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Trends and prospects in dairy protein replacement in yogurt and cheese

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ABSTRACT

There is a growing demand for nutritional, functional, and eco-friendly dairy products, which has increased the need for research regarding alternative and sustainable protein sources. Plant-based, single-cell (SCP), and recombinant proteins are being explored as alternatives to dairy proteins. Plant-Based Proteins (PBPs) are commonly used to replace total dairy protein. However, PBPs are generally mixed with dairy proteins to improve their functional properties, which makes them dependent on animal protein sources. In contrast, single-Cell Proteins (SCPs) and recombinant dairy proteins are promising alternatives for dairy protein replacement since they provide nutritional components, essential amino acids, and high protein yield and can use industrial and agricultural waste as carbon sources. Although alternative protein sources offer numerous advantages over conventional dairy proteins, several technical and sensory challenges must be addressed to fully incorporate them into cheese and yogurt products. Future research can focus on improving the functional and sensory properties of alternative protein sources and developing new processing technologies to optimize their use in dairy products. This review highlights the current status of alternative dairy proteins in cheese and yogurt, their functional properties, and the challenges of their use in these products.

1. Introduction

Proteins are among the main macronutrients necessary for the human body's function [1,2]. They can interact with other food components, contributing to the textural characteristics of a product through their functional properties. Animal proteins are the most consumed proteins globally, and their demand has been attributed to population growth. The human population is expected to increase to almost 10 billion by 2050 [3], implying that 50% to 60% more food will be needed for human consumption that year [4]. This situation would be environmentally detrimental, and finding alternative protein sources with nutritional and functional characteristics close to animal proteins and fewer harmful environmental effects is critical. Several studies have made substantial efforts to replace food animal proteins with alternative protein sources [5]. However, the main challenge is obtaining new products free of animal proteins without compromising their sensory and nutritional properties since all alternative proteins are structurally and functionally different from animal ones.

The global population consumes many dairy products because of their nutritional and high-protein content. Milk is the main base of

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these products and is principally made of caseins. Casein has no defined structure, but its complicated posttranslational modifications enable its self-assembly into casein micelles stabilized by phosphate clusters, and their behavior in food is very versatile [6]. The first approaches to mimic milk and dairy protein replacement have been achieved using PBPs [7,8]. PBPs, such as soy, oats, peas, coconuts, almonds, and rice, contain globular proteins that, when denatured, gelatinized and form a stable protein network similar to yogurt and cheese from cow's milk [9,10]. These proteins have functional and nutraceutical components such as dietary fibers, minerals, vitamins, and antioxidants [11]. However, research has shown that PBPs are not as nutritious as cow's milk and have an unpleasant flavor associated with the action of lipoxygenase (e.g., soy and kidney bean) [12–14]. Dairy analogs made from PBPs often have poor mechanical properties, requiring coagulating salts, thickeners, and emulsifiers in addition to casein and milk fat to obtain the desired texture and flavor [15–17].

Although the demand for PBPs is increasing, their inherent drawbacks do not allow them to meet the world's animal protein demand. PBPs production has also shown adverse environmental effects. Their high demand reduces soil fertility levels, contamination of water resources, and deforestation [15,18,19]. Furthermore, their conversion into animal protein is inefficient [15]. For example, to produce 1 kg of milk for human consumption, 7 kg of vegetal protein are needed [20]. In addition, to reach the same protein value as 1 L of cow's milk, it is necessary to consume 13% more soy milk [14].

Another alternative protein for animal protein replacement are SCPs. These proteins are biomass from microbial species such as algae, bacteria, fungi, and yeasts, which can be extracted and purified for human consumption [18,21]. These microorganisms can utilize industrial and agricultural waste as a carbon source (although small quantities of glucose and ammonia are still needed), are fast-growing, do not require arable land, and their production is not season or weather-dependent [21,22]. SCPs are high in protein and essential amino acids like lysine and methionine, which are deficient in most plant and animal foods [23]. However, their consumption in human foods can increase uric acid in the blood due to the high nucleic acid levels [22].

Recombinant dairy proteins are other alternative dairy proteins of great interest. These proteins are produced through recombinant DNA technology, which involves inserting the dairy protein gene into a microorganism and thus achieving protein synthesis [13]. Recombinant DNA has also been used to produce nutraceuticals and therapeutic enzymes and even improve fermentation and production processes [24]. β-lactoglobulin production as a recombinant protein is an exciting research field in the dairy industry [25,26]. This protein is essential in many foods because it has excellent functional properties, such as emulsification, and foaming capacity, significantly contributing to the product's physical and sensory characteristics [25,27]. β-lactoglobulin is a highly heat-stable protein, meaning it can withstand high temperatures during processing without denaturing or losing its functional properties [27]. Moreover, β-lactoglobulin can form stable complexes with bioactive compounds, which explains the growing interest in its application in the medicine and the pharmaceutical industry [28]. Other studies aim at obtaining bovine caseins as recombinant proteins. Nevertheless, there are few scientific publications about producing this type of recombinant milk protein [29].

The functional properties of proteins play a crucial role in their incorporation into food products. A thorough understanding of a protein's functional properties can help predict its behavior in a food matrix and the type of food that can be produced [1,20]. Although alternative proteins mentioned have been demonstrated to have excellent functionalities for application in dairy products, studies indicate that mixing them with animal dairy protein improves their functional properties and enhance their application in food systems [30–32].

Here we provide an overview of alternative protein sources for dairy products, providing concise information on replacing dairy protein with PBPs, SCPs, and recombinant protein in products such as yogurt and cheese.

2. Dairy proteins

Dairy proteins are essential in dairy products' rheological and textural properties. Depending on their physicochemical

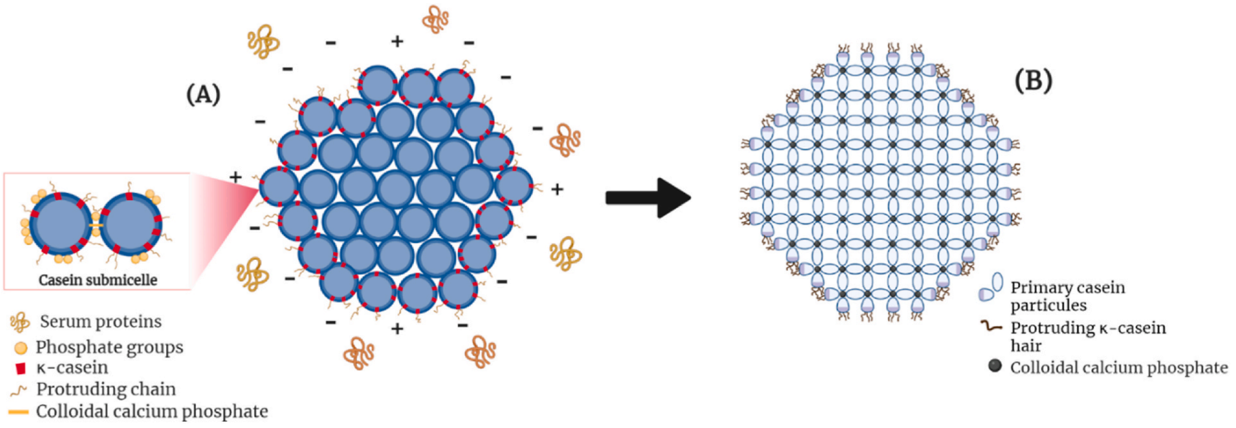

Fig. 1. Illustration of casein micelles and their aggregation. A) Initial model of the casein micelle adapted from Walstra [37]. B) Recent model of the casein micelle adapted from Huppertz et al. [6] that, unlike Walstra's model, is composed of non-spherical primary casein particles cross-linked by calcium phosphate nanoclusters.

characteristics, these proteins can form gels and generate a continuous network of protein, fat, and water aggregates that make up products such as yogurt and cheese. Bovine milk contains 80% caseins and 20% whey proteins [6,31].

Caseins are composed of four main groups: α s1-casein, α s2-casein, β-casein and κ-casein. The molecular weights of these casein fractions range from 19 to 25 kDa, with hydrophobic and hydrophilic motifs [31]. They are bound together in a colloidal association as micelles (See Fig. 1A and B) due to the ability of the phosphorylated serine residues to bind calcium and the relatively amphiphilic character of the partially glycosylated κ-casein. The κ-casein is mainly a stabilizing layer on the micelle surface [33,34]. Several models illustrate the micellar structure of cow's milk casein. However, the exact structure is still debatable [35,36]. The initial casein micelle model shows spherical casein submicelles with a calcium phosphate cross-linked hairy layer where the molecular chains of the C-terminal end of the κ-casein protrude from the micelle surface, forming a hairy layer that prevents further aggregation of the submicelles by steric and electrostatic repulsion [37] (See Fig. 1A). However, the recent Huppertz model [6] shows that non-spherical primary casein particles cross-linked by calcium phosphate nanoclusters (See Fig. 1B).

Dairy products such as yogurt and cheese are derived from the gelation resulting from the destabilization of caseins due to their isoelectric point adjustment and enzymatic cleavage. In yogurt, the addition of lactic acid bacteria to ferment lactose to lactic acid reduce the pH towards the isoelectric point (4.6), causing the hydrolysis of the milk proteins and the formation of soluble aggregates that results in forming a continuous network of caseins [15,33]. During fermentation, viscosity increases, and bacterial metabolites are produced, contributing to yogurt's flavor and health properties. In cheese, κ-casein is cleaved by rennet (chymosin) at the Phe105-Met106 linkage to form para-κ-casein, which reduces the net negative charge and steric repulsion, generating aggregation between casein micelles [15,34,38]. In both dairy products, caseins are present in a network in which fat globules, water, minerals, and dissolved solutes are all interspersed [39].

The structural versatility of caseins also allows them, under certain conditions, to interact with various compounds in food products. These interactions occur primarily through hydrogen bonding, electrostatic interactions, or hydrophobic associations [30,40]. Therefore, simulating the molecular properties of caseins has become a significant challenge, especially in the development of analog dairy products and consumer-acceptable in terms of taste and nutritional profile.

Whey proteins are derived from whey, a co-product that remains after the cheese-making process. Whey proteins are highly water-soluble, with unique functionalities and nutritional properties. These proteins consist mainly of β-lactoglobulin (1.3 g/L), α-lactalbumin (1.2 g/L), glycomacropeptide (1.2 g/L), immunoglobulins (0.7 g/L), bovine serum albumin (0.4 g/L) lactoferrin (0.1 g/L), and lactoperoxidase (0.03 g/L). These proteins are mainly obtained by precipitation, chromatography, and membrane separation processes [41,42].

In the dairy industry, whey proteins can be used as an ingredient in yogurt and cheese-making due to their functional properties [41]. For example, adding these proteins to yogurt can improve the consistency and viscosity of the product and help prevent the separation of free whey. In the dairy industry, whey proteins are used as functional foods with high nutritional value and as a substitute for various ingredients to enhance the sensory qualities of food [43].

Due to their high molecular complexity and functionality, mimicking dairy proteins has become challenging. Recently, there have been approaches to total dairy protein replacement in products such as yogurt and cheese. However, the effects of incorporating alternative dairy protein on the human health and functionality of foods have been questioned.

3. Dairy alternative proteins

Alternative proteins are novel and technological-functional sources that imitate and replace animal proteins. The demand for dairy alternative protein has been increasing in the dairy industry. High population growth, environmental problems, allergies, and lactose intolerance have led to the development of dairy analogous or animal-free dairy products. Given that alternative dairy proteins can be of plant, microbial or recombinant origin, it is essential to consider their nutritional value and digestibility. Protein Digestibility Corrected Amino Acid Score (PDCAAS) allows the comparison of protein quality concerning amino acid amount required for human consumption. These alternative protein sources have been extensively studied, so it is possible to briefly describe their nutritional characteristics, advantages, and disadvantages as alternative food ingredients (See Table 1). Each of these alternative proteins will be described below. The SCP and recombinant proteins have been the most novel alternative proteins and are discussed in more detail.

3.1. Plant-based proteins (PBPs)

Plant-based ingredients have imitated many physicochemical and sensory characteristics associated with animal-origin foods, such as milk and milk derivatives. PBPs used for making dairy products are commonly derived from legumes (e.g., soy and pea), nuts (e.g., coconut and almond), and cereals (e.g., oats and rice) [11,15,44].

Soy protein is the most studied PBP for replacing animal protein due to its high nutritional and functional value. It represents 3.5% of soymilk, which has 2% fat, 2.9% carbohydrates, and 0.5% ash. This chemical composition depends on the processing conditions and bean variety [45]. Soy protein in soymilk is emulsified with soy milk lipids, which are composed of polyunsaturated fatty acids such as linoleic acid (54%), oleic acid (22%), and linolenic acid (7.5%) [46]. These proteins are potentially anticarcinogenic and contain beneficial molecules such as isoflavones and saponins that reduce cholesterol and cardiovascular diseases [11,47]. Besides, humans digest soy protein as fast as milk's, with a PDCAAS between 90–99% (See Table 1).

Soybean proteins are mainly composed of two globulins: β-conglycinin (7S) and glycinin (11S). The β-conglycinin is a glycoprotein with a molecular mass of 150–200 kDa, while glycinin is a hexamer with a molecular mass of 300–380 kDa. Both proteins have a higher molecular weight than milk proteins and account for more than 80% of soybean protein content [31,48]. It is worth noting that the composition and structure of these proteins determines the functional properties of soy-based products. Furthermore, these proteins have complex quaternary structures, and unlike caseins, they are not phosphoproteins [45].

Pea milk is a good source of proteins, fats, fibers, vitamins, minerals, antioxidants, and phytosterols. This milk has also been evaluated as an analog dairy beverage. However, the presence of beany flavor has limited its applications [11].

Coconut milk is an oil-in-water emulsion where proteins (globulin and albumin) and phospholipids stabilize the emulsion by adhering to the surface of the coconut oil droplets as emulsifiers, preventing phase separation [49]. It is rich in vitamins and minerals such as iron, calcium, potassium, magnesium, and zinc.

Almond, oat, and rice milk contain six times fewer proteins than cow milk [16]. Almond milk is rich in fats (44–61%), low in carbohydrates (2–8%), and possesses hexameric globulins (16–26%) of molecular weight around 275–450 kDa [50]. Almond milk is used as an alternative milk beverage for people with cow's milk allergy and lactose intolerance [44]. Oat milk is a water-soluble extract of oatmeal, which can be included in lactose-free, gluten-free, and cow's milk protein-free diets [51,52]. The globulins present in oats are insoluble at a pH between 4.0 and 7.0, limiting their use in many food products [52]. Rice is one of the most consumed cereals worldwide. Rice milk is hypoallergenic and lactose-free, like almond milk and oat milk [44], and its protein is made up of glutelin, globulin, albumin, and prolamin [31]. There is ongoing research into other protein sources, for example, protein extracts from leaves (i.e., Rubisco) [53] or aquatic plants (i.e., duckweed) [54].

Plant-based milk can be produced by directly extracting the soluble material, either by soaking the plant material with water and then grinding or by dry grinding the plant material and extracting the protein in water by methods such as pH shifting, filtering, and removing the slurry of insoluble material [9] (See Fig. 2). The plant-based milk obtained can be enriched with other ingredients such as oil, flavorings, sugar, and stabilizers, depending on the desired product. Then, a heating stage must be carried out to improve the suspension and microbial stability and increase the nutritional value of the plant-based milk. For example, heating soy milk can deactivate harmful physiological substances, induce denaturation of soy protein, soften soy tissue, and eliminate the odor of raw soy [48]. Aydar et al. [17] indicate other methods for producing milk from legumes, cereals, and nuts in more detail.

PBPs can aggregate at low pH, forming gels by heating, denaturation, or enzymatic action with transglutaminase, and have hydrophobic interactions [48]. The gel-forming ability is particularly critical because a viscoelastic gel plays a major role in adhering particles, immobilizing fat, and entrapping water within the protein matrix of food [55]. Making cheese or yogurt from PBPs is not very different from that made from milk proteins. Yogurt is produced by inoculating two starter cultures of lactic acid bacteria, such as *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* to plant-based milk (See Fig. 2). Lactic acid bacteria's growth and acid production in plant-based milk is limited due to the low sugar content. Therefore, fortification with additional sugars like glucose, fructose, or lactose is necessary. Fermentation is generally carried out at 41 °C for 12 h until pH 4.2 is reached [56,57].

Among the cheeses from plant-based milk is tofu, the most common traditional cheese type from soy milk. This cheese is obtained by coagulating proteins with an acid or an alkali salt (e.g., citric acid and calcium sulfate) at low pH, which causes aggregation and gelation of proteins due to a decrease in electrostatic repulsion. This process forms a gel network sustained by hydrophobic interactions and hydrogen bonds [45].

Plant-based dairy alternatives have gained tremendous popularity among consumers due to their sustainable production compared to bovine milk [49]. However, their use during fermentation and coagulation of dairy products can weaken the food structure and cause aqueous phase separation during storage [58]. To avoid this, it is necessary to incorporate gelling agents, thickeners, or stabilizers for fabricating yogurt and cheese-like products [15,16,49]. Because of this, several researchers have focused on developing products based on milk proteins supplemented or partially substituted by PBPs [30]. Another reason that limits the use of these proteins as a food ingredient is their low content of unsaturated amino acids, such as lysine, methionine, and cysteine [45,59].

3.2. Single-cell protein (SCP)

Single-cell protein (SCP), also known as microbial protein, bioprotein, or biomass, is edible unicellular microorganisms (algae, bacteria, yeast, or fungi) that can be used for animal and human consumption [21,23]. SCP is a bulk of dead and dry-cell microorganisms (i.e., algae, bacteria, and fungal) grown on various carbon sources, primarily agricultural and industrial co-products [21,23,60].

The nutritional value of the SCP is specified by the content of crude protein, lipids, nucleic acids, and other nonprotein nitrogen compounds, vitamins, carotenes, xanthophylls, and minerals, as well as by essential amino acids profile and occurrence of essential amino acids such as lysine and methionine [23]. SCP content depends on the microorganism species, type of substrate, cell growth stage, nutrient sources, and environmental growth conditions [18,21]. These proteins have been implemented within the dairy industry as sources of amino acids or probiotics to improve the functional properties of products such as yogurt and cheese, making biomass part of the feed as a partial substitute for protein [61].

Using SCPs as an alternative protein source has many advantages, such as the short generation time and easy conversion through biotechnological methods, and they can also benefit from many substrates. In addition, these proteins have a lower land use impact, production does not depend on the seasons and climatic conditions, and therefore continuous production is possible anywhere in the world [62]. However, using microbial-based proteins in food for human consumption requires the release of cellular proteins by destroying the indigestible cell walls and reducing the content of nucleic acids. Although, protein extracts from SCP are less explored and are a topic of current research interest.

3.2.1. Algae SCP

Algae are eukaryotic organisms that can be observed in different morphological forms, unicellular and filamentous, to a few meter

Table 1
Physicochemical characteristics, advantages, and limitations of some alternative proteins.

Alternative proteins		Nutritional characteristics	Advantages	Limitations	References
Plant-based protein	Soy	• Protein: 40% • Crude fat: 20%. • Carbohydrate: 33–35%. • PDCAAS: 90–99%.	• Effect against chronic diseases and cancer. • Good emulsifying and water and oil absorption capacity. • High lysine and isoflavone concentration.	• Off-flavors due to lipoxygenases content. • Low in methionine and cysteine. • High allergenicity.	[11,19,47]
	Pea	• Protein: 23–30%. • Crude fat: 1.5–2%. • Carbohydrate: 35–40%. • Fiber (%): 60–65%. • PDCAAS: 73–89%.	• Gluten-free. • Low allergenicity. • High levels of lysine and a good source of antioxidants.	• Low levels of methionine and cysteine.	[138]
	Coconut	• Protein: 3.6–5.5%. • Crude fat: 20–44%. • Carbohydrate: 6–15%. • Fiber (%): 2–9%. • Total EAA: 71–77%. • PDCAAS: 86–94%.	• High content of monounsaturated fatty acids.	• Increase the HDL (high-density lipoprotein) levels. • High saturated fats. • Low in methionine, isoleucine, threonine, and tryptophan.	[44,139]
	Almond	• Protein: 21%. • Crude fat: 49%. • Carbohydrate: 21%. • Fiber: 12.5%. • PDCAAS: 32–34%.	• Source of alpha-tocopherol and manganese. • Low in calories.	• High allergenicity limits its use as a beverage. • Low protein solubility at acidic pH.	[11,44]
	Oat	• Protein: 9–20% • Crude fat: 5–9%. • Fiber: 2–8%. • PDCAAS: 41–60%.	• Gluten-free. • Therapeutic benefits. • High fiber content. • Hypocholesterolemia properties. • Source of antioxidants and polyphenols.	• Use limited in liquid or semi-solid products because of their high starch content. • Limited lysine content. • Low foaming capacity due to high lipid content. • Low protein solubility at acidic pH.	[11,52,126]
	Rice	• Protein: 7–76%. • Crude fat: 0.2–20%. • Carbohydrate: 18–76%. • Fiber (%): 0.06–13%. • Total EAA: 41%. • PDCAAS: 90%.	• High levels of leucine, phenylalanine, and methionine.	• Use limited in liquid or semi-solid products because of their high starch content. • Low protein content. • Limited lysine content. • Emulsion stability is very low due to the high starch content. • Low protein solubility at acidic pH.	[11,140,141]
Single-Cell Protein	Algae	• Protein: 51–93%. • Crude fat: 4–22%. • Carbohydrate: 1–17%. • Nucleic acid: 3–8%.	• Antioxidant and anti-inflammatory activity. • Sources of vitamins and minerals. • High levels of leucine, lysine, and valine). • Low allergenicity.	• Unpleasant flavor (fishy flavor) and odor. • Non-homogeneous appearance. • High nucleic acid content. • Intense color. • Low efficiency and high production costs. • High cellulose content.	[5,21,70,84,142]
	Bacteria	• Protein: 50–83.5%.	• High growth rate.	• High nucleic acid.	[21,84,86]

(*continued on next page*)

Table 1 (*continued*)

Alternative proteins	Nutritional characteristics	Advantages	Limitations	References
	• Crude fat: 1–10%. • Carbohydrate: 6.83%. • Nucleic acid: 8–14%.	• Highest methionine content.	• Small cell sizes can potentially hinder purification steps. • Endotoxins content. • High risk of contamination.	
Fungal (filamentous fungi and yeast)	• Protein: 30–60%. • Crude fat: 2–3%. • Carbohydrate: 38–54%. • Fiber (%): 3–4%. • PDCAAS (%): 94–95%. • Nucleic acid: 3–12%.	• High protein content. • More resistant to shear stress. • Rapid growth. • Insulinotropic protein. • High B vitamin content. • High lysine content.	• Mycotoxins content • Cytotoxicity • Low methionine content.	[21,92,143]
Recombinant protein	Unknown	• Therapeutic properties. • Effective bioprocess optimization.	• Posttranslational modifications. • Co-secretion of proteases. • Potential functionality changes. • Low yield. • Mutations.	[13,25,144]

*%Protein: g/100g of dry basis.

plants. They usually live in aquatic and semi-aquatic habitats, have a cellulose cell wall, are autotrophs with a simple structure, and are capable of photosynthesis. The most significant interest in algae is their potential use as a protein source based on their amino acid composition. The quality of algal proteins is high and comparable to conventional vegetable proteins. However, algae's protein content varies according to the season and the species [63].

Microalgae are a highly nutritious food source for both humans and animals due to their high protein content, which is around 70% [21,23,64]. In addition to protein, microalgae are rich in essential nutrients such as omega-3 fatty acids, vitamins A, B, C, and E, as well as mineral salts [21,64,65]. Their high concentration of fatty acids are a healthy replacement for those derived from other oils like rapeseed, soy, sunflower, and palm oil rapeseed, soy, sunflower, and palm oil [66].

Microalgae are used in several industries (food, cosmetics, and pharmaceuticals, among others) due to their concentration and quality of polysaccharides, lipids, proteins, and pigments [67,68]. Microalgae have been found to positively affect the functional properties (emulsifying capacity, and water-holding capacity, among others) of food products, as they can improve the food structure and texture after processing [5,68]. However, incorporating microalgae into food products can also impact their sensory properties, such as color and flavor, affecting consumer acceptance [5,68].

The green color of some microalgae may make milk and dairy products visually appealing to some consumers, while their distinctive flavor may be less desirable to others [5,69]. Moreover, algae imply digestion issues since their cell wall (10% cellulose) is not digestible by humans [64]. A route to avoid discoloration and reduced digestibility is the extraction of the microalgae proteins (e.g., Rubisco), which adds additional processing steps and still needs to be explored further regarding functionality [53]. On the other hand, some studies have incorporated microalgae into dairy products, mainly as probiotics.

Spirulina and *Chlorella* are the most widely used algae in fortified foods, and they are identified as GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration) [70]. These have been used as a protein source in dairy products to improve these physicochemical, sensory, and nutritional characteristics [71,72]. For example, da Silva et al. [73] developed a yogurt containing 1.5 g of encapsulated *Spirulina platensis*, presenting a homogeneous appearance with attractive color, better antioxidant activity, and an excellent nutritional profile concerning protein and fat content and energy value. Similar results were obtained by Barkallah et al. [74], who observed that yogurt a containing about 0.25 g of *S. platensis* showed good texture properties and sensory acceptability. Moreover, Patel et al. [75] developed a probiotic yogurt enriched with *Spirulina* fresh biomass carotenoids, in which the incorporation of 7% microbial protein resulted in a highly acceptable product. It should be noted that incorporating *S. platensis* in yogurt also accelerates the fermentation process, promoting the growth of probiotic bacteria [61,75]. *S. platensis* has also been effectively incorporated into cheeses. Golmakani et al. [76] demonstrated that adding 0.5% or 1.0% of *S. platensis* improved probiotic properties in feta-type cheese without negatively impacting its sensory properties.

In turn, *Chlorella* has been incorporated into different types of cheeses. Jeon [77] researched the addition of *Chlorella* (0.5–1%) to the physical and sensory properties of processed cheese, while Heo et al. [78] studied the addition of *Chlorella* powder (0 to 2.0%) to the lactic acid bacteria growth, ripening speed, and sensory properties of Appenzeller cheese. Although both studies suggested that the appropriate amount of *Chlorella* to be added for obtaining acceptable quality cheeses was only 0.5%, Tohamy et al. [79] demonstrated that adding 2% and 4% *Chlorella* to a spreadable processed cheese results in a consumer-acceptable cheese analog. Table 2 shows some outcomes from several studies, including those mentioned above.

Seaweeds are another protein source added to milk, cheese, yogurt, and ice cream to boost their protein content and nutritional profile [80]. O'Sullivan et al. [81] explored the potential of seaweed extracts from *Ascophyllum nodosum* and *Fucus vesiculosus* as functional ingredients in yogurt. The results showed that adding 0.25% and 0.5% of Ascophyllum nodosum improved the antioxidant activity of the yogurt without negatively affecting its sensory properties. Similar results were obtained by del Olmo et al. [82], who studied the incorporation of five types of seaweed (*Himanthalia elongata*, *Laminaria ochroleuca*, *Porphyra umbilicalis*, *Ulva lactuca*, and

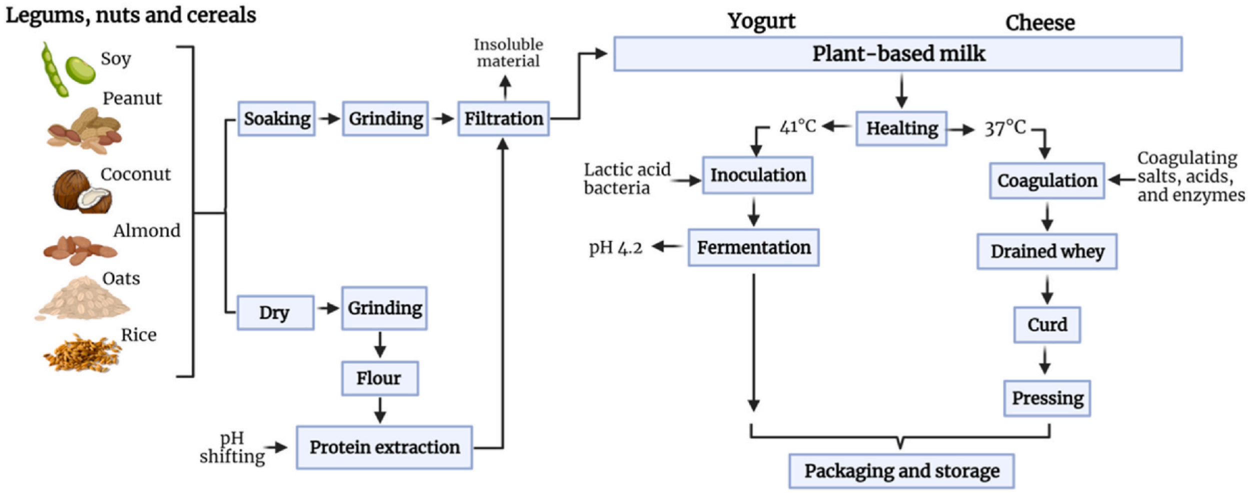

Fig. 2. Flow chart for obtaining plant-based milk and making yogurt and cheese.

Undaria pinnatifida) in the Iberian cheese. These authors demonstrated that adding 1% of dehydrated seaweed positively impacted cheese's antioxidant activity, texture, flavor, and odor. Overall, the use of seaweed in dairy products has also gained popularity in recent years due to its potential health benefits and functional properties.

3.2.2. Bacterial SCP

Bacteria have the highest contribution in making dairy products compared to algae and fungi. Lactic acid bacteria such as *Lactobacillus, Bifidobacteria*, and *Streptococcus* are the probiotic bacteria most commonly associated with yogurts and cheeses [83]. Bacterial SCP contains about ~80% protein on a dry weight basis and a nucleic acid content of about 8–12% [21,23,84].

Although the use of bacteria for feed or food has been limited due to the high nucleic acid content and high cost associated with the proteins' harvesting processes [64,85], companies such as Uniprotein, FeedKind, and Pruteen produce SCP bacterial from methanotrophic bacteria (*Methylococcus capsulatus* and *Methylophilus methylotrophus*) through methane fermentation to animal feed [21]. Another prominent company is Superbrewed Food, which produces a bacterial SCP with total safety for human consumption [86]. Superbrewed Food produces bacterial protein powder from *Clostridium* through precision anaerobic fermentation. This bacterial SCP is used as a postbiotic ingredient for dairy products.

3.2.3. Fungal SCP

Yeasts and filamentous fungi probably have the most favorable characteristics for use as a significant food source. These have a crude protein content of approximately 50% dry weight [21,23,64]. SCP production for human consumption from yeast has been preferred over SCP production from bacteria due to their use in bread and beer production [87]. The FDA (Food and Drug Administration) permits the usage of dried yeast from *Saccharomyces cerevisiae, Pichia pastoris, Kluyveromyces lactis*, and *Candida utilis* in human food [88], provided that the total folic acid content of the yeast does not exceed 0.4 mg/g of yeast [89,90].

It has already been demonstrated that *S. cerevisiae* is a promising source to obtain SCP. Its biomass can be used as a protein source in several food products and supplements [91–93]. *S. cerevisiae* is Crabtree-positive, which allows aerobic ethanol production when glucose is in excess. P. pastoris is an ascomycetous yeast that has been extensively researched for the fermentation of xylose that produces ethanol, L-lactic acid, and other compounds [94]. In addition, its ability to utilize methanol as a carbon source has been essential in SCP production. *P. pastoris* is the primary system for producing many secreted and intracellular recombinant proteins on an industrial scale, and the yields obtained are high compared to other eukaryotic systems. They secrete recombinant proteins into the culture medium, avoiding the toxicity caused by accumulated intracellular material and simplifying protein purification [95].

In contrast to other species, *K. lactis* metabolizes xylitol, cellobiose, lactose, and pentose sugars containing xylose and arabinose [90]. *K. lactis* can grow on whey, an inexpensive dairy co-product [96]. Therefore, many studies have focused on using this residue as a carbon source to obtain SCP from *K. lactis* [97–99]. *C. utilis* is an excellent platform for SCP production for animal and human food [88,100,101]. It has a high content of bioactive compounds allowing it to be part of many formulations of fermented milk beverages to improve functional and structural properties in these products [102]. In addition, *C. utilis* can produce SCP from different substrates, such as fruit wastes, potato wastewater, and molasses [103,104].

In turn, filamentous fungi are extensively used in SCP production. Many strains, such as *Aspergillus*, *Fusarium*, *Chaetomium*, *Rhizopus*, and *Trichoderma*, are used commercially. These SCP are known as mycoproteins and can substitute meat. For example, Quorn, obtained from *Fusarium venenatum*, is a food commercialized in the market as a product for human consumption from filamentous fungi's biomass [21].

3.3. Recombinant protein

Recombinant DNA, also called gene cloning or molecular cloning, allows gene expression in a species, cell line, or heterologous host that does not have that gene naturally and is different from the original organism. The host organism can be bacteria, yeast,

Table 2
Summary outcomes algae incorporation in yogurt and cheese.

Dairy product	Alternative protein	Addition	Incorporation process	Outcomes	References
Probiotic yogurt	*Spirulina*	0.25% w/w	Powder	Highest antioxidant activity, texture attributes like control yogurt, and sensory acceptability.	[74]
		1% w/w	Microencapsulation	Bioactivity higher and increased homogeneity	[73]
		7% w/w	Powder	Product with high carotenoid content and high acceptance.	[61]
	Chlorella	0.2% w/w	Powder	Lowest viscosity, good taste, and better acceptability.	[145]
Feta-type cheese	*Spirulina*	0.5–1.0% w/w	Powder	Probiotic activity maintenance, texture attributes like control cheese, and sensory acceptability.	[76]
Processed cheese	*Chlorella*	0.5% w/w	Powder	Physicochemical composition like the control cheese and better-quality characteristics.	[77]
Appenzeller cheese		0.5% w/w	Powder	Highest sensory acceptability.	[78]
Processed cheese		2% w/w	Powder	Highest firmness, lowest meltability, and highest acceptability.	[146]
Spreadable processed cheese		2 or 4% w/w	Powder	Highest minerals content and antioxidant activity	[79]

mammalian, and plant cells. Recombinant DNA is helpful in producing a wide range of heterologous or recombinant proteins, enzymes, hormones, antibiotics, and functional foods [105].

The most commonly used hosts in recombinant proteins are bacteria and yeast. The maximum amounts of recombinant proteins produced by bacteria and yeast are 14 g/L and 30 g/L, respectively [105]. *E. coli* is the first bacteria choice for the production of recombinant proteins due to its rapid growth, easy manipulation, and cost-effectiveness, in addition to the vast knowledge that currently exists about its genetics, biochemistry, and molecular biology [106–108]. Several dairy proteins, such as α-lactalbumin and β-lactoglobulin κ-casein, α_{s1}-casein, and β-casein, have been synthesized by *E. coli* [13].

Yeasts have characteristics that make them suitable to host organisms to produce heterologous proteins. They enable genetic manipulation and post-translational modifications, have rapid growth, high cell densities, and need an inexpensive medium to grow [95,107,109]. *S. cerevisiae* was the initial yeast system used for heterologous protein secretion [90,108,109]. However, this yeast requires highly sophisticated equipment for its fermentation needs, which makes it an impractical host for the large-scale production of foreign proteins.

In recombinant protein production, other beneficial host yeasts are *P. pastoris*, *K. lactis*, and *C. utilis*. *P. pastoris* is Crabtree-negative, which means that it does not lose carbon in ethanol production under anaerobic conditions. Therefore, it yields more biomass and recombinant protein [95,108]. In the dairy industry, whey proteins such as α-lactalbumin and β-lactoglobulin have been obtained by recombinant proteins from *P. pastoris*. The patent US9924728B2 describes the production of these recombinant proteins and their application in food products [29]. *K. lactis* has been studied in the production of heterologous proteins. This yeast can express and secrete different high molecular weight peptides and proteins, such as β-galactosidase and chymosin, which have been produced on a large scale. Ooyen et al. [110] and Spohner et al. [26] reviewed all currently available genetic techniques and molecular tools to use *K. lactis* as a host for protein expression. Currently, most companies and startups use yeast as a host for recombinant dairy protein production (See Table 3).

Recombinant protein from fungi has also been used in the dairy industry. For example, Perfect Day, Inc. uses *Trichoderma reesei* to produce β-lactoglobulin through precision fermentation. They obtained a homogenous white powder that can be used in foods [111], although it is not used alone. Formo and New Culture combine recombinant dairy protein with plant-based fats, carbohydrates, salts, vitamins, calcium, and minerals to produce cheese (See Table 3).

However, the success of recombinant protein synthesis is determined by post-translational modifications (PTMs). PTMs are chemical modifications in polypeptide chain after protein biosynthesis (e.g., phosphorylation, acetylation, ubiquitylation, methylation, and glycosylation, among others), which occur during translation and are necessary for protein folding and significantly affect their functional properties. PTMs in bacteria, for example, are relatively low, causing their recombinant proteins to misfold, unfold, or degrade rapidly [112,113]. On the other hand, PTMs from yeast containing non-human N-glycans of a heterogeneous high-mannose type [107–109]. These hypermannosylations interact with the asialoglycoprotein receptor in blood cells resulting in the rapid clearance of recombinant proteins in the bloodstream [114]. This has become a challenge in terms of the functionality of therapeutic recombinant proteins.

N-glycosylations in animal or yeast proteins can lead to allergenic glycan structures. For example, the digestion of caseins produces peptide residues that provide epitopes that, in some mammals, produce allergenic effects upon binding to immunoglobulin E [115]. However, although there are products based on recombinant dairy proteins from yeast (see Table 3), the digestion and absorption of

Table 3

Examples of recombinant dairy protein companies and startups.

Company/Startup	Microorganism	Recombinant protein	Product	Website
All G Foods		Casein and whey protein	Whey protein, milk, cheese, yogurt, infant formula, and nutraceuticals.	https://allgfoods.com/
Better Dairy	Yeast	Dairy proteins		https://betterdairy.co.uk/
Bon Vivant			Milk and cheese	https://bonvivant-food.com/en/
Change Foods	Yeast	Dairy proteins	Cheese	https://www.changefoods.com/
De Novo Dairy		Milk proteins	Milk	https://denovodairy.com/
Eden Brew	Yeast	Milk proteins	Milk, cheese, and Ice cream.	https://www.edenbrew.com.au/
Formo		Milk proteins	Cheese	https://formo.bio/
Fooditive Group	Yeast	Casein		https://www.fooditivegroup.com/
Imagindairy		Milk proteins	Milk and cheese	https://www.myhelaina.com/
Maya Milk		Dairy proteins	Milk	https://www.mayamilk.com/
New Culture		Dairy proteins	Milk and Cheese	https://www.newculture.com/
Nutropy		Milk protein	Cheese	https://nutropy.com/
Phyx44		Dairy proteins	Milk	https://www.phyx44.com/
Proprotein	Yeast	Casein	Cheese	https://proprotein.eu/
Real Deal Milk	Yeast	Casein and whey protein	Milk and dairy products	https://www.realdealmilk.com/
Remilk	Yeast	Casein	Dairy products	https://www.remilk.com/
Those Vegan Cowboys		Dairy proteins	Milk and cheese	https://thoseveganco wboys.com/

these proteins and their impact on the immune system are not well known.

4. Production of SCP and recombinant protein

A wide range of research has focused on studying optimal conditions to produce a greater quantity and better SCP and recombinant protein. SCP production is influenced both by the type of microorganism used and factors such as the composition of the culture medium and environmental conditions: carbon and nitrogen source, inoculum size and age, aeration, temperature, pH, and dissolved oxygen [18,41,60]. However, the degree of SCP production depends mainly on the substrate's fermentation (See Fig. 3A) [23,60]. Most substrates of interest are readily available food co-products such as whey, orange peels, sugar cane, rice husks, cassava waste, corn cobs, coconut waste, and mango waste [23,85,98]. These substrates must be non-toxic to humans, animals, and plants, abundant, regenerative, high nutritional value, and inexpensive [84]. In addition, their use in SCP production depends mainly on the type of microorganism used since each substrate has a different nutritional composition. Therefore, each microorganism reacts differently [23,60]. Previous research shows many substrates that can be used to produce SCP for different microorganisms [21,23,64].

Two types of fermentation processes can produce SCP: submerged fermentation and semi-solid-state fermentation [23,116]. Submerged fermentation is the most commonly used type of fermentation for SCP production. The substrate is always fermented in a liquid containing the nutrients necessary for the growth of the microorganisms. The substrate is kept in the fermenter, operating continuously, while the biomass produced is continuously harvested [116]. In the semi-solid fermentation process, the substrate is used in a solid state; therefore, the low moisture content limits the number of microorganisms that can carry out this fermentation. In general, the production of SCP by semi-solid fermentation implies a lower protein yield, and compared to submerged fermentation, this type of fermentation requires a high capital investment [23,116].

After fermentation, the harvested biomass in SCP and recombinant protein production must undergo further processing steps such as washing, cell disruption, protein extraction, and purification (See Fig. 3A) [64]. As a result, many advances have been made in SCP production processes to increase their productivity and value [117]. All SCPs obtained from algae, bacteria, fungi, and yeasts have a nucleic acid content in different proportions depending on the microorganism [21,23,64]. Therefore, the nucleic acid content must be reduced to below 2% w/w when SCP is produced for human consumption [21,98]. The SCP contains purine compounds that, when

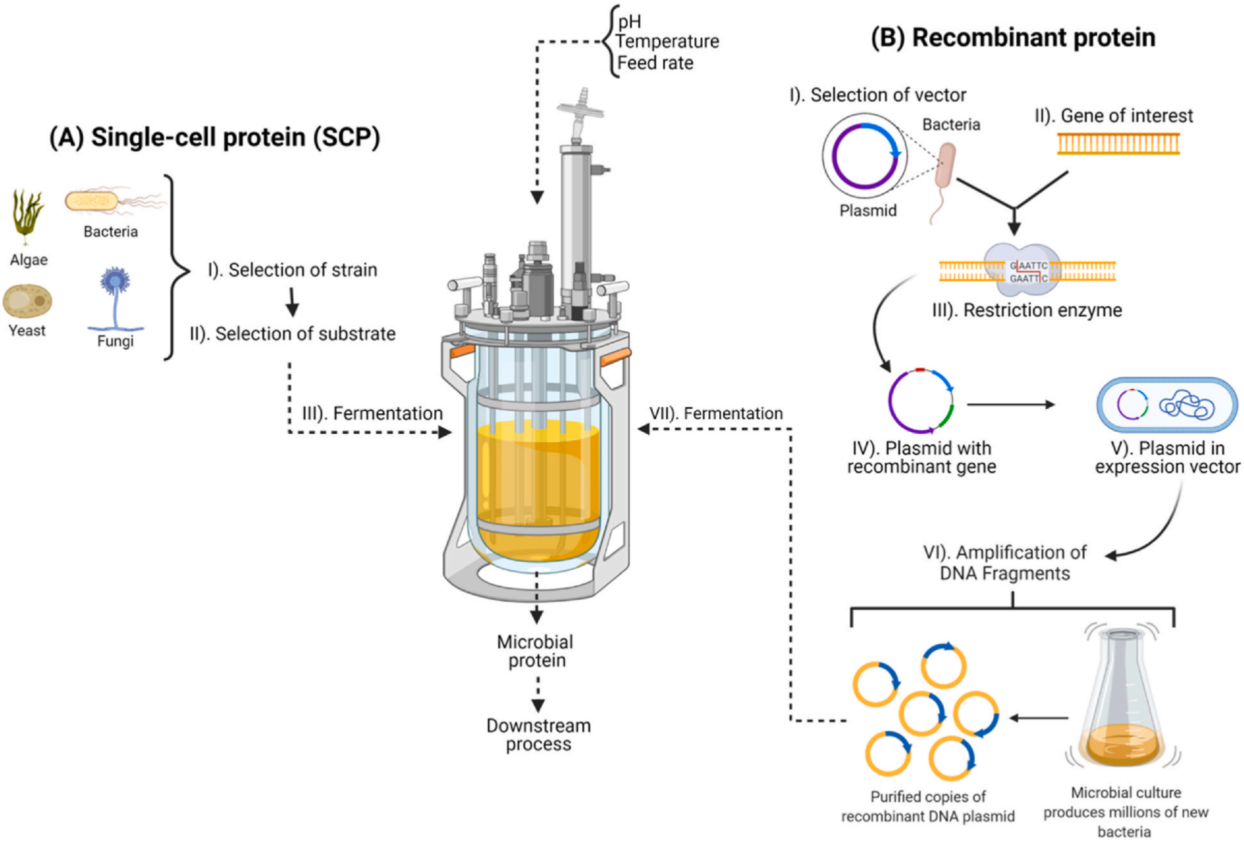

Fig. 3. Production of SCP and recombinant protein. (A) Production of SCP; strain and substrate are selected. (B)Production of recombinant protein; gene from a source organism is cleaved with a restriction enzyme and inserted into a cloning vector (plasmid). The cloning vector-inserted DNA construct is introduced into a host cell, and those cells that carry the construct are identified and grown. Both processes require adequate fermentation conditions and downstream processes.

metabolized in the human body, produce excess uric acid, which can lead to kidney stones or gout [21,23,64]. This nucleic acid reduction can be accomplished by thermal heating, alkaline hydrolysis, or chemical extraction [21]. In addition, all these microorganisms have a cell wall, which prevents access to their nutrients during digestibility. Therefore, for the use of these SCPs in food for human consumption, it may be advisable to break the cell wall using mechanical (ultrasound, high-pressure homogenization, bead milling or microfluidics), physical (freezing-press, osmotic shock, thermolysis), chemical (antibiotics, chelating and chaotropic agents, detergents, solvents, or hydroxides and hypochlorites), or enzymatic methods (autolysis) [118,119].

To obtain a recombinant protein, the starting point is selecting a vector and a gene of interest or recombinant DNA (See Fig. 3B). The gene is cloned into the multiple cloning site of the expression vector under a promoter that regulates gene expression. The gene interest is cut out with restriction enzymes, and the host plasmid is digested with the same restriction enzymes. The plasmid is transformed into an expression vector (e.g., a bacteria) capable of producing recombinant proteins. Recombinant protein production is induced at a specific growth stage by precision fermentation. Once the recombinant gene is expressed and the recombinant polypeptide chain is folded into the protein of interest, it is released from the cell to be captured and purified for downstream processing (See Fig. 3B) [120].

Currently, the use of genetically modified microbes to produce recombinant proteins can improve SCP production. Genetic engineering expands the variety of substrates and their efficiency when used by SCP-producing microorganisms, improving the nutritional properties of the biomass [21]. For example, efficient lactose conversion requires adequate expression and stability of foreign genes in the yeast host. *S. cerevisiae* must be genetically modified to metabolize lactose [121].

5. Functional properties of alternative proteins

Alternative proteins are proteins capable of replacing animal proteins in foods. These alternative proteins have resulted in foods and food components that in the food industry have improved the nutritional properties of foods from animals [31]. However, applying these proteins as an alternative ingredient depends on their functional characteristics and interactions with other components [1,20].

Functional properties determine the viability of incorporating any protein source into a food system [71]. Such properties of

Table 4
Summary of the functional property profile of the alternative proteins.

Source		Treatment	Functional properties profile summary	References
Plant-based protein	Soy protein isolated	–	Solubility: 14.9%, OAC: 1.8 g/g, WAC: 12.4 g/g, ES: 100%, FC: 171%, and FS: 67.7%. All properties were evaluated at pH 7.	[147]
	Soy protein isolated	–	Solubility at pH 12: >80%, OAC: 1–2 g/g, WAC:5–6 g/g, EA: 100–120 m^2/g, ES:15–18 min, and FS: 82.44%.	[148]
	Soy protein isolated	–	Solubility: 41.80% at pH 7.5, EA: 45 m^2/g, ES: 75–80 min, FC: 30–35%, and FS: 55–60%.	[149]
	Pea protein isolated	–	Solubility: 5%, OAC:1 g/g, WAC: 3.1 g/g, ES: 80.7%, FC: 81.1%, and FS:27.1%. All properties were evaluated at pH 7.	[147]
	Pea protein concentrates	–	Highest solubility: 60–70% at pH 12. OAC:1–2 g/g, WAC:3–3.5 g/g, EA:110–120 m^2/g, ES: 12–15 min, and FS: 89.74%.	[148]
	Coconut protein		Solubility: 10–15% at pH 7, EA: 49.94 m^2/g, ES: 65.28 min, FC: 3.68%, and FS: 100%.	[150]
	Coconut protein	Non-enzymatic deamidation	Highest solubility: 45%–50% at pH 7, EA: 67.43 m^2/g, ES: 24,49 min, FC: 7.32%, and FS: 100%.	[150]
	Almond protein isolated	Defatted	Solubility: 80%–90% at pH 3–4.	[151]
	Oat protein isolated	–	Solubility: 9%–21%, EA: 29–35. %, ES: 32–37%, FC: 75–125%, FS: 60–75%. All properties were evaluated at pH 7.	[152]
	Oat protein isolated	Defatted	Solubility: 17.5–27.5%, EA: 35–46%, ES: 35–45%, FC: 125–200%, and FS: 50–60%. All properties were evaluated at pH 7.	[152]
	Oat protein isolated	CO2-defatted	Functional properties values are highest at pH 10.5 and lowest at acidic and neutral pH. Solubility: 180–200%, FC: 80%, EA: 15.4 m^2/g and ES: 411.3 min.	[153]
	Rice	–	Solubility: 5%, EA: 50–75%, ES: 25–50%, and FS: 140%. These functional properties could increase with alkali and heat treatment and dextran addition.	[154]
	Rice protein concentrate	–	Solubility: 10%, OAC: 1–1.5 g/g, WAC: 1.46 g/g, and FS: 50%. EA and ES were low at pH 7.	[148]
Single-Cell protein	Chlorella vulgaris protein isolated	–	Solubility: 0–5% and ES: 77%. These properties were evaluated at pH 7.	[155]
	Chlorella vulgaris protein isolated	Ultrafiltration	Solubility: 30–40% and ES: 79%. These properties were evaluated at pH 7.	[155]
	Spirulina protein isolated	–	Solubility: 77.67% at pH 12. OAC: 1.85 mL/g, WAC: 2.14 mL/g, FC: 10.74 mL, FS: 19.26 min, EA: 56.32%, and ES:71.51%.	[125]
	Spirulina protein isolated	–	Solubility: 90.54% at pH 12. OAC: 0.59 mL/g, WAC: 2.96 mL/g, FC: 12.53 mL, FS: 32.40 min, EA: 51.54%, and ES: 65.20%.	[125]
	Saccharomyces cerevisiae	–	OAC: 1.72 mL/g, WAC: 2.10 mL/g, FC:10.49%, FS: 17.4%, EA: 52.36%, and ES:70.54%.	[92]
	Saccharomyces cerevisiae	Mechanical disruption	Solubility: 29.66%, OAC: 8.82 mL/g, WAC: 7.69 g/g, EA: around 50%, ES: close to 100%, and FS: 120%. All properties were evaluated at pH 7.2.	[143]

proteins include solubility, foaming, water absorption capacity, oil absorption capacity, and emulsification. Solubility measures the dissolvability of a solute in a solvent [122]. Protein solubility is influenced by factors associated with amino acid composition (molecular size, ratio of polar to apolar amino acids, among others), and in turn, this influences other functional properties of proteins [71,122]. According to Chen et al. [123], proteins with lower solubility exhibit lower foaming capacity (FC) and emulsion activity (EA) but higher water absorption capacity (WAC). FC is formed when a film or skin surrounds air cells [122]. Several studies have confirmed that FC proteins are fundamentally related to their film-forming properties at the air-water interface [124,125]. EA is defined as the volume of oil emulsified per gram of protein sample, while the emulsion stability (ES) is the time in which the emulsion separates in two phases (water-oil). WAC, or Water-holding capacity, measures the total amount of water absorbed per gram of a protein powder [122]. This property is critical in solid foods because low WAC results in a dry texture in the product [126]. Oil absorption capacity (OAC) measures the amount of oil absorbed per protein weight. The amount of oil absorbed depends on the content of nonpolar amino acids of the protein [122].

Functional properties can vary for the type of proteins, processing operations, extraction, composition, pH, and temperature, among others [20,122,123]. However, treatments such as enzymatic hydrolysis, acylation, succinylation, and supercritical carbon dioxide, can improve functional properties [52,126]. On the other hand, it is not easy to compare a protein's reported functional properties since isolation can denature the proteins. Furthermore, protein extracts are usually a mixture of multiple proteins, so their composition can change with extraction conditions, batch-to-batch variety, and growing conditions. In addition, residual nonprotein components such as lipids, carbohydrates, phenolic compounds, and salts/sugars can affect overall functionality. General information on the functional properties of alternative proteins is limited, especially those of SCPs. Table 4 shows the functional properties profile summary of some alternative proteins reviewed.

6. Sustainability of alternatives proteins

Animal protein production has long been a significant contributor to global warming. For example, livestock production accounts for 14.5% of global greenhouse gas emissions, in addition to its high consumption of water resources [127]. With the increase in population worldwide, animal protein has generated an urgent need to seek new animal protein replacement alternatives in the most consumed foods, such as dairy. The dairy industry produces large volumes of wastewater and high treatment costs of up to 3374 US $/cubic meter of treated effluent [128]. Therefore, one way to promote global sustainable development is to replace animal protein with plant-based, SCP, and recombinant proteins. However, for an alternative protein to be sustainable, it must be environmentally friendly, economically, and socially sustainable and provide food and nutritional security [18].

In an environmental context, PBPs generate fewer greenhouse gas (GHG) emissions than animal-based proteins (See Table 5). However, the production of PBPs decreases soil fertility, contaminates water resources, and contributes to deforestation and desertification of large cultivated areas [18,19,129]. Jeske et al. [15] indicate that soy and almond plantations contribute to climate change and endanger biodiversity. On the other hand, the plant crop is highly affected by biotic stress and is vulnerable to insects, weeds, and fungal pests [130]. Furthermore, the strict dependence on light as the only energy source, the strict limitation on CO_2 as the only carbon source, and the non-use of organic substrates or wastes limit the sustainable productivity of PBPs for food use. PBPs processing, i.e., purity of the protein extracts (protein isolate versus concentrate) as well as water requirements for the fractionation (wet versus dry fractionation), has an additional strong impact on the overall ecological footprint [131]. Despite this, some PBPs have functional properties that make them well-suited for a partial or total addition to dairy products [7,8,30]. In addition, they have good nutritional properties, although the quality of PBPs are inferior compared to milk protein [14,15].

SCPs have a lower environmental impact than conventional proteins (animal and plant) (See Table 5). In the best case, SCP is generally produced in tanks or reactors using agro-industrial co-product as a substrate [18] but still often relies on glucose as a carbon source and ammonia as a nitrogen source. The latter is often not considered in calculations for ecological footprint and strongly impacts the overall sustainability of these sources. For recombinant milk proteins, it was suggested that they would compare to individual extracted milk proteins concerning the ecological footprint [132]. These proteins' nutritional value depends on the type of microorganism and its physicochemical properties (protein, amino acid, carbohydrates, nucleic acid, among others) [64]. However, the use of SCP in food for human consumption is limited due to the high content of nucleic acids, which leads to kidney stones [23,64]. Nevertheless, these limitations are not new and can be adequately controlled by physical and chemical treatments. However, these will also have a negative impact on the overall CO_2 footprint of these protein sources.

Table 5

Environmental impact of some protein sources.

Protein sources	Land (m^2/kg of product)	Energy consumption (kWh/kg of product)	Water (m^3/kg)	GHG Emissions (kg CO_2eq/kg product)	Reference
Dairy	3.32–3.41	48.79–59.1	0.63	4.38–4.95	[156,157]
Beef	7–420	–	1.45	9–129	[157,158]
Chicken	3.85–3.89	51.64–63.4	0.66	5.2–5.82	[156,157]
Pork	7–15	–	1.79	4–11	[157–159]
Soy meal	1.06–1.44	27.78–36.9	0.03	2.65–2.78	[156,157]
Insects	1.5–1.52	2.84–3.02	0.1	32.0–40.4	[156]
SCPs	0.5	10	0.25	2–4	[159]
Mycoprotein	0.79–0.84	60.07–76.8	~0.5	5.55–6.15	[156,160]

In economic terms, obtaining PBPs can be costly and with low yields. Berghout et al. [133] indicate that PBPs such as lupin require a large amount of hexane and water to extract and purify the protein. Therefore, technological innovations are needed to reduce the cost of their production. Kumar et al. [51] and Pojić et al. [134] have reviewed eco-innovative technologies that contribute to the environment and can be applied to extract PBPs for human consumption. Much of the cost of SCP production is based on the type of substrate. One way to reduce costs is by selecting cheap substrates or biodegradable agro-industrial co-products, such as whey, with nutrients needed for microorganism growth and protein production [23].

7. Opportunities and challenges

A significant contribution to the future protein nutrition of humanity will have to come from new proteins, i.e., proteins that are currently not used or are not very common in human foods. A major challenge for the food industry is the replacement of animal proteins by plant, insect, SCP, or recombinant proteins. Knowing and improving the functionality of these proteins would allow understanding of their behavior when included in a food formulation. However, there are few documented functional properties of the alternative proteins mentioned. In addition, most of these works focus on information about proteins of plant origin due to their greater use in replacing animal proteins. Although plants are considered good nutritional sources and possess excellent functional properties for use in food, their implications on the environment and their high cost of production make research on other more sustainable protein sources necessary. With the inevitable increase in population, agricultural land, water, and non-renewable resources are expected to become scarce, so PBPs can no longer be considered the only substitute for animal protein. Therefore, having an exploratory focus on microbial-based proteins for novel food development seems to be a realistic and beneficial approach for future generations.

The incorporation of insect protein is emerging as a future trend in the food industry, including its incorporation in dairy products [135,136]. Insects are a sustainable and efficient source of protein, requiring less water and land than traditional livestock [137]. Additionally, their nutritional composition and functional properties could provide several benefits, such as improving foods' texture and sensory properties. However, several challenges are associated with using insect proteins in dairy products. For example, David-Birman et al. [136] studied the effect of incorporating soldier fly larvae in ice cream and found that it decreased the ice cream's overrun and changed its color, resulting in altered texture, including hardness and adhesiveness. Therefore, further studies are needed to address the technical, functional, and sensory aspects of incorporating insect proteins into dairy products despite the potential benefits.

Considering that society is increasingly aware of its food and nutritional and healthy contribution, SCPs can meet these requirements. These proteins reduce the environmental impact of protein production costs, and their nutritional properties can contribute to developing and improving a food's properties [2]. Although SCPs have many limitations in their use as food due to the high nucleic acid content, there are several physical and chemical treatments for reducing or eliminating these. Additionally, recombinant DNA in the production of SCPs allows for improvements in biomass production, increasing nutritional characteristics, optimizing the use of agro-industrial co-products, and obtaining more functional recombinant proteins.

The few existing studies on the incorporation of biomass from microorganisms in dairy products focus on the use of algal. Although algae are nutritionally compared to PBPs, their incorporation into yogurt and cheese has been hampered by sensory problems.

On the other hand, the total substitution of dairy protein for recombinant protein has not been documented. Therefore, there is an urgent need in this research field to explore using these proteins in food products for human consumption, especially in incorporating these proteins in dairy products.

From the above, this review highlights several opportunities and challenges: First, to analyze the characteristics and functional properties of unicellular proteins, such as yeasts, to understand their role in dairy products. Second, to analyze the characteristics of these proteins in food systems. It could be through partial or total substitution of milk protein by single-cell proteins in products such as yogurt and cheese. Third, analyze mixtures of biomass of microorganisms, and include mixtures with proteins of vegetable origin, such as soy protein, which has shown greater acceptance within the dairy market. Finally, include the genetic modification of microorganisms through recombinant DNA to improve their functional properties. These scopes will give greater knowledge about the behavior, functionality, and incorporation of SCP and recombinant proteins in dairy matrices.

8. Conclusions

Globally, the demand for food continues to increase, and this trend significantly impacts the environment regarding food production. Global food is projected to increase by 50%–60% by 2050 , driving the scientific and professional community to discover and apply processes, technologies, and methods contributing to food safety and supporting the sustainability of food systems. One such avenue of exploration is the development of new alternative proteins, such as plant-based and microbial proteins, which are being explored due to their potential environmental, economic, and social benefits. Microbial proteins (SCP and recombinant protein) are particularly promising due to their rapid production and can be obtained from agro-industrial co-products without requiring arable land. Moreover, microbial genetic modification may lead to a better future for SCP production. However, it is crucial to ensure their safety for human consumption through appropriate evaluation and regulation. Currently, the information on using SCP and recombinant protein in dairy products remains limited or less explored compared to PBPs. Therefore, further research is needed, particularly on the functional properties of these alternative proteins and their potential uses as protein substitutes.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of competing interest

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

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