative purposes and if the procedures for execution of the fermentation process are not strictly followed (expert opinion).

Also, the presence of rhizoxins can be a food safety concern. Research has shown that some strains of *Rhizopus*, like *R. microsporus*, are associated with a bacterial endosymbiont *Bulkholderia rhizoxinica*, which produces rhizoxin (Lackner, Hertweck, and Madhani 2011, Birol and Gunyar 2021). When the fungus is infected by bacteria (i.e., it penetrates the fungus) and then given antibiotics, it can be cured of *B. rhizoxinica*. When this happens, the cured fungus stops sporulating, meaning *B. rhizoxinica* takes over

the control of the sporulation processes. The consequences for food safety are that the infected molds secrete the bacterial toxin rhizoxin and possibly other toxins (expert opinion).

Conclusions

This study reviewed food safety hazards related to alternative proteins for meat and dairy replacers for the European Union market. The emphasis was on the current trends in the industry, including processing methods and the safety of plant-based proteins. Also, trends and the food safety of alternative proteins from insects, cultured meat, seaweed, and single-cell proteins (SCPs) were described.

Understanding the safety of alternative proteins is paramount for ensuring food safety. For plant-based proteins, typical food safety hazards related to the plant or product itself or processing are relevant to monitor in meat and dairy replacers. For example, mycotoxins and plant toxins may be present in the plant product itself. In addition, spore-forming bacteria, *Listeria* spp., and *Salmonella* spp. might be present after processing into plant-based proteins. Furthermore, given the intended increase in protein alternatives in consumers' diets, the risk of allergens is apparent. However, there are knowledge gaps on allergens in proteins from mushrooms, the use of green leaves, insects, seaweed, and SCPs. In general, the occurrence of processing contaminants and anti-nutritional compounds in plant-based alternatives for meat and dairy replacers were seen as potential hazards, although information is limited.

Since current trends show that plant-based proteins are increasing globally, evaluating the hazards related to these types of proteins should take precedence in EU monitoring programs. At the same time, it remains critical that the trends toward upcoming alternative protein sources and the effects of processing are further explored. Although hazards in alternative proteins like insects and seaweed are currently being addressed in parts of the EU, many knowledge gaps were identified for these alternative proteins that need further attention. This is especially relevant for the other upcoming protein alternatives, i.e., cultured meat and SCPs, as more research on the potential hazards related to these proteins is recommended.

Data on the effects of processing on some hazards was limited. However, it is important to evaluate the effects of processing alternative proteins on food safety in order to better understand the risks for consumers. Overall, our findings can aid industry and governmental authorities in understanding current trends and start prioritizing hazards for future monitoring of alternative proteins for meat and dairy products.

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Appendices

Appendix A. Search strings for select literature

Legume search string for Scopus

(TITLE (legume* OR pulse OR pulses OR bean* OR pea OR peas OR cowpea* OR chickpea* OR lentil* OR bambara* OR lupin* OR soy* OR glycine OR phaseolus OR vigna OR azukia OR dolichos OR rudua OR lablab OR pisum OR cicer OR ononis OR orobus OR faba OR lens OR ervum OR lupinus OR cajan* OR cytissus) AND TITLE (pollutant* OR "chemical hazard*" OR contaminant* OR toxin* OR "agricultural chemical*" OR agrochemical* OR "chemical compound*" OR "chemical substance*" OR residu* OR pesticide* OR "plant protection product*" OR organophosphate* OR organochlorine* OR carbamate* OR pyrethroid* OR "heavy metal*" OR element* OR "mineral oil*" OR mycotoxin* OR pharmaceutical* OR "vet* drug*" OR "vet* medic*" OR antibiotic* OR antimicrobial* OR antiparasitic* OR anti-parasitic* OR nsaid* OR sedative* OR hormone* OR steroid* OR "beta*agonist*" OR "marine toxin*" OR "algae toxin*" OR "plant toxin*" OR "Pyrrolizidine alkaloid*" OR glycoalkaloid* OR radionuclide* OR dioxin* OR pcb* OR perfluor* OR pfas OR "flame retardant*" OR pah* OR "polycyclic aromatic hydrocarbon*" OR *plastic* OR norovirus OR salmonella OR "clostridium perfringens" OR campylobacter OR "staphylococcus aureus" OR listeria OR "Escherichia coli" OR "hepatitis A virus" OR "bacillus cereus") AND TITLE-ABS-KEY ("public health" OR haccp OR "consumer protection" OR "food safety" OR "risk assessment*" OR "risk analys*" OR hazard* OR "human health*" OR "health impact" OR risk* OR uptake* OR *transfer* OR accumulation* OR concern OR toxic*) AND TITLE ("food protein" OR protein OR "plant* protein" OR "purified protein" OR "altern* protein" OR "protein source" OR "white protein")) AND PUBYEAR > 2010

Legume search string for CAB Abstract

(legume* or pulse or pulses or bean* or pea or peas or cowpea* or chickpea* or lentil* or bambara* or lupin* or soy* or glycine or phaseolus or vigna or azukia or dolichos or rudua or lablab or pisum or cicer or ononis or orobus or faba or lens or ervum or lupinus or cajan* or cytissus).ti. AND (pollutant* or "chemical hazard*" or contaminant* or toxin* or "agricultural chemical*" or agrochemical* or "chemical compound*" or "chemical substance*" or residu* or pesticide* or "plant protection product*" or organophosphate* or organochlorine* or carbamate* or pyrethroid* or "heavy metal*" or element* or "mineral oil*" or mycotoxin* or pharmaceutical* or "vet* drug*" or "vet* medic*" or antibiotic* or antimicrobial* or antiparasitic* or anti-parasitic* or NSAID* or sedative* or hormone* or steroid*

or "beta*agonist*" or "marine toxin*" or "algae toxin*" or "plant toxin*" or "Pyrrolizidine alkaloid*" or glycoalkaloid* or radionuclide* or dioxin* or PCB* or perfluor* or PFAS or "flame retardant*" or PAH* or "polycyclic aromatic hydrocarbon*" or \$plastic* or norovirus or salmonella or "clostridium perfringens" or campylobacter or "staphylococcus aureus" or listeria or "Escherichia coli" or "hepatitis A virus" or "bacillus cereus").ti. AND ("public health" or HACCP or "consumer protection" or "food safety" or "risk assessment*" or "risk analys*" or hazard* or "human health*" or "health impact" or risk* or uptake* or \$transfer* or accumulation* or concern or toxic*).ti,ab,id. AND ("food protein" or protein or "plant* protein" or "purified protein" or "altern* protein" or "protein source" or "white protein").ti. limit 2 to yr="2011 -Current"

Green leaves search string for Scopus

TITLE-ABS-KEY("Green leave*" OR "green leaf*" OR "beet leave*" OR "beet leaf*" OR "potato haulm*" OR "potato leave*" OR "potato leaf*" OR "tobacco leaf*" OR "tobacco leave*" OR "soybean leaf*" OR "soybean leaves*" OR "cauliflower leaf*" OR "cauliflower leaves*" OR "cauliflower stem*" OR "spinach leaf*" OR "spinach leaves*" OR "spinach by-product*" OR "pea leaf*" OR "pea leaves*" OR "pumpkin leaf*" OR "pumpkin leaves*" OR "amaranth leaf*" OR "amaranth leaves*" OR "cowpea leaf*" OR "cowpea leaves*" OR "cabbage leaf" OR "cabbage leaves*" OR alfalfa OR ryegrass) AND TITLE-ABS-KEY(pollutant* OR "chemical hazard*" OR contaminant* OR toxin* OR "agricultural chemical*" OR agrochemical* OR "chemical compound*" OR "chemical substance*" OR residu* or pesticide* or "plant protection product*" or organophosphate* or organochlorine* or carbamate* or pyrethroid* or "heavy metal*" or element* or "mineral oil*" or mycotoxin* or pharmaceutical* or "vet* drug*" or "vet* medic*" or antibiotic* or antimicrobial* or antiparasitic* or anti-parasitic* or NSAID* or sedative* or hormone* or steroid* or "beta*agonist*" or "marine toxin*" or "algae toxin*" or "plant toxin*" or "Pyrrolizidine alkaloid*" or glycoalkaloid* or radionuclide* or dioxin* OR PCB* or perfluor* or PFAS or "flame retardant*" or PAH* or "polycyclic aromatic hydrocarbon*" or *plastic* OR norovirus OR salmonella OR "clostridium perfringens" OR campylobacter OR "staphylococcus aureus" OR listeria OR "Escherichia coli" OR "hepatitis A virus" OR "bacillus cereus") AND TITLE-ABS-KEY("public health" OR HACCP OR "consumer protection" OR "food safety" OR "risk assessment*" OR "risk analys*" OR hazard* OR "human health*" OR "health impact" OR risk* or uptake* or *transfer* or accumulation* or concern or toxic*) AND TITLE("food protein" OR protein OR "plant* protein" OR "purified protein" OR "altern* protein" OR "protein source" OR "white protein") AND PUBYEAR > 2010

Green leaves search string for CAB Abstracts

("Green leave*" or "green leaf*" or "beet leave*" or "beet leaf*" or "potato haulm*" or "potato leave*" or "potato leaf*" or "tobacco leaf*" or "tobacco leave*" or "soybean leaf*" or "soybean leaves*" or "cauliflower leaf*" or "cauliflower leaves*" or "cauliflower stem*" or "spinach leaf*" or "spinach leaves*" or "spinach by-product*" or "pea leaf*" or "pea leaves*" or "pumpkin leaf*" or "pumpkin leaves*" or "amaranth leaf*" or "amaranth leaves*" or "cowpea leaf*" or "cowpea leaves*" or "cabbage leaf" or "cabbage leaves*" or alfalfa or ryegrass).ti,ab,id. AND (pollutant* or

"chemical hazard*" or contaminant* or toxin* or "agricultural chemical*" or agrochemical* or "chemical compound*" or "chemical substance*" or residu* or pesticide* or "plant protection product*" or organophosphate* or organochlorine* or carbamate* or pyrethroid* or "heavy metal*" or element* or "mineral oil*" or mycotoxin* or pharmaceutical* or "vet* drug*" or "vet* medic*" or antibiotic* or antimicrobial* or antiparasitic* or anti-parasitic* or NSAID* or sedative* or hormone* or steroid* or "beta*agonist*" or "marine toxin*" or "algae toxin*" or "plant toxin*" or "Pyrrolizidine alkaloid*" or glycoalkaloid* or radionuclide* or dioxin* or PCB* or perfluor* or PFAS or "flame retardant*" or PAH* or "polycyclic aromatic hydrocarbon*" or \$plastic* or norovirus or salmonella or "clostridium perfringens" or campylobacter or "staphylococcus aureus" or listeria or "Escherichia coli" or "hepatitis A virus" or "bacillus cereus"). ti,ab,id. AND ("public health" or HACCP or "consumer protection" or "food safety" or "risk assessment*" or "risk analys*" or hazard* or "human health*" or "health impact" or risk* or uptake* or \$transfer* or accumulation* or concern or toxic*). ti,ab,id. AND ("food protein" or protein or "plant* protein" or "purified protein" or "altern* protein" or "protein source" or "white protein").ti,ab,id. limit 2 to yr="2011 -Current"

Appendix B. Interview guides

General

- (1) Which plant-based proteins are most promising in your perspective?
- (2) Which other alternative proteins can we expect to rise in the near/far future?
- (3) What are the prerequisites to increase the market share for alternative proteins?
- (4) Which bottlenecks do you foresee preventing a significant market share for alternative proteins?
- (5) Which processing techniques are (expected to be) used related to insect proteins/fermented products? Are there differences between raw ingredients used?
- (6) Which food safety hazards do you foresee related to plant-based proteins?
- (7) What are the current knowledge gaps?
- (8) Who else should we contact regarding the safety of plant-based proteins?

Plant-based proteins

- (1) Which plant-based proteins are you researching?
- (2) What do we know at this stage about food safety hazards with respect to plant-based products?
- (3) Which plant-based products are most hazardous (pea, oats, etc.)?
- (4) Which upcoming/new food safety hazards do you foresee with respect to the ingredients used to produce the alternative proteins from plant-based products?
- (5) Which processing techniques are (expected to be) used related to plant-based products? Are there differences between ingredients used (e.g., peas, oats, nuts)?
- (6) Which (new) food safety hazards do you foresee with respect to these processing techniques?

- (7) Which analytical methods are currently available in-house to tackle the hazards mentioned in 2, 3, and 5?
- (8) Do you foresee increased food safety risks due to increased dose (e.g., more soy use as an alternative protein source), cross-contamination, other issues, etc.?
- (9) What are the current knowledge gaps?
- (10) Who else should we contact regarding the safety of plant-based proteins?

Plant-based protein processing

- (1) Which plant-based proteins are you researching?
- (2) What processing techniques are used for making plant-based products?
- (3) What techniques are expected to be used in the next 3-5 years?
- (4) What do we know at this stage about food safety hazards with respect to processing plant-based proteins?
- (5) Which upcoming/new food safety hazards do you foresee with respect to the processing alternative proteins from plant-based products?
- (6) What are the current knowledge gaps?
- (7) Who else should we contact regarding the safety of plant-based proteins? And for that of processing?

Alternative proteins and processing

- (1) Which plant-based proteins are most promising in your perspective?
- (2) Which other alternative proteins can we expect to rise in the near/far future?
- (3) What are the prerequisites to increase the market share for alternative proteins?
- (4) Which bottlenecks do you foresee preventing a significant market share for alternative proteins?
- (5) Which processing techniques are used to make alternative protein products (plant-based, fermented products, cellular agriculture, etc.)?
- (6) What do we know at this stage about food safety hazards with respect to processing alternative proteins (plant-based, fermented products, cellular agriculture, etc.)?
- (7) Which upcoming/new food safety hazards do you foresee with respect to the processing alternative proteins from plant-based products?
- (8) What are the current knowledge gaps?
- (9) Who else should we contact regarding the safety of plant-based proteins?

Toxicological hazards

- (1) In our research, we focus primarily on plant-based products. What do we know at this stage about food safety hazards with respect to plant-based products?
- (2) Which plant-based products are most hazardous (pea, oats, etc.)?

- (3) Which upcoming/new food safety hazards do you foresee with respect to the ingredients used to produce the alternative proteins from plant-based products or from other protein sources?
- (4) Do you foresee increased food safety risks due to increased dose (e.g., more soy use as an alternative protein source), cross-contamination, other issues, etc.?
- (5) Which processing techniques are (expected to be) used related to plant-based products? Are there differences between ingredients used (e.g., peas, oats, nuts)?
- (6) Which (new) food safety hazards do you foresee with respect to these processing techniques?
- (7) Which analytical methods are currently available in-house to tackle the hazards mentioned in 2, 3, and 5?
- (8) What are the current knowledge gaps?
- (9) Who else should we contact regarding the safety of plant-based proteins?

Allergens

- (1) Which plant-based proteins are you researching?
- (2) Which plant-based proteins can cause allergic reactions?
- (3) Do you foresee increased food safety risks due to increased dose (e.g., more soy used as an alternative protein source), cross-contamination, other issues, etc.?
- (4) Which upcoming/new food safety hazards do you foresee with respect to the ingredients used to produce the alternative proteins from plant-based products?
- (5) Which processing techniques are (expected to be) used related to plant-based products? Are there differences between ingredients used (peas, oats, nuts, etc.)?
- (6) Which (new) food safety hazards do you foresee with respect to these processing techniques?
- (7) Which analytical methods are currently available at WUR to tackle the hazards mentioned in 2, 3, and 5?
- (8) What are the current knowledge gaps?
- (9) Who else should we contact regarding the safety of plant-based proteins? And specific to allergenicity?

Anti-nutritional factors

- (1) Which plant-based proteins are you researching?
- (2) Which plant-based proteins are of concern considering anti-nutritional factors?
- (3) What do we know at this stage about anti-nutritional factors with respect to plant-based products?
- (4) Which upcoming/new food safety hazards do you foresee with respect to the alternative proteins from plant-based products?
- (5) What are the current knowledge gaps?

- (6) Who else should we contact regarding the safety of plant-based proteins?

Insects and fermented products

- (1) What do we know at this stage about food safety hazards with respect to insect proteins or fermented products?
- (2) Which upcoming/new food safety hazards do you foresee with respect to the raw ingredients used to produce insect protein/fermented products?
- (3) What are the prerequisites to increase the market share for insect proteins/fermented products?
- (4) Which bottlenecks do you foresee preventing a significant market share for insect proteins/fermented products?
- (5) Which processing techniques are (expected to be) used related to insect proteins/fermented products? Are there differences between raw ingredients used?
- (6) Which (new) food safety hazards do you foresee with respect to these processing techniques?
- (7) Which analytical methods are currently available in-house to tackle the hazards mentioned in 1, 2, and 4?
- (8) What are the current knowledge gaps?
- (9) Who else should we contact regarding the safety of plant-based proteins? And for that of insects/fermented products?

Company with fermented products

- (1) Can you briefly introduce your company? What is your role? Which plant-based proteins are you developing?
- (2) How do you view your business in the future? How is food safety included?
- (3) Which bottlenecks do you foresee preventing a significant market share for these products?
- (4) What do we know at this stage about the safety of these plant-based alternatives?
- (5) Which upcoming or new hazards do you foresee with respect to the raw ingredients used to produce these products?
- (6) Which processing techniques are (expected to be) used?
- (7) Which (new) food safety hazards do you foresee with respect to these processing techniques?
- (8) What are the current knowledge gaps?
- (9) What tools/solutions/improvements/innovations do you need to improve your product's quantity and/or quality?
- (10) Who else should we contact regarding the safety of plant-based proteins?