Non-animal proteins as cutting-edge ingredients to reformulate animal-free foodstuffs: present status and future perspectives

Fatma Boukid[1#], Cristina M.Rosell[2], Sara Rosene[3], Sara Bover-Cid[1], Massimo Castellari[1]

[1] Institute of Agriculture and Food Research and Technology (IRTA), Food Safety and Functionality Programme, Food Industry Area, Finca Camps i Armet s/n, 17121, Monells, Catalonia, Spain

[2] Institute of Agrochemistry and Food Technology (IATA-CSIC), C/ Agustin Escardino, 7, Paterna, 46980, Valencia, Spain

[3] General Mills, 1 General Mills Blvd, Golden Valley, MN, USA

#: corresponding author (email: fatma.boukid@irta.cat)

ABSTRACT:

Consumer interest in protein rich diets are increasing, with more attention being paid to the protein source. Despite the occurrence of animal proteins in the human diet, non-animal proteins are gaining popularity around the world due to their health benefits, environmental sustainability, and ethical merit. These sources of protein qualify for vegan, vegetarian, and flexitarian diets. Non-animal proteins are versatile, derived mainly from cereals, vegetables, pulses, algae (seaweed and microalgae), fungi, and bacteria. This review's intent is to analyze the current and future direction of research and innovation in non-animal proteins, and to elucidate the extent (limitations and opportunities) of their applications in food and beverage industries. Prior knowledge provided relevant information on protein features (processing, structure, and techno-functionality) with particular focus on those derived from soy and wheat. In the current food landscape, beyond conventionally used plant sources, other plant proteins are gaining traction as alternative ingredients to formulate animal-free foodstuffs (e.g., meat alternatives, beverages, baked products, snack foods, and others). Microbial proteins derived from fungi and algae are also food ingredients of interest due to their high protein quantity and quality, however there is no commercial food application for bacterial protein yet. In the future, key points to consider are the importance of strain/ variety selection, advances in extraction technologies, toxicity assessment, and how this source can be used to create personalized food.

Keywords: plant proteins, microbial proteins, functionality, food design, food safety

1. Introduction

Food proteins are essential nutrients for human health, used in the body for building bones, muscles, enzymes, hormones, and regulating immune function (Mitchell et al. 2015; Dougkas and Östman 2016; Zambrowicz et al. 2013; Groen et al. 2015). In recent years, high protein diets are growing more popular, with more deliberation on the source of protein that is being consumed (Banovic et al. 2018; López-Barrios, Gutiérrez-Uribe, and Serna-Saldívar 2014; Pal and Suresh 2016; Sokolowski et al. 2019). Animal proteins, the largest share of the global protein market, are derived mainly from milk, eggs, meat, and seafood. Non-animal proteins are derived from a wide selection of plant sources such as pulses, legumes, cereals, and other alternative sources (i.e. fungi, bacteria and algae). Based on a survey [1825 participants in 5 EU countries] on consumer acceptance to the main protein sources in food products, dairy-based protein was the most accepted protein source (75% of the respondents found its consumption acceptable or very acceptable), followed by plant-based protein as the most accepted alternative and more sustainable protein source (58%), with single-cell protein (20%), insect-based protein (9%) and *in vitro* meat-based protein (6%) (Grasso et al. 2019) at the bottom of consumer preference.

Currently, the plant protein market of is experiencing rapid growth, owing to factors such as population growth, a rise in health consciousness, increasing welfare concerns over animal production of ingredients, rising meat prices, changes in lifestyle (vegetarian, vegan and flexitarians), ethical concerns, and sustainability (Aschemann-Witzel, Varela, & Peschel, 2019; Chihi, Mession, Sok, & Saurel, 2016; Dagevos & Voordouw, 2013; De Backer & Hudders, 2015; Henchion, Hayes, Mullen, Fenelon, & Tiwari, 2017; Lan, Chen, & Rao, 2018; Meticulous Research®, 2019a). Likewise, the global demand for microbial protein alternatives is also expanding to include a wider variety of renewable and sustainable sources of protein, mainly algae and fungi (Mintel 2019a). Despite its high content of protein (up to 92%), the commercial exploitation of bacteria has been focused mainly on animal feed and not yet for human consumption (Ritala et al. 2017; Yang et al. 2017).

The global plant-based protein market is projected to grow at a compounded annual growth rate (CAGR) of 8.1% from 2019, to reach a value of $14.32 billion by 2025 (Meticulous Research®, 2019). There is an increase of different types of plant proteins in response to demand for more applications with in the food and beverage marketplace (meat, poultry, seafood, bakery, meat analogue, dairy and dairy alternatives, cereals and snacks, beverages, etc.), animal feed, nutrition and health supplements, cosmetics and pharmaceuticals (Meticulous Research®, 2019)**.** In short, the food and beverage segment has commanded the largest use of plant-based protein ingredients in 2019 (Meticulous Research®, 2019a)**,** and North America has the largest share of the overall plant-based protein market (Meticulous Research®, 2019)**.** As summarized in Table 1, the main marketed plant proteins are from soy, wheat, pea, potato, rice, and corn (Meticulous Research®, 2019a). Lately, due to the high demands, agro-industrial by-products are also proposed as an important source of plant proteins, although the recovery efficiency is still under research (Gençdağ, Görgüç, and Yılmaz 2020). Based on their purity, these proteins are commercialized in different forms: i) protein rich flour (54% protein), produced by milling and air classification of plant, algae or fungi; ii) protein concentrates (65−72% protein), prepared by removing soluble components from the flour; iii) protein isolate (≥90% protein), which is a highly refined or purified ingredient created by removing non-protein components; and iv) other forms including hydrolyzed and textured (Nishinari et al. 2014). Proteins isolates are a highly sought-after ingredient category due the high demand of premium proteins as food dietary supplements for athletes, bodybuilders, and vegetarians (Markets and Markets, 2019b).

*****Table 1*****

No doubts, food developers have been facing serious challenges substituting animal proteins with plant-based options without hampering the end-quality of the product (nutritional and technological features and consumers' perception) (Malek, Umberger, and Goddard 2019; Smetana et al. 2015; Jose, Pouvreau, and Martin 2016; Nepocatych et al. 2019). Nevertheless, these alternative proteins are the current research hotspot with emphasis on their compositional and techno-functional properties for the development of innovative ingredients and acceptable high protein-based products to meet consumer expectations (Hoehnel et al. 2019; Lafarga et al. 2018; Lafarga, Álvarez, et al. 2019; Aschemann-Witzel and Peschel 2019; Sousa et al. 2019).

The inclusion of protein ingredients as a food is not new, initial research dates to the late forties, where the objective was optimization of production lines. Some preliminary studies focused on the nutritional aspects (chiefly amino acids profile) of plant proteins (*e.g.* peanut, soy and wheat proteins) (Kelley and Baum 1953; Hove, Carpenter, and Harrel 1945; Arthur et al. 1948). Researchers went further, investigating isolation procedures of proteins, particularly on soybean for a better amino acids composition in the sixties (Byers 1961; Pomeranz 1965; Szmelcman and Guggenheim 1967) and to partially replace animal proteins in food applications, such as the meat industry by the seventies (Hanafy, Seddik, and Aref 1970; Childers 1972; Milner 1974). At that time, the use of vegetal proteins was undesirable because, in some cases, it was closely related to fraudulent actions in animal protein replacement. In the following decades, focus of research was on the application of protein from different sources, such as legumes and aquatic plants in the eighties and nineties (Gueguen 1983; Radmer and Parker 1994). Following studies started testing the impact of processing on functionalities, bioactivity, and sensory properties of these proteins, as well as how processing conditions can be improved to optimize incorporation in food formulations (Wäsche, Müller, and Knauf 2001; Tömösközi et al. 2001). Non-animal proteins inclusion in human foods started many decades ago, with varying objectives. The evolution of this research is important, as it accelerated future innovations.

Therefore, this review aims i) to critically analyze the meaningful advances in non-animal proteins, ii) to provide updated insights for the dynamic global market of non-animal proteins, iii) to define the characteristics of non-animal proteins in the market; iv) to identify the challenges of developing food products with targeted nutritional, technological and sensory features, and v) address the upcoming research and innovation trends and challenges.

2. Extraction and fractionation treatments

Protein extraction can be carried out either through wet or dry processing. Wet extraction is the most commonly patented process for protein extraction (Anson and Pader 1955). This process is still widely used at industrial level, where proteins are solubilized under alkaline or acidic conditions, followed by: centrifugation (to remove insoluble material such as starch and fiber), isoelectric precipitation, washing, centrifugation (to remove soluble material such as sugars, soluble fibers and fats), neutralization, and drying (Taherian et al. 2011; Papalamprou, Doxastakis, and Kiosseoglou 2010). Noteworthy, the formation of protein–phenolic complexes may influence protein structure, solubility, hydrophobicity, thermal stability, and isoelectric point (Jakobek, 2015; Ozdal, Capanoglu, & Altay, 2013; Eczyk, Swieca, Kapusta, & Gawlik-Dziki, 2019). These factors will affect protein extraction yield and ingredient properties including digestibility and bioaccessibility (Ozdal, Capanoglu, and Altay 2013; Jakobek 2015). From protein extraction technologies initially applied for in patents, several innovations have been reported; due to the rapid technological advance, only the most novel or recent technologies are discussed further.

Wet processing techniques can enable the production of proteins isolates with high purity (90%), where protein recovery can be further increased through the use of solvents like methanol, ammonium sulfate and/ or acetone improving protein precipitation (Adenekan et al. 2018). The use of solvent and thermal treatment can induce protein denaturation, thereby reduce their techno-functionality (Wu, Myers, and Johnson 1997; Jafari et al. 2016; Zhao et al. 2018). Another drawback is the high use of water and energy as well as high industrial wastes, which negatively impact the environment and sustainability (Ruiz et al. 2016; Chéreau et al. 2016). In the frame of circular economy, waste streams are usually destinated for animal feed, such as okara from soy protein extraction. Since the extraction of proteins is challenging, several innovative processes (physical, chemical and biological) have been developed to enhance both functionality and aroma profile of non-animal proteins, removing the beany flavor (Gao et al. 2020). The combination of electroacidification and ultrafiltration were used for soy protein extraction resulting in enhancing the solubility of both isolates and concentrates (Mondor et al. 2004). Ultrasound treatments enhanced the conjugation process, resulting in higher grafting extents, solubility, and emulsifying properties (Ma et al. 2020; Huang et al. 2020). Although ultrasound significantly improve the soy protein extraction yield by 4.2%, it has not been commonly commercialized for industrial extraction due to required high energy inputs (Preece, Hooshyar, Krijgsman, Fryer, & Zuidam, 2017b). Unlike traditional single frequency ultrasound, multi-frequency ultrasonic pretreatment was more effective in modulating protein structure (*e.g.* of rice protein, zein, and gluten protein) (Jin et al., 2015; Li et al., 2016; Salimi Khorshidi, Ames, Cuthbert, Sopiwnyk, & Thandapilly, 2019; Yang et al., 2017) and shorten the extraction time when selected the adequate dual frequency combination (Golly et al. 2020). Chemical methods can be used through alternative solvents, such as supercritical fluids (Russin et al. 2011) and biochemical methods (enzymes or enzymes assisted extraction) (Bildstein et al. 2008; Suphat Phongthai et al. 2018). Certain potato protein fractions are isolated via chromatography and therefore are more soluble (Giuseppin, Laus, and Schipper 2014). Recently, enzymatic extraction assisted with microwave or vacuum processing was proposed for obtaining plant proteins with phenolic compounds from food waste sesame bran, combining the technofunctional properties of the proteins with the bioactivity of antioxidant compounds (Görgüç, Özer, and Yılmaz 2020b; Görgüç, Özer, and Yılmaz 2020a). For some proteins, like rice protein, extraction reviewed methods include alkaline, enzymatic, and physical, enlightening the complete understanding of protein functionality (Amagliani et al. 2017a; Phongthai, Homthawornchoo, and Rawdkuen 2017). Twin-screw extrusion has been tested as extraction technology for obtaining alfalfa proteins, outcomes show the importance of the liquid/solid ratio (Colas et al. 2013). Electrospinning techniques have been used to produce nanofibers, creating proteins isolates for both food packing and biomedical applications. As carriers of hydrophilic drugs, alginate/soy protein isolates nanofibers loaded vancomycin (Wongkanya et al. 2017) thereby offering a controlled drug release, antibacterial activity, and compatibility with cells (Kim & Netravali, 2017; Xu, Jiang, Zhou, Wu, & Wang, 2012). Likewise, a protein concentrate from *Spirulina* in combination with polyethylene oxide enabled the formation of nanofibers suitable for food packaging (Moreira et al. 2018). The protein extraction from oilseeds is even more challenging and remains a multi-staged and inefficient process. But, recently a simple method is proposed consisting of an aqueous extraction to obtain protein-oleosome extract with a posterior separation of the protein and oil as intact oleosomes from the oil-in-water emulsion (Ntone, Bitter, and Nikiforidis 2020). In all described extractions methods, plant proteomics could help identify an evaluate and proteins, select the best extraction method, based on the protein source (Luthria et al. 2018; De Sousa Barbosa et al. 2013).

Dry fractionation enables the production of protein concentrates with lower purity (50-70%) while preserving the native protein functionality. There are two main methods for extracting plant proteins: air classification and electrostatic separation (Assatory et al. 2019). These processing methods comprise of two key steps, milling and air classification (Assatory et al. 2019), enabling the separation of protein rich fraction (fine particles) from starch rich fraction (coarse particles) based on the differences in density and particle size (Boye, Zare, and Pletch 2010; Schutyser and van der Goot 2011). The critical parameter during air classification is the cut-point of protein-starch separation, which depends on the source type (Boye, Zare, and Pletch 2010; Schutyser and van der Goot 2011). Moreover, some pretreatments are deemed mandatory to increase the yield and functionality of the resulting protein fraction. In the case of oil rich seeds (*e.g.* soy), a defatting step reducing the oil content in the flour prior extraction facilitates particles dispersion, improving the detachment of proteins from starch granules (Pelgrom et al. 2015; Schutyser and van der Goot 2011). Drying is also commonly used as a pretreatment in the case of peas or lupin (Berghout et al. 2015; Pelgrom et al. 2015).

Electrostatic separation is increasing in occurrence as solvent free and dry option for protein fractionation that can replace air classification (Assatory et al. 2019). Electrostatic separation considers the differences in dielectric properties between protein and carbohydrates (Aryee & Nickerson, 2012; Wang, Zhao, De Wit, Boom, & Schutyser, 2016). Proteins can be charged to a higher extent (due to the presence of ionizable groups) than carbohydrates (with low proton affinity and ionizability) (Tabtabaei et al. 2016). For instance, electrostatic separation increased the protein content of lupin fractions from 35% to 59%, but did not have any relevant impact on pea flour, suggesting that this process is closely related to the protein source (Pelgrom et al. 2015). Lupin protein concentrate (65.1%) was obtained through coarse milling, to detach protein bodies and avoid powder agglomeration, followed by electrostatic separation, showing promise for scaling-up at an industrial level (Waglay et al. 2019)., Further investigations are needed to identify optimal process conditions, considering both the structure of protein and its interactions with starch.

Despite the vast research focused on increasing yields in protein extraction, we are still facing many challenges for the viability of protein extraction, ensuring the economy of the process. Even more challenging seems the recovery of protein from green leaves (RuBisCO), although non-commercial attempts have been reported (Tamayo Tenorio et al. 2016).

3. Characteristics of non-animal proteins: structure, techno-functionality, and health related aspects

The proper processing, extraction, and isolation of proteins can strongly influence their nutritional value and functionality (Stone, Karalash, et al. 2015; Contreras et al. 2019; Rodsamran and Sothornvit 2018; Amagliani et al. 2017a; Pojić, Mišan, and Tiwari 2018). Based on recent literature, the applied processing (conventional or innovative; chemical, physical or biological; cold or hot; single or combined) must be carefully chosen, due to their impact on protein quality, and consequently on their potential application (Pojić, Mišan, and Tiwari 2018; Z. Wang et al. 2016; Wattanasiritham et al. 2016; Waglay et al. 2019; Burger and Zhang 2019; Lu et al. 2016; Katherine E. Preece et al. 2017). This section summarizes the fundamental compositional components, nutrition, and biofunctionality of most commercialized non-animal protein alternatives.

3.1. Soy protein

Soy protein composes ~40% of total soybean seed and comprised chiefly by storage proteins, albumins, and globulins. According to their sedimentation coefficients, soy protein can be classified into four main categories, 2 S (Svedberg units, S), 7S, 11S, and 15S fractions (Xu et al., 2017). Among these four proteins, the two major fractions are 7S globulin (conglycinin, ~150 and 200 kDa) and 11S globulin (glycinin, ~300–380 kDa) (accounting for 35% and 52% of total soy protein, respectively), followed by 2S (8%) and 15S (5%) (Hsiao et al. 2015; A. Singh et al. 2015). Soy protein provides a well-balanced amino acid composition (18 amino acids), containing all the essential amino acids (Gorissen et al. 2018). Soy bioactive peptides, deriving mainly from β-conglycinin and glycinin, may induce several physiological responses such as antioxidative, antimicrobial, antihypertensive, anticancer, and immunomodulatory effects (Agyei 2015; Coscueta et al. 2016). They also contribute in the reduction of cholesterol, the risk of hyperlipidemia, and cardiovascular diseases (Dan Ramdath et al. 2017; McGraw et al. 2016). Concerns over the allergenicity of soy protein started in the nineties, and with advanced technologies of detection and quantification, have been better characterized (Zeiger et al. 1999; Huijing Li et al. 2016). Glycinin and β -conglycinin are considered as major allergens, with more than 42 identified epitopes (Taylor et al. 2015; Holzhauser et al. 2009; Shengdi Hu et al. 2013). Soy allergies can provoke symptoms ranging from mild to severe (enterocolitis atopic eczema and immediate IgE-mediated reactions) (Shriver and Yang 2011; Huijing Li et al. 2016). Several mitigation strategies (*e.g.* microwave, ultrafiltration, high pressure processing, pulsed electrical fields, irradiation, ultrasound, genetic or chemical modifications) were investigated to reduce the allergenic potential of soy protein, without complete elimination of the epitopes (Meinlschmidt et al. 2016; Katz et al. 2014). Soy protein has excellent functional features such as gelling, emulsifying ability (at pH 6.5 and pH 8.2), and water- and oil- holding capacity (Barac, Pesic, Stanojevic, Kostic, & Bivolarevic, 2015; Li et al., 2019; Wu, Hua, Chen, Kong, & Zhang, 2017). Compared to fish protein, soy protein exhibits a decrease in gel stiffness and viscoelasticity (C. Wu et al. 2020; C. Wu et al. 2018; C. Wu et al. 2019). Soy protein showed great encapsulation capacity to enhance substance (e.g.; curcumin and resveratrol) solubility and to form nanocomplexes (Chen, Li, & Tang, 2015a, 2015b; Liu, Li, Zhang, & Tang, 2019; Pujara, Jambhrunkar, Wong, McGuckin, & Popat, 2017). This protein has good film-forming capacity, developing homogeneous, edible, and biodegradable films with good barrier and mechanical properties and controllable water solubility (Galus, 2018; Han, Yu, & Wang, 2018; Zhao et al., 2016).

3.2. Wheat protein

Based on their solubility, wheat protein can be subdivided into: water/salt-soluble proteins (albumins and globulins) and water/salt-insoluble ones or gluten (glutenin and gliadin) (Scherf, Koehler, and Wieser 2016). Wheat proteins are relatively rich in sulfur amino acids (Shewry et al. 1986), with the presence of ACE inhibitory peptides and dipeptidyl peptidase inhibitor, as well as other bioactive peptides (with anti-thrombotic, antioxidant, hypotensive, and opioid activities) (Karami et al. 2019). Gluten is rich in glutamine, proline, and contains small amounts of lysine, methionine, threonine, and other essential amino acids. Due to the high content of glutamine (30% to 35%) and proline (10% to 15%), gluten can trigger immune reactions, mainly celiac disease for genetically predisposed subjects, where over 30 amino acid sequences were identified as epitopes (Sollid et al. 2012; Ozuna and Barro 2018). Subsequently, numerous methods were used to reduce the allergenicity of gluten including physical (*e.g.* microwaving or thermal treatments), chemical (*e.g.* addition of polyphenols), and biological approaches (*e.g.* germination, enzymes or fermentation) (Boukid, Mejri, Pellegrini, Sforza, & Prandi, 2017; Boukid, Prandi, Buhler, & Sforza, 2017; Gobbetti, Giuseppe Rizzello, Di Cagno, & De Angelis, 2007; Pérot et al., 2017; Susanna & Prabhasankar, 2011). These studies underline that lactobacilli and fungal combination of proteases allowed a total abolishment of gluten in wheat flour, while enzymes like transglutaminase reduced the binding with the interferon (but not fully inhibited), and microwave changed the structure of proteins but did not impact the antigenic capacity of gluten. Commercially, gluten (around 80% of wheat proteins) is extracted from wheat flour and labelled as “vital wheat gluten” when its technological properties are maintained after hydration. Glutenin is associated with dough elasticity, while gliadin is associated with viscosity and extensibility (Shewry et al. 2002). Vital gluten is added as an ingredient to dough to improve its baking quality in terms of water absorption capacity, cohesiveness, viscosity, and elasticity (Ortolan et al. 2017; Bardini et al. 2018). Wheat gluten has film forming properties, enabling the formation of semipermeable membranes to be used for encapsulating agent or as food coatings or edible films (Ansorena, Zubeldía, and Marcovich 2016).

3.3. Pea protein

Peas protein (~ 25 % of pea seed) are divided into globulins (70–80%) and albumins (10–20%) (Lan et al. 2019). Globulins can be subdivided into legumin (hexameric protein, 300–400 kDa, 11S) and vicilin (trimeric protein, 150–170 kDa, 7S), with minor amounts of convicilin proteins (composed of three ∼70 kDa sub-units, 7S) (Chihi, Sok, & Saurel, 2018; Mohamed Lazhar Chihi et al., 2016; Lam, Can Karaca, Tyler, & Nickerson, 2018; Lan et al., 2019). Pea protein hydrolysate exhibited the presence of peptides with heath promoting properties thanks to their bioactive activities (e.g. antihypertensive, antidiabetic, and antioxidant) (Huan Li and Aluko 2010; Roy, Boye, and Simpson 2010; Chalamaiah, Yu, and Wu 2018). Recently, AKSLSDRFSY peptide was characterized from pea protein hydrolysate as an angiotensin, converting enzyme 2 up-regulating property in vascular smooth muscle cells (Liao et al. 2019). A randomized cross-over meal test study comparing animal (pork/veal) based meals and vegetable (peas/beans) based meals indicated the higher satiation reached with vegetable proteins (Kristensen et al. 2016). Likely, the higher fiber content of the vegetable meals results in higher satiating effect reached with lower protein intake. Pea protein was reported to trigger allergic reactions including anaphylaxis (Sanchez-Monge et al. 2004). Pis s 1 and Pis s 2 have been suggested as potential major pea allergens deriving from vicine and convicine (Popp et al. 2020). Legumin and vicine have quite similar isoelectric point (4.5) and denaturation temperature (82.7-85.5 °C) (Mession, Roustel, and Saurel 2017; Djoullah, Husson, and Saurel 2018). The ratio between legumin/vicilin depend on several factors (variety, origin, isolation and production methods) that can strongly impact the functionality of pea proteins (e.g. water-binding capacity, oil-binding capacity, foam properties, gelation and emulsion stability) (Chao, Jung, & Aluko, 2018; Chihi et al., 2018; Ladjal Ettoumi, Chibane, & Romero, 2016; Stone, Avarmenko, Warkentin, & Nickerson, 2015). Pea protein exhibits comparable emulsification and foaming properties as soy protein, but lower gels formation capacity that can be improved by applying enzymatic treatments (Silva et al. 2019; Barac et al. 2015; Stone, Karalash, et al. 2015). Also, pea proteins showed good film forming properties in combination with plasticizers (e.g. polyols), conferring the formation of an excellent oxygen barrier properties for encapsulation (Varankovich et al. 2015; Hedayatnia et al. 2019).

3.4. Potato protein

Potato proteins can be divided into three main groups, patatin (39–43 kDa; ∼40%), protease inhibitors (4.3-20.6 kDa; ∼50%), and other high molecular weight proteins (mainly oxidative enzymes, ∼10%) (Schmidt et al., 2017; Waglay, Achouri, Karboune, Zareifard, & L'Hocine, 2019; Waglay & Karboune, 2017). Compared to other plant proteins from cereals, potato proteins contain important amount of lysine, which is generally lacking in such crops (Gorissen et al. 2018; Jesper Malling Schmidt et al. 2018). Potato proteins are associated with several health benefits including lowering allergic response (Steiß, Simon, and Langner 2015) and satiety (Y. Wu et al. 2019); antimicrobial (Bártová, Bárta, and Jarošová 2019), antioxidant (Udenigwe et al. 2016) and anticancer effect (M. Zhang and Mu 2018) as well as blood pressure and blood serum cholesterol control (Lea et al. 2016). Enzymatic hydrolysis of potato proteins was used to produce soluble proteins with potential bioactivity such as DIKTNKPVIF and a dipeptide IF (Marthandam Asokan, Yang, and Lin 2018). Potato protein allergies are much less common, patatin was identified as a major cross-reactive protein triggering atopic dermatitis (Schmidt, Raulf-Heimsoth, & Posch, 2002). Potato proteins have interesting functional features such as solubility, foaming, emulsifying, and gelling abilities, which are dependent on the extraction method used (Hoehnel et al., 2019; Schmidt, Damgaard, & Greve-Poulsen, Sunds, Larsen, Hammershøj, 2019; Schmidt et al., 2018; Seo, Karboune, & Archelas, 2014; Waglay et al., 2019; Waglay, Karboune, & Khodadadi, 2016). Patatin has excellent foaming and emulsifying abilities (Schmidt et al., 2018). Patatin also could interact with polyphenols, which react with salivary proteins. This complexation is used as a non-allergenic alternative to animal proteins, in wine fining, reducing the astringency (Gambuti, Rinaldi, and Moio 2012). Furthermore, potato proteins were reported to have antifreeze functions with potential applications in medical, agricultural, industrial, and biotechnological fields (Wallis, Wang, and Guerra 1997). Potato protein is one of the most appreciated plant-based proteins for consumers, due to its association with starch and in turn positive connotation to food texture (Aschemann-Witzel and Peschel 2019).

3.5. Rice protein

Based on solubility, rice proteins can be categorized into albumin, globulin, prolamin, and glutelin. Rice proteins are also easily digestible, highly bioavailable, and contain more essential amino acid lysine than other cereal proteins source of essential amino acids such as lysine (Amagliani et al. 2016; Liu et al. 2016; Suphat Phongthai et al. 2018). Due to its essential amino acid profile, rice protein can play an important role in infant nutrition (Wang et al. 2019; Amagliani et al. 2017a). Rice proteins are considered hypoallergenic and contain specific bioactive peptides that can elicit beneficial effects including anti-oxidative, anti-hypertensive, anti-cancer, and anti-obesity activities (Amagliani et al. 2019; Amagliani et al. 2017a). Allergenic proteins have been isolated from a rice salt-soluble fraction, with a molecular mass ranging from 14 to 16 kDa, and were associated to the baker's asthma (Nakamura and Matsuda 1996). In term of functionality, native rice proteins have limited capacity to stabilize oil-water emulsions, have limited emulsifying properties, and low solubility (solubility <2% w/v; pH=4-7) thereby limiting its complete exploitation at industrial level (Amagliani, O'Regan, Kelly, & O'Mahony, 2017a; Gomes & Kurozawa, 2020; Wang, Yue, Xu, Wang, & Chen, 2018). Several techniques (chemical, biochemical, and physical) are adopted to modify rice protein native structure to improve their functional properties (Gomes and Kurozawa 2020). However, such treatments are challenging and may hinder the functional and nutritional properties of proteins (Li, Wang, Sun, Li, & Chen, 2019; Liu et al., 2016; Wang et al., 2019; Wang et al., 2016).

3.6. Corn protein

Corn proteins are mainly comprised of zeins (60% of all the proteins) (Gezer, Liu, & Kokini, 2016; Liu, Cao, Ren, Wang, & Zhang, 2019). Zein can be classified in α, β, γ, and δ-zeins, where α-zeins are the most abundant (70%-85% of total zein) (Z. Liu et al. 2019; Turasan et al. 2018). These proteins differ in structural (having different amino acids chains and molecular weight) and solubility properties (Hu, Wang, Fernandez, & Luo, 2016). Zein is rich in glutamic acid (21–26%), leucine (20%), proline (10%), and alanine (10%), yet deficient in tryptophan and lysine (Dhillon et al. 2016). This deficiency can be compensated to obtain a balanced nutritional product such as a blend zein-potato protein (Glusac et al. 2018). Zein can be considered to be a potential source of bioactive peptides with inflammatory, antihypertensive, hepatoprotective, anti-obesity, antimicrobial, and antioxidative activities (Liang et al. 2019; Liang et al. 2018). At a functional level, the high amount of nonpolar amino acid residues is responsible for the highly hydrophobic properties characteristics of zein, which results in low solubility in water (Glusac et al. 2018; Dong et al. 2017). Zein has a strong ability to entrap a large number of hydrophobic compounds (Chen et al., 2018; Dai et al., 2018; Wei, Sun, Dai, Zhan, & Gao, 2018), great ability to stabilize emulsion and foam, (Blanco, Smoukov, Velev, & Velikov, 2016; Boostani et al., 2019; Cao, Liu, Zhang, Wang, & Ren, 2020; Pan, Tikekar, Wang, Avena-Bustillos, & Nitin, 2015; Teklehaimanot & Emmambux, 2019; Wang et al., 2016) as well as film-forming and fiber-forming capacities (Chen et al., 2015; Gezer, Brodsky, Hsiao, Liu, & Kokini, 2015; Kasaai, 2018). Commercially, a corn protein isolate (70-90% protein) has been recently launched as the first food-grade non-zein corn protein, targeting bakery and meat analog applications (Cargill 2020).

3.7. Algal protein

Algal proteins are derived from various edible algae (macroalgae or microalgae), microalgal species (such as *Spirulina* spp., *Chlorella* spp. and *Dunaliella salina*) being the most used due to their high content of protein (Grossmann, Hinrichs, and Weiss 2019; Aiello et al. 2019; Medina et al. 2015; Caporgno and Mathys 2018; Lupatini, Colla, et al. 2017). With respect to algal biomass, the development of algal proteins ingredients (isolates or concentrates) are still limited due to the high technology costs related to production. Extracting and purifying algal proteins is a challenging task, particularly maximizing yield without hindering the nutritional and functional properties. This explains why the commercialization of algal biomass is more common than isolated protein ingredients. In recent years, several processing strategies (e.g. bead millings, ultrasound technology, pulsed electric field, and freezing) have been developed for cell wall disruption, and thereby increased the availability of algal proteins entrapped within resistant cell walls (Lupatini, de Oliveira Bispo, et al. 2017; Bleakley and Hayes 2017; Yücetepe, Saroğlu, and Özçelik 2019; Vernès et al. 2019; Teuling et al. 2017; Agboola et al. 2019; Yucetepe et al. 2018). Nutritionally, algal proteins are rich several essential amino acids such as lysine, methionine, threonine, tryptophan, histidine, leucine, isoleucine, valine, and phenylalanine, depending on the strain (Lupatini, de Oliveira Bispo, et al. 2017; Waghmare et al. 2016). For instance, *Spirulina platensis*, one of the richest protein sources of microbial origin (46%–63% DB, dry matter basis), has a protein level comparable to meat (71–76% DB) and soybeans (~ 40% DB) (Lupatini, de Oliveira Bispo, et al. 2017). In the US, GMO algal proteins may have customized amino acid profiles. Algal peptides were investigated for several biological activities such as anti-cancer, anti-obesity, antioxidant, antimicrobial, antihypertensive, and immunomodulatory activities (Fan et al. 2018; Gargouri, Magné, and El Feki 2016; Aiello et al. 2019; Moreira et al. 2019; Bhosle et al. 2015). Although few adverse effects are associated with algae, some allergic reactions were reported towards seaweed and *Spirulina* (Le, Knulst, and Röckmann 2014). However, concern over algae allergenicity is still not fully deciphered for species not approved as "novel foods" or algal deriving ingredients such protein isolates. Functionally, algal proteins present promising properties, such as foaming, emulsifying, gelling, and water and oil absorption (Benelhadj et al. 2016; Yücetepe, Saroğlu, and Özçelik 2019; Teuling et al. 2019; Pereira, Lisboa, and Costa 2018). Algal protein concentrates (e.g. *Spirulina platensis*) had higher water/ oil absorption capacities, foaming capacity, and foam stability than other algae and plant proteins (Yücetepe, Saroğlu, and Özçelik 2019; Benelhadj et al. 2016). Noteworthy, foaming capacity was comparable with those of egg white protein indicating algal proteins as valuable vegan alternative to include in food formulation (Lupatini Menegotto et al. 2019). Solubility of algal proteins showed high variability as a function of species, extraction methods, protein isolate concentration, and ionic strength. *Arthospira platensis* had comparable solubility to that of commercial concentrate of whey protein (73.9 ± 3.5%) and soy protein (50%) (Benelhadj et al., 2016; Chen et al., 2019; Pereira et al., 2018). Regardless of the pH conditions, algal protein isolates were able to form a stable emulsion, the emulsifying activity index (30 m^2/g) was higher than amaranth protein isolates (15.3–17.7 m^2/g), soy protein isolates, (10.86 m^2/g) and napin protein isolates (12.8–19.4 m^2/g) (Chen et al., 2019; Hu, Cheung, Pan, & Li-Chan, 2015; Lupatini Menegotto et al., 2019; Teuling et al., 2019).

3.8. Fungal protein

Fungal protein, or mycoprotein, refer to protein ingredients derived from the cultivation processes of fungi (yeast or filamentous molds) in plant biomass (Stoffel et al. 2019). In general, mycoprotein is an interesting source of good-quality proteins, with good acceptance among consumers (Finnigan, Needham, and Abbott 2016). Fungi (*Fusarium venenatum*) contain all essential amino acids and the net protein (45% DB) has high biological value compared to milk (J. Lonchamp et al. 2019; Julien Lonchamp, Clegg, and Euston 2019). The essential amino acids composition is similar to milk, human muscle, and *Spirulina platensis*, thus better than the majority of plant-based proteins (van Vliet, Burd, and van Loon 2015; Dunlop et al. 2017). Additionally, *in vivo* trials on healthy young men showed that 60 g of mycoprotein allowed an optimal response regarding muscle protein synthesis (Dunlop et al. 2017). Several health benefits have been associated with the substitution of meat for mycoprotein, including improvements in blood cholesterol concentration and glycemic response, (Souza Filho et al.

2019) increase satiety, and high digestibility (Bottin et al. 2016). However, some studies reported the association of mycoprotein with allergic and gastrointestinal symptoms (Hoff et al. 2003; Jacobson and DePorter 2018; Van Durme, Ceuppens, and Cadot 2003). Symptoms can range from mild nausea to life-threatening emesis (Jacobson and DePorter 2018). Future research on the functionality of mycoprotein is warranted, as there is no available literature in this regard. While algal proteins may be perceived as savory and umami, fungal proteins are perceived as mild tasting with low off-flavor, limiting their utilization to certain types of food products (Pojić, Mišan, and Tiwari 2018). Mycoprotein mainly found its place in the market as a healthy substitute to meat such as Quorn Foods (Marlow Foods Limited and 3fbio Ltd).

4. Food Applications: opportunities and challenges

Non-animal proteins are gaining popularity in their versatile forms (isolates, concentrate, flour, hydrolysates or textured) in food industries as: i) functional ingredients to enhance the nutritional value or ii) main ingredient for developing non-meat alternatives, or iii) additives with peculiar functional properties that may enhance the technological features of food products. In fact, in the search of cleaner labels, consumer preferences shift towards plant-based foods, and food perception improves when specifying the type of protein (Aschemann-Witzel and Peschel 2019).

4.1. Meat analogues

Meat analogues, also called meat substitutes or meat alternatives, have been trending upward among vegetarian and non-vegetarian consumers, leading to a boost of their market share of the total meat market (Weinrich and Elshiewy 2019; Siegrist and Hartmann 2019). The global meat substitute market is projected to grow at a CAGR of 7.9% during the forecast period of 2019-2024 (Mordor Intelligence, 2019). Meat analogues are designed with on plant proteins, instead of animal proteins, to have similar aesthetic properties (e.g. structure, texture, flavor, color, and appearance) to meat (Chiang et al. 2019; Bedin et al. 2018), applying in many cases extrusion to obtain texturized vegetable proteins (Zhang et al., 2019). Technologically, designing appealing meat substitutes is still challenging (Vandenbroele et al. 2019).

Many analogues are traditionally made from plant-based proteins such as soy protein or wheat gluten, and more recently pea protein (Grahl et al. 2018). In meat analogues applications, plant-based proteins play crucial roles of structuring and binding, with functional properties (e.g. water and oil holding capacities, solubility, emulsification, foaming, and gelation properties) that are closely associated with the type of protein (e.g. amino acid sequence and structure) and the environmental factors (e.g. pH, temperature, and ionic strength) (Contreras et al. 2019; Amagliani et al. 2017a; Hoehnel et al. 2019;

Alves and Tavares 2019). Soy protein ingredients are most commonly used in creating fibrous structure (Schreuders et al. 2019). Based on purity, several forms of soy protein ingredients are available in the market including textured soy proteins (50–55% protein), concentrated proteins (65–70% protein), and isolated proteins (85–90%) (Bedin et al. 2018; K. E. Preece et al. 2017a). Even though a high degree of purification of proteins is not required in meat analogue production, the use of soy isolates is the most appreciated due to the absence of beany taste and pronounced off- flavors (Morales et al. 2015; Marlies Geerts et al. 2018)**.** Both textured and concentrated protein can be used as alternatives to soy isolates due to their lower cost (Pietsch, Bühler, et al. 2019). Wheat gluten is also used in creating similar structural anisotropy to meat due to its binding and film-forming capacities, enabling the formation of fibrous proteinaceous materials (Krintiras et al. 2016; Schreuders et al. 2019; Pietsch, Schöffel, et al. 2019). Blends of gluten (30%) and soy concentrates (70%) showed great efficiency in the formation of a strong fibrous structure due to disulfide bonding (Dekkers et al. 2018; Chiang et al. 2019). Water distribution within the blend was heterogenous due to greater water absorption capacity of soy proteins compared to gluten (Dekkers et al. 2018; Schreuders et al. 2019; Schreuders et al. 2020). Pea protein is gaining interest as an alternative for soy protein, due to lower concerns over allergenicity and safety (e.g. genetically modified seeds), as well as its high adaptability to grow under different climate conditions (Geerts, Mienis, Nikiforidis, van der Padt, & van der Goot, 2017; Peters, Vergeldt, Boom, & van der Goot, 2017; Tulbek, Lam, Wang, Asavajaru, & Lam, 2016).

Beside plant proteins, novel sources of proteins (algae and fungi-based) are finding their way as binder, filler, and flavoring ingredients in the formulation of meat analogues (Grahl et al. 2018; Smetana et al. 2015). Likewise, algae protein offers an alternative protein for those with a soy allergen, with the additional benefit of improving the amino acids profile (Marti-Quijal et al. 2018). Meat analogues can be reformulated with mainly total algal biomass and other non-purified forms of proteins. Microalgae integration increased the contents of vitamins B and E in the extrudate, where over 95% was retained in the final product (Caporgno et al. 2020). Incorporating *Spirulina platensis* biomass (10%, 30% or 50%) in a texturized soy base resulted in products with black color and intense flavor (earthy notes and an algal odor). Particularly, 50% addition hindered the texture, where the elasticity, fibrousness, and firmness of the extrudates were decreased (Grahl et al. 2018).

Several studies focus on meat substitute production from fungal origin, where they detailed the processing, used strains, and formulation to that of commercial product, Quorn™ (Finnigan et al., 2016; Lonchamp et al., 2019; Jacobson, 2018; Ritala et al., 2017). In brief, mycoprotein is produced by an edible fungi (*Fusarium venenatum*) and is the basis of Quorn™ meat substitutes (Souza Filho et al. 2018). Quorn™ not only contains protein but also high quantities of fiber and starch, which provides positive textural and nutritional attributes to meat-analogs. Beside fungi, egg albumin can be added as a flavoring agent and protein binders to the formulation of vegetarian meat substitute, for vegans, potato protein is used instead of egg albumen (vegan Quorn^{TM}).

4.2. Dairy-free beverages

Recently, milk consumption has been declining due to lifestyle trends, allergic reactions, lactose intolerance, and health concerns associated with animal based products (Abbring et al. 2019; Zingone et al. 2017). In turn, the consumption of plant alternatives have risen, for their lactose-free nature responding to consumers suffering from intolerance and animal-free nature suitable for consumers following a vegan diet (Lawrence, Lopetcharat, and Drake 2016; Chalupa-Krebzdak, Long, and Bohrer 2018). More than half of dairy consumers also purchase (non-dairy) plant-based beverages either to reduce (not completely eliminate) their consumption of animal deriving products (McCarthy et al. 2017), or for health promoting functional beverages (Qamar et al. 2019).

Most plant-based beverages are deriving from soy, rice, almond, and coconut. From a nutritional viewpoint, soy protein has a total protein content comparable to cow's milk (Lacerda Sanches, Alves Peixoto, and Cadore 2019) and contains all the essential amino acids for the human body (Jeske, Zannini, and Arendt 2018; Jeske, Zannini, and Arendt 2017). Soy based beverages might present some drawbacks such as an off-flavor due to action of lipoxygenase on unsaturated fatty acids. With the increasing prevalence of soy allergies (about 0.5% of the global population), more plant alternatives are needed (S. Wang, Chelikani, and Serventi 2018; Sethi, Tyagi, and Anurag 2016). Beverages based on pea protein isolate (3% w/w) had a rich aroma profiles (21 aroma compounds) generated by the reaction pathways of lipid oxidation and the Maillard during the Ultra High Temperature (UHT) treatment. Results showed that pea protein-based beverage aroma profile was characterized with beany, potato, pasta, and cooked green bean aroma attributes, but no changes were reported as a result of storage (Trikusuma, Paravisini, and Peterson 2020).

Plant proteins offer interesting nutritional and functional benefits for the development of innovative infant formulas. In the European Union, protein sources allowed in infant and follow-on formulas are exclusively cow’s milk protein, goat's milk proteins, soy protein isolates, and hydrolyzed proteins following clinical evaluation (Bocquet et al. 2019). In the case of children suffering from cow's milk protein allergy, soy protein-based formulas have been widely used as an alternative. However, up to 14% infants suffering from cow milk allergy also have negative reactions to a soy protein based formula (Bocquet et al. 2019). Hydrolyzed rice protein formulas can be used as a plant-based alternative to cow's milk protein-based. However, this substitution may not be suitable nutritionally considering the different chemical composition of milk and plant-based beverages. These formulas are, therefore, fortified with vitamin D3 (cholecalciferol) and free lysine, threonine, and tryptophan to enhance their nutritional value, making them mor similar to human milk (Bocquet et al. 2019). In a non-dairy infant formula, plant proteins (pea, rice, or potato) were included as a fortifying agent (50%) to whey proteins. Protein degree of hydrolysis and amino acid bioaccessibility were very similar between the control (100% whey protein) and pea, but lower for rice and potato proteins-based infant formulas (Roux et al. 2020). Therefore, the source of proteins must be carefully considered to meet nutritional requirements for infants (Le Roux et al. 2020).

For fermented beverages, the fortification using different plant proteins (0.5%; soy protein isolate, pea protein isolate, wheat gluten, and rice protein) improved protein and amino acid contents. During storage, this fortification increased viscosity. Soy protein isolates-based beverages showed rich essential amino acids profiles particularly lysine, leucine, isoleucine, methionine and threonine. Also, the taste of these drinks have improved, particularly those made from pea proteins isolates (Akin and Ozcan 2017). More research is required to understand the behavior of these proteins during processing and storage and to ensure the physical stability and reconstitution abilities of these products (Le Roux et al. 2020). Including enzymes, or mixing two or more types of plant-based milk can be a starting point to develop a product with a high nutritive value equivalent as cow's milk (Akin and Ozcan 2017; Sethi, Tyagi, and Anurag 2016).

Milk and dairy products are not commonly used as delivery vehicles of microalgal biomass or microalgae-derived compounds. A yoghurt fortified in lipids extracted from *Pavlova lutheri* was found efficient in enhancing the nutritional properties (increasing the Omega 3 content) without altering the functional properties. However, the final product was not appreciated by consumers for the relevant change in color (decrease in lightness and increase in greenness and yellowness) (Robertson et al. 2016).

4.3. Bread

Bread is staple food that can be a suitable vehicle for protein fortification as summarized in Table 2. The inclusion of plant-based proteins in this food was primarily added for increasing the protein intake in the human diet, and secondary for the specific functionality of some proteins (Hoehnel et al. 2019; M. Liu et al. 2018).

*****Table 2*****

4.3.1. Gluten-containing bread

In bakery, vital gluten is mostly used in low amounts to increase the strength of protein network of flours with low protein content for bread making. This addition will improve the mixing tolerance and handling of doughs to form a more cohesive dough network (Bardini et al., 2018; Boukid et al., 2018; Boukid, Carini, Curti, Pizzigalli, & Vittadini, 2019). Consequently, during baking, the dough network will be able to trap and retain the gases formed in baking, resulting in enhanced bread volume and improved yield, color, crumb uniformity, crumb firmness, and sensory properties, as well as protein level (Giannou and Tzia 2016; Ortolan et al. 2017; Ortolan and Steel 2017).

Even though the addition of non-wheat proteins enhances the nutritional profile of bread, it leads to a dilution of gluten and starch (dilution effect) (Hoehnel et al. 2019). The selection of the protein source and amount, with appropriate functionalities significantly affect their potential interactions with wheat flour components, thereby the final structure of the dough and quality of the bread (Zhou, Liu, and Tang 2018). The substitution of wheat flour with 15% of non-wheat proteins (pea, potato, and zein isolates) and gluten affected gluten-aggregation, pasting, and bread characteristics depending on protein source (Hoehnel et al. 2019). Potato and pea protein isolates weakened the gluten-network in doughs contrary to zein. Consequently, gluten and zein based breads had the highest specific volumes and low crumb hardness, compared to those made from pea protein isolates, which showed lower values than the control (Hoehnel et al. 2019). Likewise, replacing wheat flour with soy protein hydrolysates (20%) negatively impact the dough properties (reduction in dough stability) compared to control (100% wheat flour). This is likely due to the interaction of soy protein with wheat flour components that hindered hydration and gluten network formation (Schmiele et al. 2017)**.** The addition of soy protein isolates (30%) decreased dough peak torque and stickiness, resulting in reduction of bread specific volume (from 2.61 to 1.31 cm^3 /g) and increased hardness (173 to 696 g) (Zhou, Liu, and Tang 2018).

To improve the nutritional quality of bread, several algal species have been added as whole algal biomass, and not as purified forms of proteins (Graça et al. 2018; Nunes et al. 2020; Lafarga, Mayre, et al. 2019; García-Segovia et al. 2017). The addition of microalgal biomass increased protein content bread from 7.40% (control) to 11.63% (bread with 10%), minerals (control: 261.7 mg/kg calcium, 196 mg/kg magnesium, and 8.72 mg/kg iron to fortified bread: 721.2 mg/kg calcium, 336.6 mg/kg magnesium, 41.12 mg/kg iron) (Ak et al. 2016). Generally, 3% addition level had a positive impact on dough rheology and viscoelastic characteristics, strengthening the gluten network without affecting fermentation (Graça et al. 2018). However, beyond 3%, the technological properties of bread can be hindered such as undesirable sensorial attributes and reduction in bread volume due to the dilution of starch and gluten (Lafarga, Mayre, et al. 2019; Graça et al. 2018). The volatile profile was also affected, where fourteen volatile compounds were detected in control group and only ten compounds were detected in bread with *Spirulina platensis* (Ak et al. 2016). Another limiting factor is a noticeable change of color in fortified breads due to algal biomass pigments (Graça et al. 2018; García-Segovia et al. 2017). Proteins ingredients, particularly isolates, can instead ensure a better result (Lafarga, Acién-Fernández, et al. 2019). The use of microalgae showed a positive effect on the inhibition of mold growth during the subsequent storage thus extending the shelf life of bread (Ak et al. 2016)**.**

4.3.2. Gluten-free bread

Plant proteins (obtained from gluten-free sources) are valuable ingredients to enhance the nutritional properties of gluten-free bread, which are largely formulated with starchy ingredients (Tomić, Torbica, and Belović 2020; Suphat Phongthai et al. 2016; Matos Segura and Rosell 2011). Plant proteins (other than gluten) have been reported advantageous due to lower allergenicity and unique techno-functional properties (Moreno et al. 2020; Mohamed Lazhar Chihi et al. 2016). Technologically, protein additions to gluten-free systems may increase the elastic modulus by cross-linking, improve the perceived quality by enhancing Maillard browning and flavor, improve structure through gelation, and supports foams (Han et al., 2019; Suphat Phongthai et al., 2016; Smith, Bean, Selling, Sessa, & Aramouni, 2017). Apart from the nutritional increase through the plant protein addition, some research has been focused on finding proteins that could mimic gluten functionality in yeast fermented breads.

The benefits of plant proteins are closely associated with their form (different purity) and amounts. The incorporation of plant protein isolates generally enhances the nutritional quality (protein quantity and quality) of gluten-free bread. Some limitations might be encountered such as the poor water solubility of plant proteins that can results in less uniform bubble distribution compared to animal proteins or a very pronounced taste (Silva et al. 2018; Silva et al. 2019; Wouters et al. 2017). Regarding gluten free doughs or batters, the inclusion of plant proteins increased the water absorption and also modified the mechanical and surface related textural properties (Marco and Rosell 2008).

Incorporating soy proteins (at a range from 2.3 to 4%) in bread formulation with high water retention may result in batters with lower surface-activity and lower stability, leading to breads with lower specific volume and a dense crumb structure (Masure et al. 2019). Higher levels (13%) of soy proteins were used for replacing gluten in rice based breads, although again with lower specific volume, which could be increased with hydroxypropylmethyl cellulose (HPMC) and transglutaminase (Marco and Rosell 2008). Soy proteins had a significant effect on the dough techno-functional properties, increasing the elastic (G') and viscous (G'') moduli, and the same effect was observed with pea proteins (Marco & Rosell, 2008). The formation of a better network for breadmaking could be reached by enzymatic crosslinking of the proteins using transglutaminase, promoting interactions either within beta-conglycinin and glycinin of soybean and the glutelin of the rice flour (Marco et al. 2008) or within the albumins and globulins of rice flour and pea protein isolates (Marco et al. 2007). Specifically, the ß-conglycinin isolated from soy showed viscoelastic properties resembling the gluten functionality (Espinosa-Ramírez et al. 2018). This protein fraction enabled a network that held the carbon dioxide released during baking in gluten-free yeast leavened breads (Espinosa-Ramírez et al. 2018).

Within the same range of addition, rice protein concentrates (2% addition level) enhanced the rheological properties of the batter and the relative elasticity of final gluten-free breads due to functional properties including oil and water binding capacity, foaming, and emulsifying ability (Suphat Phongthai et al. 2016). These breads (fortified with 2% rice protein concentrate) had the highest specific volume, enhanced the crumb porosity, and enhanced sensory attributes (Suphat Phongthai et al. 2016). With respect to the volatile profiles, rice protein based bread crusts had high content of 2-acetyl-1-pyrroline enabling a pleasant aroma (Pico et al. 2019) Tomić et al. 2020). Enriched millet flour-based bread with proteins (pea and rice protein concentrate; 10%) and transglutaminase (0.5, 1.0 and 1.5%), improved the technological quality of bread (structure strengthening, specific volume, and sensory quality), while the enzyme effect was masked (Tomić, Torbica, and Belović 2020). Protein fortification also reduced bread hardness and noted a complete loss of the bitter taste originating from millet (Tomić, Torbica, and Belović 2020). Breads fortified with 30% pea proteins presented lower specific volume and weight loss during baking, and higher hardness than those obtained with 100% starch (Sahagún and Gómez 2018a). This addition reduced the rapidly digestible starch fraction and increased the slowly digestible starch, resulting in a bread with lower glycemic index compared to the control (Sahagún et al. 2020). Zein (5%) was included in a gluten-free formulation based on raw maize flour (70%) and pre-gelatinized maize flour (30%). Prior to dough-making, the zein was premixed with water to form a viscoelastic mass, rather than including dry zein, to improve its extensibility and gas-holding capabilities. The zein fibrils appeared to entrap the maize flour particles, which enhanced bread crumb cell structure and increased loaf volume. However, the crumb cell walls were much thicker than in wheat bread and comprised clumps of starch granules (Khuzwayo, Taylor, and Taylor 2020).

Brown algae added at levels ranging from 2 to 10% increased the antioxidant activity of white rice flour-based bread. Increasing level of addition resulted in undesirable change of color (decrease in lightness and yellowness of breadcrumb), decreased in hardness, and exhibited a low degree of staling. The addition of algae at 4% inclusion enabled the highest specific volume compared to the control. Up to 4% was also accepted by consumers, while higher levels resulted in unpleasant taste (Różyło et al. 2017).

4.4. Pasta

4.4.1. Gluten containing

Pea proteins (added in a range between 0 to 12.5%) were assessed as possible ingredients in wheat noodles (Wee et al. 2019). Both native and denatured (by heating 5% w/w native pea protein suspension at 85 °C for 30 min in a water bath and freeze-drying for a minimum of 48 h) forms were considered. This study revealed that denatured pea protein reduced *in vitro* glucose release due to a lower degree of gelatinization and greater binding of protein to the starch matrix. In turn, native protein had less impact on degree of gelatinization and glucose release in noodles. The form of protein (denatured or native) did not significantly influence product texture or sensory perceptual properties (Wee et al. 2019).

Microalgal proteins have been also implemented for enriching pasta. El-Baz et al., (2017) prepared pasta by adding low amounts (below 3%) of *Dunaliella salina* powder to enhance its nutritional value, particularly protein content, minerals, phytochemicals, and unsaturated fatty acids (El-Baz, Abdo, and Hussein, 2017). Incorporation of the microalgal powder improved water absorption, resulting in an increase of the pasta volume and weight, but also losses in cooking. Sensory evaluation revealed that 1% addition did not affect flavor, mouthfeel, or overall acceptability. The acceptability and mouthfeel were negatively affected at higher levels, and the pasta was darker in color. Much higher levels were tested with *Spirulina platensis* (up to 15%), affecting cooking quality (increase in weight and volume) without affecting cooking loss. Apart from pasta color, specifically pasta luminosity and yellow index decreased, and green index increased (Özyurt et al. 2015). Sensory evaluation indicated that pasta enriched with 10% *S. platensis* was the most appreciated in terms of flavor and appearance.

4.4.2. Gluten free

Beside enhancing protein quantity and quality, the fortification of gluten-free pasta with protein plays an important technological role in determining the structure, texture, and sensory properties of the final product (Suphat Phongthai et al. 2017; Laleg et al. 2016; Linares-García et al. 2019). The most frequently used proteins in gluten-free pasta are from animal origin, mainly egg protein, milk protein, and whey protein as they can improve textural characteristics (springiness, resilience and adhesiveness), cooking properties (low cooking loss), and the digestibility of pasta (Muneer et al. 2018; Linares-García et al. 2019).

For plant proteins, soy protein is among the most used proteins for formulating animal-free and gluten-free pasta. Incorporating soy protein isolate (up to 10%) decreased the starch retrogradation of rice flour-based spaghetti and resulted in a more porous structure compared to control (100% rice flour), and 5% addition gave the best eating quality and overall acceptability (Detchewa et al. 2016). Banana flour-based pasta was enriched with soy protein or egg white (5, 10, and 15%) and compared to conventional pasta (100% semolina) and banana pasta (100% banana flour) (Rachman et al. 2019). Cooking properties of banana pasta (optimum cooking time, swelling index, water absorption index, and cooking loss) was enhanced with increasing protein levels, particularly with soy protein addition, improving the extensibility (Larrosa et al. 2016; Suphat Phongthai et al. 2017; Rachman et al. 2019) and preventing structure disintegration (Suphat Phongthai et al. 2017). Pea and rice protein isolates have been used for enriching quinoa pasta, formulated with extruded and non-extruded quinoa (red and white) flour. The addition of pea protein (12%) increased protein content (27.9%) and pasta firmness (Linares-García et al. 2019). Pasta enriched with *Spirulina platensis* biomass at 2% addition was acceptable without altering cooking and texture properties, phenolic compounds, chlorophyll, and carotenoids, and antioxidant activity increased (Fradinho et al. 2020).

Noodles not only have been tested with the purpose of protein enrichment, but also protein-based noodles have been developed and studied. When gluten-free noodles were processed into pasta-like sheets with pea protein isolate (>90% proteins) at high levels, doughs showed high crosslinking resulting in stronger protein networks (high strength and extensibility) (Muneer et al. 2018). The use of zein was effective in increasing dough stability and rice noodle firmness, regardless of the particle size or amylose content of the flour (M. Kim et al. 2019; Jeong et al. 2017). Thus, the ability of zein to generate a viscoelastic protein network above its glass transition temperature enabled the production of gluten-free rice doughs. Overall, the type of protein, level of protein, and protein interaction with the properties of the main ingredient(s) can impact the end-quality of pasta/noodles (Rachman et al. 2019).Gluten-free noodles formulations can include different ingredients such as rice flour and starch, maize, quinoa, millet, banana, hydrocolloids, enzymes, or blend of different flours and starches. Therefore, comparison of different studies is complex (tricky) due to the high diversity of ingredients that might radically change the properties of the formulated products (see summary Table 3).

*****Table 3*****

4.5. Baked goods

As summarized in Table 4, several types of baked goods have been enriched with protein, impacting their nutritional, technological, and sensory quality depending on the main ingredient, type, and amount of protein, as well as the presence or absence of gluten.

*****Table 4*****

4.5.1. Gluten-containing

Fortification of gluten-containing cookies typically incorporate dairy proteins (*e.g.* whey protein or casein) (Gani et al. 2015; Wani et al. 2015). The application of plant proteins showed contradictory outcomes, likely due to the range of formulations (Tang and Liu 2017; Gani et al. 2015; Wani et al. 2015). Partial substitution of wheat flour with whey and soy protein (0–30%) resulted in relevant effect on rheological quality depending on the type and amount of protein (Tang and Liu 2017). Increasing the level of soy protein from 5 to 30% resulted in higher water absorption, opposite to whey protein concentrate. Biscuits enriched with 5% and 10% of soy protein were smaller, while those made with 30% soy protein were wider, but all of them had good overall acceptability scores (Tang and Liu

2017). Tang and Liu (2017) reported that whey protein provoked an increase of expansion, but this effect was not observed in others studies (Gani et al. 2015; Wani et al. 2015).

Different species of microalgal biomass (*Spirulina platensis*, *Chlorella vulgaris*, *Tetraselmis suecica*, and *Phaeodactylum tricornutum* at 2 and 6%) were used to substitute wheat flour in cookies formulation (Batista et al. 2017). Increasing level of fortification increased protein, phenolic contents and antioxidant potential (Singh et al. 2015; Batista et al. 2017). Cookies prepared with *Spirulina platensis* and *Chlorella vulgaris* showed higher protein contents compared to *Tetraselmis suecica*, and *Phaeodactylum tricornutum*. Regardless of the specie, the addition of 2% strongly affected sensory aspects of cookies (*e.g.* smell, taste, and overall acceptability) due to the presence of sulfuric compounds, diketones, α-ionone, and β-ionone. Cookies enriched with 2% *Spirulina platensis* recorded the highest acceptance score (Batista et al. 2017); whereas adding up to 6% of *Chlorella* without affecting the sensorial properties was possible if the biomass was suitably pre-treated (*e.g.* defatting) (Sahni, Sharma, and Singh 2019). This suggests that suitable pre-treatments can ensure the mitigation of the undesirable components responsible for off-flavors, thereby favoring incorporation at higher levels. Another option might be the inclusion of hydrocolloids such as guar gum. For instance, high levels of fortification (>7% *Spirulina platensis* and >30% sorghum flour) negatively affected the textural and sensory attributes of flavor and graininess. However, when guar gum was added to the formulation (*Spirulina platensis* 7%, sorghum flour 30% and guar gum 1%), it was possible to maintain a good quality (P. Singh et al. 2015).

4.5.2. Gluten-free

Dairy and soy protein are the most used protein sources in gluten-free products (Sahagún and Gómez 2018b; Mancebo, Rodriguez, and Gómez 2016). However, available scientific literature is scarce, and it is not possible to compare the results of the different studies, which are based on different combinations of main ingredients (*e.g.* rice flour, starch, maize flour) and different proteins.

The substitution of rice flour by soy protein (up to 10% addition level) affected the quality of cookies, improving them (decrease in the hardness) when adding 7.5% soy protein along with glycerol monostearate (0.5%) (Sarabhai et al. 2015). Soy protein isolate inclusion resulted in light crust color of cookies, due to its lower lysine amounts, as compared to whey protein which participate in Maillard reaction (Sahagún and Gómez 2018b). The combination of protein and emulsifier enabled the formation of gluten free cookie dough similar to the structure of that based on gluten proteins (Sarabhai et al. 2015).

The protein addition in this type of product not only affects the technological quality, but also has a significant impact on the nutrient value. The substitution of maize flour with soy protein isolate (5-30%) increased the protein content of cookies from 8.69 (5%) to 29.11 (30%); while the calorific value decreased from 468 (control) to 383 cal/100 g (30%). Cookies enriched with 20% soy protein were well accepted by consumers, but increasing levels of substitution decreased the overall acceptability of the enriched products (Adeyeye, Adebayo-Oyetoro, and Omoniyi 2017).

Different mixtures of rice flour, maize starch, and pea protein (up to 20%) were used to develop protein rich cookies. Pea protein incorporation increased hydration properties of the mixture and dough consistency, leading to smaller, softer, and darker cookies compared to the control. Fortified cookies (20% pea protein) showed higher acceptability (the best scores for texture and odor). Therefore, protein and starch can be used to adjust the desired cookie characteristics depending on the needs of manufacturers (Mancebo, Rodriguez, and Gómez 2016).

Recently, a comparative study was performed to evaluate the effect of different types of protein (pea, potato, egg white, and whey) (15–30%) on cookies (Sahagún and Gómez 2018b). The hydration properties of protein-supplemented doughs were lower than the control, except for pea protein. Subsequently, G' and G'' values for pea and potato protein were like the control, while egg white and whey protein had lower values. As a result, egg white produced harder cookies, whey protein produced wider cookies, potato protein produced darker cookies, and pea protein did not affect cookie parameters, but consumers preferred pea protein cookies (30% addition level) (Sahagún and Gómez 2018b).

4.6. Snacks and bars

The addition of protein from plants has made a great impact on sports/performance nutrition bars. According to the Mintel Global New Products Database (GNPD), in the 12 months prior to July 2019, 14% of total European launches in sports/performance and nutrition markets featured a vegan/no animal ingredients claim, a five percentage point increase since 2014 (Mintel 2018). The “high-protein” claim was amongst the top three claims made by snack bars globally in 2019 (Mintel, 2019). This market expansion is going beyond traditional soy and dairy proteins to new and innovative alternatives including pea protein and microalgae protein (Mintel, 2019). Pea protein isolates were used to formulate extruded rice snacks, where 30% inclusion resulted in high initial expansion but delayed melt solidification, resulting in melt shrinkage and non-uniform final extrudate structures. However, extrudates containing 20% pea proteins isolates had the highest final expansion, and no significant shrinkage was observed (Philipp et al. 2018). The incorporation of 2.6% *Spirulina platensis* provided an increase of 22.6% in protein, 28.1% in lipids, and 46.4% in minerals compared to 0% *Spirulina platensis* -based snacks (Lucas et al. 2018). Also, the enriched products had adequate physical and structural properties, which resulted in 82% acceptance index (Lucas et al. 2018; Lucas et al. 2017). Similar results were found in the case of maize extrudates enriched with *Spirulina platensis* (2-8%), where protein content increased (average 0.6%) with each 1% increase in *Spirulina* *platensis* concentration. However, sensorial acceptance was reduced in products enriched with the higher percentages of *Spirulina*, due deterioration of properties such as color and crispness (Tańska, Konopka, and Ruszkowska 2017).

Snack bars enriched with 2% and 6% *Spirulina platensis* presented no significant difference compared to the control (0% *Spirulina platensis*) (Lucas et al. 2019). These additions (2% and 6%) provided a protein increase of 11.7% and 29.9% respectively. The physicochemical (texture and color) and microbiological parameters remained stable during storage (30 days) (Lucas et al. 2019). Overall, snacks seem a suitable vehicle for health-beneficial components of microalgae and other sources of protein (See Table 4).

4.7. Other products and beverages

Non-animal proteins have been used for reformulating innovative beverages (Table 5). Textured soy protein was incorporated into egusi (white seed melon- *Cucumeropsis mannii*) soup and stew-sauce, which are typical Nigerian foods. The swelling ratio ranged from 2.05 to 5.39 depending on the brand when texturized soy protein was used, which influenced the acceptability of the sensory perception of the enriched soups and sauces. In this case, the addition of 70% textured soy protein granules were accepted by the consumers (Alamu and Busie 2019).

Babault et al. (2015) reformulated sport drinks by adding different protein isolates (85% protein content). A comparative *in vivo* study (n=161 males) was conducted to compare whey protein vs pea protein supplementation on muscle thickness and strength during a 12-week resistance training program. The study used sports drinks (300 mL) containing 25 g of protein (pea isolates or whey protein concentrate), or a placebo (no protein added). Increases in thickness were significantly greater in the pea group as compared to placebo, whereas there was no difference between whey and the two other products. Muscle strength also increased with time with no statistical difference between groups. Since no difference was obtained between the two protein groups, the authors suggested that vegetable pea protein could be used as an alternative to whey-based dietary products (Babault et al. 2015).

A shake for elderly developed using a low amount of *Spirulina* increased the protein content from 41.3 (0% *Spirulina platensis*) to 43.4% (0.75% *Spirulina platensis*). Sensorial analysis (based on a 9-point hedonic scale) revealed that the product containing *Spirulina platensis* was appreciated and recorded an acceptance score (7.7) within the range of that of the control (7.9) and higher than that of commercial (6.9) (Santos et al. 2016).

Smoothies enriched with *Spirulina platensis* (2.2%) showed the higher acceptance scores compared to those enriched with *Chlorella vulgaris*; this can be explained by the strong marine odor and flavor of

Chlorella compared to *Spirulina platensis*. The enriched smoothies (2.2% *Spirulina platensis*) showed stable quality including sensory properties during storage (5 °C for 14 days) (Castillejo et al. 2018).

The incorporation of microalgal biomass (*Spirulina*, *Chlorella* or *Tetraselmis*; at concentrations ranging from 0.5 to 2.0%) increased viscosity, antioxidant capacity, and phenolic content of a broccoli-based soup. Increasing the level of addition of microalgae (all species regardless of addition level) reduced the sensorial acceptability compared to broccoli-only soup (91.1%), where the most accepted was that formulated using 0.5% addition level of *Tetraselmis* (82.2% acceptance rate based on a 5-point hedonic scale) (Lafarga, Acién-Fernández, et al. 2019).

*****Table 5*****

5. Trends in the market of animal-free proteins

The non-animal protein market is continuously growing, with no signs of slowing. It is expected to represent one-third of all protein fortification by 2054 (Mintel 2019a). Perceived health benefits are the main driver for consumer purchase, while concerns about animal ethics or the environmental impact of animal products are secondary drivers.

Generally, animal protein sources provide higher protein contents and the required amino acid contents to qualify as high quality proteins compared to most plant-based proteins (Gorissen et al. 2018; van Vliet, Burd, and van Loon 2015). However, serious concerns are rising over the high prevalence of allergies and intolerances (lactose) and increased incidence of cardiovascular diseases, various cancers, and mortality risks (Burger and Zhang 2019; Virtanen et al. 2019; O'Sullivan et al. 2016). Also, consumers may have concern over the association of the spread of diseases through meat (*e.g.* bovine spongiform encephalitis and multidrug-resistant bacteria). Although many plant protein sources are considered deficient in essential amino acids particularly lysine and leucine (Gorissen et al. 2018; van Vliet, Burd, and van Loon 2015), they may provide health benefits due to their association with the reduction of body mass indices (BMIs), blood pressures, blood cholesterol, incidence of the cardiovascular diseases, and diabetes (Sokolowski et al. 2019; Navruz-Varli and Sanlier 2016; De Souza et al. 2017; Lopez et al. 2019; Turner-McGrievy et al. 2020; Cramer et al. 2017; Martini et al. 2018).

Environmental concerns include climate change, resource scarcity, environmental sustainability, and rainforest clearing (Janssen et al., 2016; Lopez et al., 2019; Schmidt et al., 2015). Global warming and sustainability concerns have been shown to deviate consumer interest from animal-based products to plant-based food products (Nadathur, Wanasundara, and Scanlin 2017; Reipurth et al. 2019; De Boer, Schösler, and Aiking 2014). Plant-based protein production is more environmentally friendly, producing considerably less greenhouse gas emissions compared with that of meat protein, and is less exhausting to natural resources (energy, water, and land inputs) (Fresán et al. 2019; Fresán et al. 2018). As a matter of fact, the production of plant foods tends to generate a smaller carbon footprint when compared to animal sources (Lynch, Johnston, and Wharton 2018; Boukid, Zannini, et al. 2019; Klamczynska and Mooney 2017; Apostolidis and McLeay 2016). Some proteins are mainly recovered from by-products, which contribute in reducing the industrial wastes and its implication on economy and environment (Cheetangdee and Benjakul 2015; Senaphan et al. 2018). Producing a unit of animal food protein induces more environmental damage than producing an equivalent unit of plant food protein (Gardner et al. 2019). Algal proteins can be obtained from a relatively sustainable source, since algae i) is a rich source of proteins; ii) do not compete with traditional food crops for land; iii) is a multiuse crop (fuel, food, feed..); and iv) mitigate greenhouse gas emissions (Tredici et al. 2015; Klamczynska and Mooney 2017; Laurens et al. 2017). Fungal proteins do not require agricultural land and may be obtained through a circular economy based on recycling agri-industrial wastes (Ritala et al. 2017; Satari and Karimi 2018; J. Lonchamp et al. 2019; Finnigan, Needham, and Abbott 2016). Algal and fungal alternative sources can be far more sustainable (lower foot printing) than animal and some plants sources (S Matassa 2016; J. Lonchamp et al. 2019; Laurens et al. 2017). Although, when the production is scaled up for commercial use, to obtain desirable product and keep consistency, costly/not sustainable technologies may be used, making them comparable in resource use to animal products.

Vegan and vegetarian diets are increasing in popularity due to ethical (animal-related), health (self-related) and environment-related motives (Janssen et al. 2016). Ethical considerations are fueled by concerns over animal welfare, animal suffering in farming, animal rights, and speciesism (Costa et al. 2019; Chuck, Fernandes, and Hyers 2016; Radnitz, Beezhold, and DiMatteo 2015; Faber et al. 2020). Vegetarians do not consume animal flesh (meat, poultry, fish or seafood) but consume other animal derived products including eggs and dairy, while vegans exclude both flesh meat and animal-derived food from their diet (Appleby et al. 2016; Faber et al. 2020; Rosenfeld and Burrow 2017). Flexitarian population following a semi-vegetarian diet will have also a great impact on the growth of non-animal proteins market (more than one in five Americans is a flexitarian) (Mintel 2019b). This diet consists on the reduction of the consumption of animal products in favor of those plant-based products, opening new opportunities for plant protein applications.

6. Safety and regulation

Generally, ensuring food safety requires the assessment of nutritional value, microbiological, toxicological, and allergenic risks. The main safety concern of proteins is their allergenicity. For grain protein, regulatory aspects are clear in this regard, where thresholds of major allergens (such as gluten and soy) have been defined (Codex alimentarius commission 2009). The General Standard for the Labelling of Prepackaged Foods (CXS 1-1985) includes provisions for the declaration of certain foods and ingredients known to cause hypersensitivity referred to as "allergen labelling" (Codex Committee On Food Labelling 2019). Furthermore, it is mandatory to declare the presence in any food or food ingredients obtained through biotechnology of an allergen transferred from any of the list of allergen products. When it is not possible to provide adequate information on the presence of an allergen through labelling, the food containing the allergen should not be marketed. In the EU, the Regulation 1169/2011 establishes that the mandatory information on the package label informs consumers on the absence or presence of a potentially allergenic food components aligning with what declared in the Codex (European Parliament 2011). Likewise, some allergic reactions to mycoprotein have been reported but no regulation are imposing the declaration of mycoprotein as an allergen on the label of meat substitute products (Jacobson and DePorter 2018). In the UK, the safety of mycoprotein was cleared in 1983 as the first novel food with no further revision in respect to its allergenicity (FAO/WHO 2000). Regarding novel foods, EU legislation included proteins deriving from algae (microalgae and seaweed) and required that the ingredients must apply and fulfil the criteria found in the context of Regulation (EU) 2015/2283, before they can be launched onto the food market (European Parliament 2015). This regulation requires that, to ensure safety, all the characteristics of the novel food that may pose a safety risk to human health are investigated and possible effects on vulnerable groups of the population must be determined. However, no clear indication was mentioned about the assessment of allergy risks related to novel protein. At present, there is no predictive and validated method for the assessment of novel protein allergenicity (Pali-Schöll et al. 2019). Therefore, the allergenicity assessment for these novel foods is focused on immediate risks to consumers due to the presence of existing IgE that could arise either from unexpected exposure to an allergen to which they are already allergic, or to a likely cross-reactive protein based on Codex guidelines (Abdelmoteleb et al. 2021). Based on the risk assessment of the Food Safety Commission of China and the guidelines set by the Codex Alimentarius Commission, the standard applied on the edible algae foods (blue algae, green algae, brown algae and red algae) set limits only to some heavy metals and pheophorbide, and no mention to potential allergens (Food Safety Commission of China and the guidelines set by the Codex Alimentarius Commission 2013). Nevertheless, some maximum residues levels are not yet set for algal proteins. Indeed, algal species are not known to have toxic metabolites, yet they can accumulate toxic elements (e.g. heavy metals) if exposed during their cultivation (Rzymski 2015; Hosseini, Khosravi-Darani, and Mozafari, 2013). Noteworthy, innovative accurate analytical tools are required to achieve regulatory and safety approval. In all cases, the general labeling requirements set in Regulation (EU) 1169/2011 and other relevant labeling requirements in EU food law must be applied for protein ingredients and their inclusion in food product (European Parliamentand Council of the European Union 2011).

7. Conclusions

This article focused on gaining insight into the non-animal proteins market and forthcoming trends (health, ethics, and environmental impact) in food and beverages. Away from the propaganda over animal versus non-animal proteins, this comprehensive review examined the most significant motivations behind consuming strictly or partially non-animal proteins. First, the expansion of protein alternatives (from plant, algae, and fungi) has been shown several times in published studies. Scientific evidence has shown animal proteins do have a better amino acid profile, but consuming more non-animal proteins does not mean compromising such a benefit. Indeed, blending proteins from different (non-animal) sources can enable additional benefits. This does not mean that plant protein alternatives are overtaking animal protein sales, but it means that the non-animal protein market will keep growing to meet the needs of the growing global population (9 billion by 2050) (The World Bank, 2016), while at the same time shifting to more sustainable protein sources.

For the future, innovation is the key to boost the growth of plant protein market, where these points must be considered:

i) Breeding: the selection new varieties or strains with peculiar properties (higher productivity, higher proteins content, and better amino acid composition, less anti-nutrients, etc) to respond to manufacturers/consumers requirements.
ii) Other plant sources such as lupin protein and oat protein might emerge because consumers probably will want additional protein sources to choose from.
iii) Innovative technologies (cost effective, green, and sustainable) will enable companies to overcome the challenges of productivity, shelf life, nutritional completeness, and sensory acceptability of the final product.
iv) Safety and allergenicity: many alternative proteins are considered novel foods, where EFSA already defined a list of edible species from algae and fungi but still their purified ingredients (proteins extracted from these species) must go through the procedure of risk assessment for regulatory and safety approval.
v) Building trust with consumers may be achieved by using recognizable ingredients in products with clean labels, are non GMO, vegetarian, vegan, contain and free-froms.

vi) Personalized nutrition is likely the future of the food industry: alternatives proteins enable a larger portfolio of ingredients, making tailor-made products possible for consumers to try non-traditional sources of proteins.

Declaration of competing interest

The authors declare no competing interests.

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Author contributions

F. Boukid collected, drafted, and wrote the review. C M. Rosell, S. Rosene, S. Bover-Cid and C. Massimo contributed in the design the framework of this review and critically revised different sections of the draft. All authors contributed to the revision of the manuscript and read and approved the submitted manuscript.

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Table 1: A debrief on the current situation of non-animal proteins market

Source	Market value	Ingredients	Food application	Leading companies	Region	References
Plant proteins						
Soy protein	expected to reach US$7.3 billion by 2025 (at a CAGR of 7.1% from 2019 to 2025)	isolates; concentrate; protein flour; textured protein	bakery and confectionery, meat extenders and substitutes, nutritional supplements, beverages	Archer Daniels Midland, DuPont, The Scoular Company, Fuji Oil Asia Pte, Cargill, and DowDupont	North America accounts for the major market share	(Meticulous Research®, 2019b).
Wheat protein	is expected to reach a value of US$1,836.480 million by 2024, from US$1,274.150 million in 2018, growing at a CAGR of 6.28%	gluten; textured protein; hydrolyzed protein	bakery and snacks, nutritional supplements, dairy products, processed meat	Archer Daniels Midland, Agridient, Amilina, Anhui Reapsun Food, Cargill, Chamtor, Crespel & Deiters GmbH, Crop Energies, Dengfeng Grainergy Agricultural Development, Jaeckering, Kroener Staerke, Manildra Group, MGP Ingredients, Inc, Permolex, Roquette, and Tereos Syrol	North America accounts for the major market share	(Research and markets, 2019b).
Pea protein	estimated at US$32.09 million in 2017, and is expected to reach US$176.03 million by 2025, growing at a CAGR of 23.6% during the forecast period (2018 - 2025)	isolates; concentrate; textured protein	bakery, meat extender and substitute, nutritional supplement, beverage, snacks	Cargill, Icorporated, DuPont, Kerry Inc., Glanbia plc, The Scoular Company, Avebe, Growing Naturals, LLC, Puris	North America is estimated to be the largest market	(Meticulous Research®, 2019a).
Potato protein	forecasted to reach US$ 168.47 million by 2024 growing at a CAGR of 7% during the forecast period (2019 - 2024)	isolates; concentrate	Beverage, Snacks & Bar, Animal Nutrition	Avebe, Tereos Group, Agridient, Agrana, PEPEES SA, Kemin Industries, Inc., Omega Protein Corporation, Roquette Foods	North America leads the market followed by Europe	(Mordor Intelligence, 2019b)
Rice protein	expected to reach 180 million US$ in 2024, from 120 million US$ in 2019, growing at a CAGR of 7.7% during the forecast period (2019-2024)	isolates; concentrate	bakery and snacks, nutritional supplements	AIDP Inc., Axiom Foods Inc., Bioway (Xi'an) Organic Ingredients Co., Ltd., Golden Grain Group Ltd., RiceBran Technologies, Nutrition Resource Inc., Shaanxi Fuheng (FH) Biotechnology Co., Ltd., and Shafi Gluco Chem Pvt., Ltd.	The market is spread across North America, Latin America, Asia Pacific, Europe, and Middle East and Africa.	(Beroeinc 2019) (Research TechSci, 2019).
Corn protein	expected to reach 80 million US$ in 2024, from 65 million US$ in 2019	Zein (conventional and organic)	Food and beverage industry, pharmaceutical, cosmetics and coating agents	Zein Products, Archer-Daniels Midland Company, Glanbia plc, AGT Food & Ingredients, Burcon Nutrascience Corporation, Penta International, E. I. Du Pont De Nemours And Company, Roquette Freres, Cargill Inc., Cosucra Groupe Warcoing, Ingredion Inc., CHS	Zein is primary available in North America, Europe and Asia-Pacific, South America, Middle East and Africa	(Global info research, 2019).

				Inc		
Non-animal proteins						
Algal protein	expected to grow at a CAGR of 7.03% to reach a total market size of US$0.838 billion by 2023, increasing from US$0.596 billion in 2018	form: powder and liquid; source: marine and freshwater algae; type: *Spirulina platensis*, *Chlorella* and other algae	Bakery & Confectionery, Beverages, Breakfast Cereals, Sauces, Dressings & Spreads, Snacks)	Allma, Cyanotech Corporation, Earth Rise Nutritionals, Energybits, Far East Bio-Tech Co., Heliae Development LLC, Myanmar Spirulina Factory, Nutrex Hawaii Inc., Roquette Klötze, and TerraVia Holdings Inc.	North America accounts for major revenue share of global algal protein market, followed by Europe	(Mordor Intelligence, 2019a).
Fungal protein	estimated at around US$ 200 million in 2018 growing at CAGR of 12%	minced and slices	food & beverage such as meat alternatives and meat extenders	Marlow Foods Ltd., Yutong Industrial CO. Limited, Shouguang FTL BIO. CO., LTD. and 3fbio Ltd	Europe, followed by North America	(Factmr 2019)

Table 2: Bread as a vehicle or non-animal proteins

Protein source	Level of addition	Effect of the addition	Reference
Gluten-containing			
Vital gluten	0 and 1% of wheat flour	- improve the mixing tolerance and handling of doughs with low protein content -improve bread volume and improved yield, color, crumb uniformity, and crumb firmness	(Bardini et al. 2018; Boukid et al. 2018; Boukid, Carini, et al. 2019)
Vital gluten, zein, pea, potato isolates	15% of wheat flour	-increase protein content of bread -pea and potato proteins weakened the dough -gluten increases the volume; faba and pea proteins maintain a similar firmness to that of the control -zein and gluten produces the best bread (high volume and lowest firmness)	(Hoehnel et al. 2019)
Vital Gluten	2%, 4%, 5%, and 6% of wheat flour	-enhance dough properties -improved bread yield, color, crumb uniformity, and firmness -	(Giannou and Tzia 2016)
Soy protein hydrolysate	0-20% of wheat flour	--reduce dough stability	(Schmiele et al. 2017)
Soy protein isolates	0-30% of wheat flour	-decrease breads specific volume and increase hardness	(Zhou, Liu, and Tang 2018).
A. platensis	11% of wheat flour	-improve the nutritional properties (proteins and mineral content) of breads	(Ak et al. 2016)
Chlorella vulgaris	1-5% of wheat flour	-up to 3% enhance bread properties, but beyond decrease bread volume and increase firmness	(Graça et al. 2018)
Gluten free			
Soy protein isolates	2.3-4% of rice flour or a mixture of potato and cassava starches	- -increase water retention and reduce batters stability -decrease specific volume	(Masure et al. 2019)
Rice protein concentrate	2% of rice flour	- enhance the rheological properties of the batter and the relative elasticity of breads	(Suphat Phongthai et al. 2016)
Rice or pea protein	5 and 10% of rice flour-corn starch	-enhance volatile profile	(Pico et al. 2019).
Pea and rice concentrate	10% of millet flour	Improve bread quality (structure strengthening, specific volume and sensory quality) and reduce firmness	(Tomić, Torbica, and Belović 2020)
Pea protein isolate	30% of starch	-descrease spefic volume and increase firmness	(Sahagún et al. 2020)
Zein	5% of a blend of maize flour (70%) and pre-gelatinized maize flour (30%)	enhance bread crumb cell structure and increased loaf volume.	(Khuzwayo, Taylor, and Taylor 2020).
Brown algae addition	2-10%	-increase the antioxidant activity -decrease bread lightness and yellowness -The addition of 4% of increase specific volume and	(Różyło et al. 2017).

results accepted by

Product	Protein source	Level of addition	Effect of the addition	Reference
Gluten-containing				
Noodle	Pea proteins	Up to 12.5%	Do not affect product texture and sensory perceptual properties	(Wee et al. 2019).
Pasta	*D. salina*	1, 2, and 3% of durum wheat semolina	-enhance its nutritional value (protein content, minerals, phytochemicals and unsaturated fatty acids) - increase of the pasta volume and weight, -increase cooking losses. -1% addition did not affect flavor, mouthfeel and overall acceptability,	(El-Baz, F.K., Abdo, S.M. and Hussein 2017)
Pasta	*Spirulina platensis*	5, 10 and 15% of durum wheat semolina	-increase in weight and volume - decrease pasta luminosity and yellow index and increasing green index -10% was the most appreciated in terms of flavor and appearance	(Özyurt et al. 2015)
Gluten-free				
Spaghetti	Soy protein isolate	0, 2.5, 5.0, 7.5, 10.0 % of rice flour	- decrease the starch retrogradation and result in porous structure	(Detchewa et al. 2016).
Pasta	Soy proteins	5, 10, and 15% of banana flour	-increase optimum cooking time, swelling index, water absorption index, and cooking loss	(Rachman et al. 2019).
Pasta	Potato, pea and rice protein isolate	6% and 12% of extruded quinoa and non-extruded quinoa (red and white) flour	-increase protein content and pasta firmness	(Linares-García et al. 2019).
Pasta	*Spirulina platensis*	1-15% of rice flour and *Psyllium* gel in a 50/50 ratio	Increase phenolic compounds, Chlorophylls, carotenoids, and antioxidant activity	(Fradinho et al. 2020).
Pasta-like sheets	Protein isolate (>90% proteins) + dietary fiber (containing 21% proteins, 37% starch and 42% fiber)	protein to fiber ratios (100/0, 90/10, 80/20, 70/30 and 50/50, respectively)	-form strong protein network (high strength and extensibility)	(Muneer et al. 2018).
Noodles	Zein	5% of rice flour	increase dough stability and rice noodles firmness	(Kim et al. 2019)
Noodles	Zein	5% and 10% of rice flours with different amylose contents (12, 19, and 26%)	- generate a strong viscoelastic protein network	(Jeong et al. 2017).

Table 3: Pasta and noodles as vehicles of non-animal proteins

Table 4: Baked goods and snacks

Product	Protein source	Level of addition	Effect of the addition	Reference
Baked goods				
Gluten-containing				
Biscuits	Soy protein isolate	0-30% of wheat flour	-increase water absorption -Biscuits enriched with 5% and 10% were smaller, while those made with 30% were wider, but all of them had good overall acceptability scores	(Tang and Liu 2017).
Biscuits	*A. platensis*	1.63, 3, 5, 7, 8.36% of wheat flour	-increase protein, phenolic contents and antioxidant activity -	(Singh et al. 2015).
Biscuits	*A. platensis*, *C. vulgaris*, *T. suecica* and *P. tricornutum*	2 and 6% of wheat flour	--2% of Spirulina was acceptable by panelists	(Batista et al. 2017)
Cookies	*Chlorella* (defatted flour)	3, 6, 9 and 12% of wheat flour	6% of chlorella was liked by panelists	(Sahni, Sharma, and Singh 2019).
Gluten-free				
Cookies	Soy protein concentrate	5, 7.5 and 10% of rice flour	-7.5% decrease hardness)	(Sarabhai et al. 2015).
Cookies	Soy protein isolate	5-30% of maize flour	--increase the protein content and decrease calorific value -20% was accepted by panelists	(Adeyeye, Adebayo-Oyetoro, and Omoniyi 2017)
Cookies	Pea proteins isolate	0, 10 and 20% of different mixtures of rice flours and maize starches	-increase hydration properties of the mixture and dough consistency -produce small, soft and dark cookies -20% was accepted by panelists	(Mancebo, Rodriguez, and Gómez 2016)
Cookies	Pea and potato protein isolates	0, 15 and 30% of corn flour	potato protein produced darker cookies, and pea protein did not affect cookie parameters, but consumers preferred pea protein cookies (30%)	(Sahagún and Gómez 2018b)
Snacks				
Extruded snacks	Pea protein isolates	0- 30% of rice starch	20% pea proteins isolates had the highest final expansion without significant effect on shrinkage	(Philipp et al. 2018).

Extruded snacks	*Spirulina platensis*	0.4, 1.0, 1.8, 2.6, and 3.2% of a mix (2:1 ratio of organic rice flour and organic corn flour)	-increase protein content -82% acceptability index	(Lucas et al. 2018)
Corn grits extrudates	*Spirulina platensis*	2-8% of total formulation	-increase protein content -decrease sensory acceptability	(Tańska, Konopka, and Ruszkowska 2017)
Snack bars based on oat and rice flakes	*Spirulina platensis*	2 and 6% of total formulation	- increase protein content -stability of physicochemical (texture and color) and microbiological parameters during storage (30 days)	(Lucas et al. 2019).

Table 5: Beverages fortified with non-animal proteins

Product	Protein source	Level of addition	Effect of addition	Reference
Egusi (white seed melon- *Cucumeropsis mannii*) soup and stew-sauce	Textured soy protein	70%	70% textured soy protein granules were accepted by the consumers	(Alamu and Busie 2019).
Sport drink	Pea protein isolates	25 g of protein in to 300 mL	-increase muscle strength and thickness	(Babault et al. 2015).
A shake for elderly	*Spirulina platensis*	0.75%	0/75% was accepted by the consumers	(Santos et al. 2016)
Smoothies	*Spirulina platensis* or *Chlorella vulgaris*	2.2%	Stable sensory properties and quality during storage (5 °C for 14 days)	(Castillejo et al. 2018).
Broccoli-based soup	*Spirulina platensis*, *Chlorella*, or *Tetraselmis*	0.5-2.0%	-increase viscosity, antioxidant capacity, and phenolic content -0.5% was the most accepted	(Lafarga, Acién-Fernández, et al. 2019)