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Review

Advancements in Research on Alternative Protein Sources and Their Application in Food Products: A Systematic Review

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Abstract: To ensure food security amid dwindling natural resources, alternative proteins (APs) have been suggested as a sustainable solution. Yet, the adoption and consumption of APs remain limited. This review aims to delve into the latest progress (following PRISMA guidelines) concerning the utilization of proteins from alternative sources, particularly focusing on their effective incorporation into food products. Our findings reveal that insect proteins can improve amino acid profiles in bakery products. However, consumer acceptance remains low due to cultural biases, with optimal sensory results being achieved at lower substitution levels (5–10%). Mushroom proteins, when incorporated into meat analogs and bakery items, enhance nutritional value and offer favorable sensory properties, making them viable replacements in meat products. Plant-based proteins, such as pea and soy proteins, increase fiber and antioxidants and improve texture in meat alternatives, although formulation adjustments are necessary to meet consumer expectations for taste and overall experience. Microalgae offer unique benefits for bakery, confectionery, and dairy products by boosting protein, fatty acids, and probiotic growth while maintaining sensory acceptability. In conclusion, this study highlights that the effective incorporation of APs into food products can help in the development of healthier, more sustainable diets. That said, the success of AP acceptance will depend on continued innovations in formulation and consumer education.

Keywords: alternative proteins; insects; algae; mushrooms; plant proteins; acceptance; substitution rate

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1. Introduction

The discourse surrounding APs in the development of new food products is prevalent in contemporary food science. Addressing the dual challenge of substantially increasing food production to support an ever-growing global population, which is projected to reach 9.8 billion by 2050, with the highest growth rates in Africa and Asia [1], while concurrently diminishing the environmental footprint of agricultural systems is imperative. Presently, agriculture accounts for 26% of global greenhouse gas emissions, with livestock contributing approximately half of these emissions [2]. Consequently, the development of animal-free protein alternatives has become a pivotal focus for innovative protein companies. The sector has witnessed a significant influx of investment, amounting to billions of dollars, with numerous new enterprises emerging. Projections indicate that the market for

APs could attain a valuation of $290 billion by 2035 [3]. The supply of adequate protein is critically important as proteins that are derived from animals and are valued for their technological functionalities are favored by consumers. Proteins are fundamental to food structure and function, as well as being vital for metabolic activities such as maintenance, growth, and cellular repair. However, protein production is resource-intensive and significantly impacts environmental sustainability [4]. Recent data link diet, health, and the environment conclusively, highlighting the need for a global food system transformation within planetary boundaries [5]. To address these issues, alternative protein sources such as plants, mushrooms, algae, and insects are gaining substantial attention. These alternatives not only provide potential environmental benefits by reducing emissions and conserving resources but also offer improved nutritional profiles of high-carbohydrate food. This concept has historical roots as APs were primarily consumed by vegans and vegetarians, with soy, legumes, and wheat protein being the main components [6]. Additionally, insects have been part of the diet in regions of Africa and Asia [7]. The AP industry faces significant challenges in cost-effective production scaling and in formulating products with properties similar to conventional foods. Different APs have different functional properties, making food processing difficult, and their long-term health impacts are not fully understood. Consumer resistance due to perceptions of unnaturalness and disgust is also a barrier to their adoption, despite potential global and societal benefits [8]. Consequently, the focus of food science and technology research is identifying new protein sources, developing more efficient extraction methods, and enhancing their functional (solubility, emulsification, foaming, and gelation) and sensory (taste, mouthfeel, and appearance) properties. Additionally, innovative processing methods, especially for texturization, and novel formulation approaches are being explored.

Therefore, the aim of this paper is to review the literature to identify the advancements in the application of proteins from alternative sources, with a particular emphasis on their successful integration into food products, the quality of such products, and their acceptance by consumers. The main selection criteria are as follows: the article was published between 2020 and 2024 and contains original research. Our primary contribution to the literature is an up-to-date and in-depth overview of the factors driving the acceptance and application of various APs in food products for human consumption. This comprehensive overview helps bridge knowledge across research domains and sets a research agenda aimed at understanding and guiding consumer acceptance of APs and exploring their applications in food products.

2. Background of Some Commonly Used Alternative Proteins in Food Product Formulation

2.1. Insect-Based Proteins

Even though insect consumption has been known for thousands of years, using insects as food ingredients in Western civilization is considered an urban trend that is evolving quickly among other AP sources such as plant, fungi, and algae protein sources [9]. In comparison to cattle, the production of insect-based proteins is much more sustainable when considering the carbon footprint, water, energy, and nutrient consumption [10]. When produced with an intention for human consumption, insects can be consumed whole. However, recent consumer trends suggest minimal processing (chopping, cooking, and seasoning), or extracting particular ingredients for formulating enriched products to yield better organoleptic properties and wider consumer acceptance. Pretreatment, drying, and formulation are the three primary procedures that are often followed in the industrial processing of edible insects [11]. During pretreatment, enzyme activity and microbial contamination are reduced in order to ensure food safety and prevent potential lipid oxidation [12]. The two most commonly used pretreatment methods are blanching and freezing. Drying (oven drying or freeze-drying) is applied to prolong the shelf life of protein powder. The final step is food product formulation. The simplest method to process insects is grinding them into a powder, while defatting or protein extraction and purification can be performed to produce more complex products [11] (Figure 1).

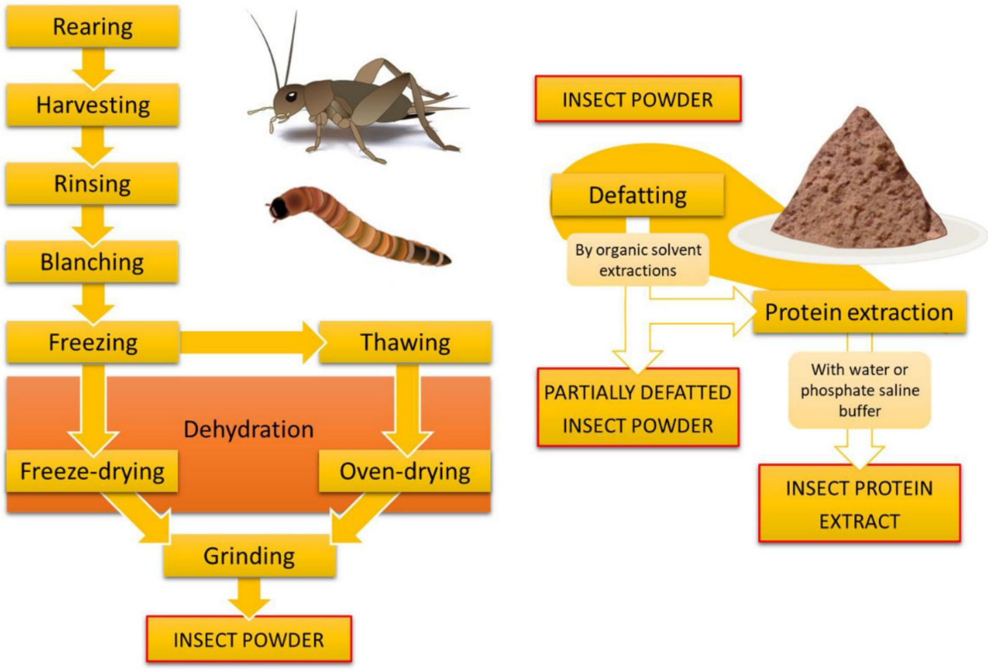

Figure 1. Common procedures for obtaining insect-based proteins suitable for food incorporation [13].

2.2. Algae-Based Proteins

The term algae is associated with a broad range of organisms, ranging from picoplankton cells to macroalgae. Algae-based protein sources refer to seaweed and microalgae, which have gained increased attention together with a general increase in their consumption as APs. The technological properties of algae-based proteins are similar to plant-based proteins, which contribute to their consumer acceptance [14]. Functional properties such as gelation, foaming, or emulsification are highly pH-dependent [15]. Algae can have higher protein content than traditional sources; some authors claimed that algae-based proteins have a lower digestibility than plant protein [16,17]. In the literature, different pretreatments, including physical and chemical processes, enzymatic hydrolysis, as well as innovative green extraction techniques like ultrasound and microwave-assisted extraction, have been reported [18] (Figure 2). The main disadvantage of algae protein sources is related to the reproducibility of their chemical composition and the process conditions due to great variations in their biomass composition. The biggest opportunity concerning algae protein lies in the production of bioactive peptides with potential health benefits.

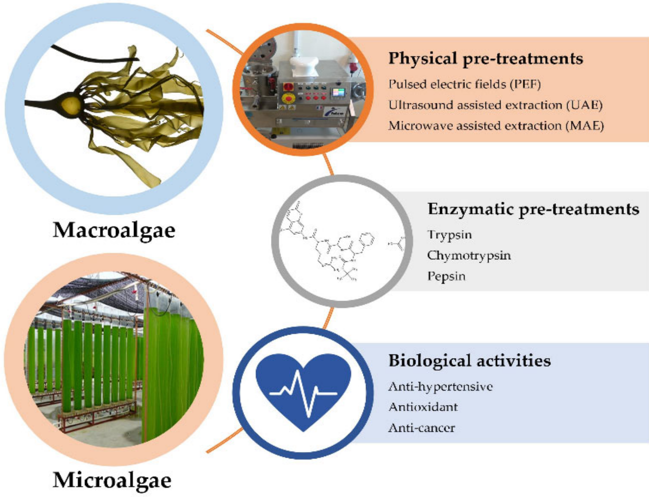

Figure 2. Processes for the generation and isolation of bioactive peptides or cryptides from algae [19].

2.3. Plant-Based Proteins

Some plant-based proteins, such as soybeans, peas, and wheat, are preferred due to their availability, low price, and familiar properties. They are widely used as meat analogs [20]. While there is rising concern regarding allergens in soybean and wheat proteins, the versatility of plant protein sources enables the utilization of other plant sources, such as pea protein, which are gaining more attention due to low their allergen potential and ability to be used in plant-based meat analogs. There are different extraction techniques available for protein separation. Among others, wet fractionation is the most commonly used and considered a standard method due to its possibility to obtain high-purity (>90%) proteins (Figure 3) [21,22].

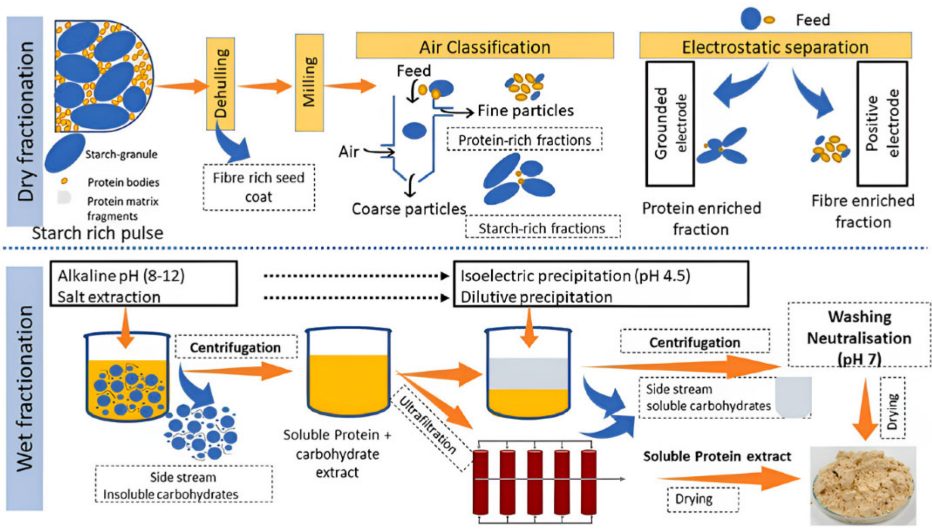

Figure 3. Plant protein fractionation techniques [23].

Some disadvantages of plant protein are poor solubility, sensitivity to pH changes and temperature, and ionic strength. Another reported disadvantage is the frequent presence of some specific bioactive compounds with anti-nutrient activity. Therefore, to reach desirable functionality and applicability, plant proteins must often be modulated. By altering their physicochemical characteristics regarding their limitations, plant-based proteins can be modified to become multipurpose components of food products. Protein modification techniques can be broadly categorized as physical, chemical, biological, and other innovative techniques [24]. In contrast, dry fractionation is performed in the absence of organic solvents, where proteins are separated using air and electrostatic separation.

2.4. Fungi-Based Proteins

There are two main directions in the production of fungi-based proteins: harvesting mushrooms (macrofungi) and the production of single-cell proteins (microfungi proteins). Fungi are eukaryotes and comprise more than 80,000 species. The term single-cell protein is used for proteins from microbial sources. Those proteins can be found in the form of biomass or extracted protein. Mushrooms are the higher fungi [25]. In edible mushrooms, protein content is ranked from 19 to 35% of dry weight, with high levels of branched-chain amino acid (BCAA) content [22,26]. Mushrooms are also known for their high content of bioactive peptides and proteins such as immunomodulatory proteins, lectins, ubiquitin-like proteins, and ribonucleases laccases [27]. The properties of fungi proteins are well-documented and have been extensively reviewed [25,28–31]. The biggest advantages of mushroom protein are its sensory properties, which are similar to meat, and a low degree of allergenicity. While mushrooms are usually consumed whole, single-cell proteins, which are obtained by filamentous fungi, are usually subjected to several processing steps (Figure 4), including fermentation, harvesting, washing, cell disruption, protein extraction, and purification [32].

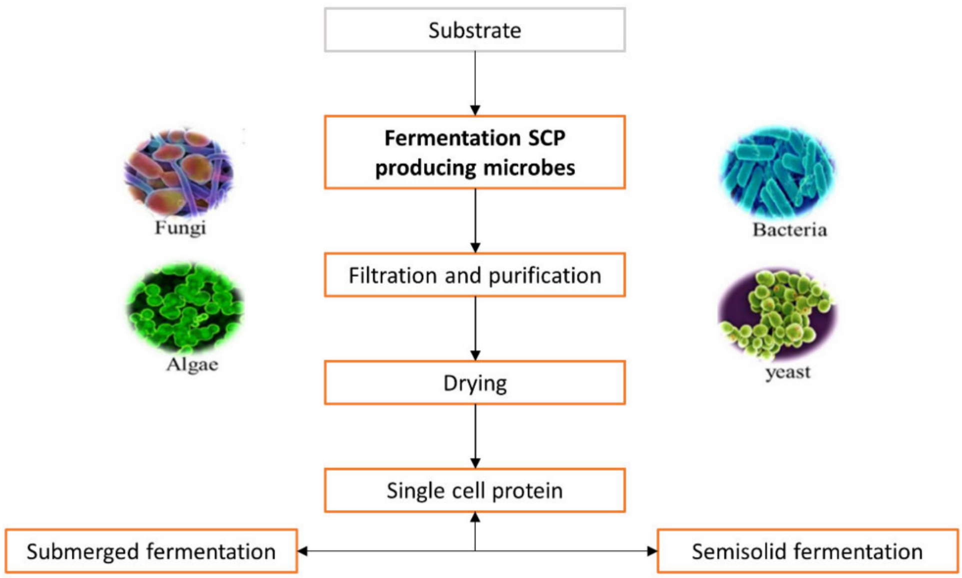

Figure 4. Processing steps during industrial production of single-cell proteins [25].

3. Materials and Methods

Following PRISMA guidelines, we conducted a comprehensive search through PubMed/MEDLINE, ScienceDirect, SpringerLink, and ResearchGate databases, covering studies published until June 2024. The search focused on randomized articles published in English that evaluated the effects of incorporating APs into bakery, dairy, and meat products on their nutritional value and sensory acceptance in humans. The review was not registered in any public registry, and accordingly, the review protocol was not prepared.

PRISMA Review Protocol

This study explored the use of APs to enhance the nutritional value of food products for humans, excluding any applications in animal feed. It primarily examined insect-based proteins, plant-based proteins, mushrooms, and algae. Sensory evaluations targeted adult participants, comparing different types of AP and their effects on nutritional and sensory qualities. This study also considered the forms and substitution rates of the APs used, focusing on research conducted over the past five years (2020–2024).

Studies that did not focus on products for human consumption were excluded. Additionally, papers irrelevant to this study based on their titles, such as those involving unrelated research, review papers, and book chapters, were not considered. Additional filters for disciplines/subject areas were used in databases that have this option (ScienceDirect and SpringerLink), where earth science, energy, education, computer science, economics, pharmacy, biochemistry, genetics and molecular biology, medicine and dentistry, immunology and microbiology, and social science areas were excluded.

The search spanned four databases: PubMed/MEDLINE, ScienceDirect, SpringerLink, and ResearchGate. Keywords for databases included "alternative protein", "food products", "enrichment", "human consumption", and "article". Initially, a database of 12,331 scientific papers was collected. Before commencing the search, articles outside the scope of this study, based on their year of publication (n = 127), and duplicate records (n = 1472) were removed. From the remaining articles (n = 10,732), studies not relevant to the systematic review title (n = 9089), book chapters (n = 471), inaccessible articles (n = 41), articles not discussing human consumption of alternative proteins (n = 276), articles not discussing relevant sources of protein (n = 141), and review papers (n = 684) (Figure 5), were excluded. Ultimately, 30 scientific papers were analyzed in this study.

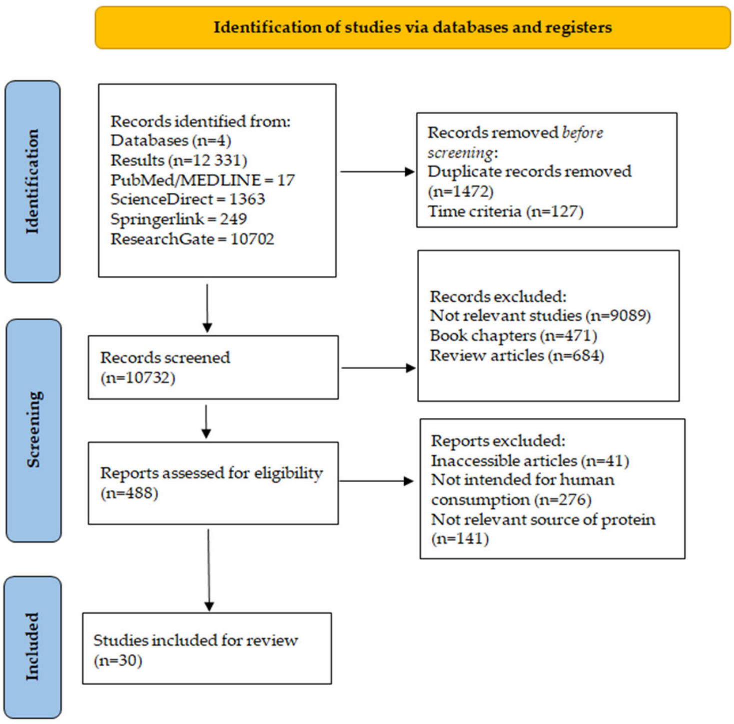

Figure 5. Utilization of the PRISMA flow diagram for conducting database searches and elucidating accessible articles for review.

4. Results and Discussion

Dietary adjustments with lower protein demand from conventional animal sources are necessary to make the shift to a healthier, more resilient, and sustainable food system to support the future of food systems, which should ensure suitable amounts of healthy food. The change from excessive dependence on a small amount of resource-intensive animal proteins to a wider range of reduced-impact protein components and food products is known as protein diversification. APs derived from plants, algae, insects, and materials derived by cellular agriculture could play a significant role in contributing to the diversity of proteins as ingredients in food products. Some technologies for producing AP are more sophisticated than others, and some protein sources are more acceptable to consumers than others [33–35]. The 30 articles investigated are listed in Tables 1–4.

Table 1. Insects as AP sources and their impact on food properties.

No.	Protein Source	Type	Enriched Product	Form of Protein	Findings	Substitution Level	Reference
1.	Mealworm (*Tenebrio molitor* L.)	Insect	Wheat bread	Powder	↑ Protein and fat content, good microbiological quality, and 5% of substitution is optimal	5, 10, and 15%	[36]
2.	Mealworm (*Tenebrio molitor* L.)	Insect	Wheat bread	Powder	Lower specific volume, higher hardness, ↑color intensity, 5% substitution is optimal	0, 5, 10, and 15%	[37]
3.	Mealworm (*Tenebrio molitor* L.)	Insect	Dough and biscuit	Powder	↑Protein content and amino acid composition; up to 15% substitution level was sensorially acceptable	0, 5, 10, 15, and 20%	[38]
4.	Migratory locust (*Locusta migratoria* L.)	Insect	Wheat bread	Powder	↑ Protein, fat, fiber, and ash contents, ↑values of essential amino acids, and 1–4% substitution had the highest acceptability	0, 1, 2, 3, 4, and 5%	[39]
5.	Cricket (*Acheta domesticus* L.)	Insect	Bread and pasta	Powder	Best cooking behavior obtained at a 5% replacement level	5, 10, and 20%	[40]
6.	House cricket (*Acheta domesticus* L.)	Insect	Wheat bread	Powder	5% cricket flour had the highest sensory acceptability and higher levels lower scores; ↑protein and fat content, and decreased carbohydrate content	5, 10, and 15%	[41]
7.	Mealworm, buffalo worm, and cricket	Insect	Wheat bread	Powder	↑Protein and amino acid content and sensory properties Up to 10% of supplementation is acceptable	10, 20, and 30%	[42]
8.	Cricket (*Acheta domesticus* L.)	Insect	Pita chips	Powder	Emotional elicitation plays a crucial role in consumer acceptance of edible cricket protein (ECP)-enhanced foods	6.9%	[43]
9.	Mealworm (*Tenebrio molitor* L.)	Insect	Gluten-free bread	Fiber and insect powder	Fibers improved bread quality and mixtures of plant fibers, with low concentrations of insect powder being particularly effective	2, 5,7, 9, and 13%	[44]
10.	Black soldier fly (*Hermetia illucens* L.) larva	Insect	Burger patties (cooked and raw)	-	Could not fully replace beef as the 100% patty could not hold its structure Partial replacement of 25% was found to be the most successful	0, 25, 50, 75, and100%	[45]
11.	Mealworm, buffalo worm, and pea protein.	Plant and insect	Bread	Powder	↑Protein and mineral content; bread with insect protein obtained a higher liking score than the one enriched with pea proteins	5 and 10%	[46]
12.	Locust (*Locusta migratoria* L.)	Insect	Cheddar cheese	Protein hydrolysate powder	Strong antimicrobial and antioxidant activities, enhanced refrigerated storage quality for up to 3 months, and improved antioxidant activity	0.0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3%	[47]
13.	Black soldier fly (*Hermetia illucens* L.) larva	Insect	Meat analogs and chicken breasts	Powder	The optimal formulation of chicken breasts was 6.7 g/100 g black soldier fly larva protein (BSFLP); the formulation for the plant-based meat analog was 21.5 g/100 g BSFLP.	Proportions of the mixture: X1 + X2 + X3 = 1	[48]
14.	Black soldier (*Hermetia illucens* L.)	Insect	Doughs and baked products	Powder	↑Essential amino acids, maintaining satisfactory rheological and physicochemical characteristics	2 and 4%	[49]

↑—increasing value. AP—alternative proteins.

4.1. Insect-Based Proteins: Food Applications and Consumer Acceptance

Most of the analyzed studies (14) used insects as protein sources (Table 1).

There are insect species that are consumed by humans, either whole or as a component of prepared foods. In the European Union (EU), edible insects are classified as novel foods, and they need market authorization before entering the market [50]. Only three insects—migratory locust, house cricket, and mealworm—are currently approved. However, in the USA and Canada, edible insects are allowed to be imported and sold if they adhere to the rules set forth by food safety agencies. Among the analyzed studies, mealworms were the most abundant insects (6), while the less commonly used insects were crickets (4), black soldier flies (3), and locusts (2). One of the main sources of daily energy intake is cereal-based items, such as bread and bakery products. Therefore, there is growing consumer interest in the consumption of cereal products with a better nutritional composition, primarily higher micronutrient composition and increased protein content [42]. Several authors reported increased protein content as a reason for adding APs to cereal products [36,42,45,49]. Mazurek et al. [42] reported that when compared to wheat bread, the amino acid score (AAS) for lysine increased from about 40% to over 70%, while only 10% of insect flour delivered from mealworms, buffalo worms, and crickets was used. Montevecchi et al. [49] reported that in bread production, using semi-whole meal flours enhanced with black soldier flour powder efficiently adds vital amino acids to the final product while preserving acceptable rheological and physicochemical properties. In addition to protein content, an increase in AP sources leads to an increase in overall nutritional value. Althwab et al. [39] found that the inclusion of migratory locust powder considerably raised the bread samples' protein, fat, fiber, and ash contents. Also, higher levels of essential amino acids were found in breads enriched with locust powder. Similar improvements in nutritional composition were reported by Mazurek et al. [42], where high concentrations of oleic acid, palmitic acid, and linoleic acid were found in the fatty acid profile, ranging from 42.95% for mealworms to 28.79% for cricket flour, 23.76% for buffalo worms to 26.02% for cricket flour, and 31.26% for cricket flour to 26.93% for buffalo worms, respectively. Gantner et al. [41] concluded that adding 5% to 15% of cricket flour increased the protein and fat content and decreased the carbohydrate content in all studied wheat bread samples. Thermal processing of insects with higher cooking temperatures increases acidity by breaking down glycogen into lactic acid. This also affects gelling and foaming properties, triggers coagulation, and increases surface hydrophobicity. Prolonged heating results in the hydrolysis of proteins and lipids. Cooking does not affect the fatty acids' composition and proteins' secondary structure [51]. Drying impacts protein solubility and oxidation, while thermal processing modifies sulfur-containing amino acids in insects to improve the acceptability, digestibility, nutritional quality, and bioavailability [52]. However, higher cooking temperatures can damage lipids and proteins. The way insects are processed can influence their nutrient content and how easily these nutrients are absorbed. For instance, methods like boiling and vacuum cooking help preserve high levels of polyunsaturated fatty acids and proteins in mealworms [53].

Even enhancing the nutritional composition of bakery products is an interesting opportunity for the cereal industry as the addition of proteins undoubtedly leads to changes in rheology, texture, and other sensory characteristics. Khuenpet et al. [37] studied the impact of adding larval-stage mealworm (*T. molitor* L.) to wheat bread. They found that an increase in larval-stage mealworm powder led to higher hardness and a lower specific volume of the produced bread. The higher fiber content of the insect powder can physically disrupt the continuity of the gluten network, causing an increase in hardness [54]. A rise in color intensity results from the Maillard reaction during baking and the initial brown hue of the mealworm powder from the larval stage. Similar findings were encountered by Xie et al. [38], where mealworm powder was added to dough and biscuit, with an impact on the pasting, shear, viscosity, and stretching behavior of wheat flour. The increased levels of proteins, lipids, and fiber dilute the starch content of the flour, thereby restricting starch swelling and gelatinization during cooking, which results in a lower maximum viscosity [55]. Insect-derived protein affects viscosity by increasing gelatinization while reducing maximum viscosity, breakdown velocity, and setback viscosity. This is caused by the high fiber and protein content in cereals, which delays gelatinization by preventing the hydration and swelling of starch granules [56].

Some authors suggested improvements for producing bakery goods with APs derived from insects. Althwab et al. [39] recommended that the functional features of binary mixture systems can be significantly enhanced by blending wheat flour with different concentrations of locust protein powder. Bresciani et al. [40] analyzed the influence of cricket powder on bread and past rheology properties. The dough rheology results showed that in order to maintain a suitable bread volume, more water should be added and the mixing and leavening times must be shortened. In fact, a product with a volume and crumb hardness comparable to the control (wheat flour) was produced by 10% cricket powder enrichment. Dough rheology is mainly affected by non-protein ingredients, like chitin from insect powder. The increased dough development with the addition of insect powder is due to the hydration properties of dietary fiber or the presence of other hydrophobic compounds, such as insect oil [57]. Lipids prevent starch swelling by forming starch–lipid complexes that cover the starch surface, making it more hydrophobic and limiting water absorption into the granules [58]. Even though cricket powder reduced extensibility, fresh pasta enhanced with cricket powder could be produced; the best cooking behavior was achieved at a 5% replacement level.

Many consumers continue to be skeptical about APs, such as insects. Despite its potential as a sustainable protein source, insect protein did not appear to appeal to any consumer group, according to a Wageningen University study that examined the factors influencing consumer acceptance of various APs. However, people's attitudes toward insect protein improved with their level of knowledge about the health and environmental advantages of insects [51]. Therefore, one of the critical factors in including insect protein in the human diet is an acceptable substitution rate. The studied substitution rates varied widely, from 5% to 30%. The results of Gantner et al. [36] showed that 5% bread enrichment with mealworm powder was optimal. The same rate was reported by Khuenpet et al. [37], where bakery products fortified with 5% of larval stage mealworm revealed the most comparable quality to the control bread sample. A 5% acceptable substitution rate was also recommended for crickets added to wheat bread. Sensory evaluation performed in a study by Gantner et al. [41] showed that the bread with 5% cricket protein powder did not differ significantly from the control bread sample. However, breads with higher levels of cricket flour had significantly lower overall scores compared to the control bread. Higher substitution rates (up to 10%) were reported for insect protein addition in wheat bread in a study by Mazurek et al. [42].

Some lower acceptable substitution rates were reported for locust protein powder. According to the results of sensory evaluation, bread samples containing locust protein powder at levels of 1–4% had great general acceptability. Tauferová et al. [44] performed a study where 2–15% of mealworm (*T. molitor* L.) powder and mealworm fibers were added to gluten-free bread. The type and proportion of fiber have a big impact on how gluten-free bread tastes and how well-liked it is by consumers. In general, fibers enhanced the quality of bread; low quantities of insect powder and plant fiber mixtures performed very well. Notably, fiber additions improve nutritional and sensory qualities, which benefits manufacturers as well as customers. For improved gluten-free products, recipes should be reformulated to take these findings into account. García-Segovia et al. [46] compared the addition of two insect protein sources of mealworm (*T. molitor* L.) and buffalo worm

(*Alphitobius diaperinus* L.) with pea plant protein (*Pisum sativum* L.). According to the study, *T. molitor* demonstrated better values than those found in earlier investigations, even though there were no appreciable differences in the technological characteristics of the two insect powders. They concluded that, although it may lower the overall quality of the bread, adding other protein sources, such as insect powders, might increase the bread's protein and potential mineral content. Surprisingly, the bread enhanced with pea proteins received a lower acceptability score than the bread enriched with insect proteins. Xie et al. [38] reported the highest acceptable substitution rates. The results of sensory evaluation in the mentioned study suggested that the biscuits produced with mealworm powders up to a 15% substitution level were sensorially acceptable. The study [42] evaluated three formulations of pita chips (plain, Italian, and Cajun), each containing 6.9% of edible cricket protein, and measured overall liking, specific emotions, and intent to purchase for the three varieties (before tasting), after tasting/before cricket protein statement, and after tasting. Promoting the advantages of edible cricket protein had an impact on the emotional profiles of the treatments, which indirectly affected product acceptability, but it did not significantly alter total treatment preferences. While improving sensory profiles and lowering negative feelings like "disgusted" can increase product acceptance, evoking emotions like "interested" and "adventurous" is what drives product enjoyment.

On top of additions to bakery products, several authors [45,47,48] used insect protein sources as protein replacements for cheese and meat products. Bessa et al. [45] aimed to determine whether black soldier fly larvae could partially or even fully replace beef in a burger patty. Comparing the black soldier fly larvae patties to the beef patties, it was discovered that the regular beef patties had more protein, fat, and moisture and maintained their texture throughout their refrigerator shelf life. Because the 100% patty could not maintain its structure under cooking or physical pressure (patty formation), black soldier fly larvae could not completely substitute beef in these patties. Of all the treatments, partial substitution of 25% for beef proved to be the most effective; perceived hardness, texture, and springiness did not change. The black soldier-containing patties were notably different in color, which raises concerns about their potential consumer acceptance, even if they had fewer cooking losses than the control patty. Raw black soldier protein patties were preferred less than raw beef patties, whereas cooked black soldier protein patties were preferred even more than cooked beef patties. Miron et al. [48] used black soldier larvae as meat analogs. Using a beef burger, a chicken breast, and a commercial plant-based meat analog as reference matrices, the impact of adding black soldier fly larvae protein (87.6 ± 2.4 g/100 g content) on the textural qualities of meat analogs was assessed and contrasted with those of meat analogs made using other protein substitutes, such as soy protein isolate and wheat gluten. The study showed that meat analogs derived from insect proteins combined with vegetable proteins can offer textural qualities comparable to those of commercial plant-based meat substitutes and chicken breasts. Also, their formulation closely mimics the textural characteristics of chicken breasts and the textural characteristics of plant-based meat analogs. Singh et al. [47] studied the effectiveness of locust protein hydrolysates in improving the quality of cheddar cheese. In addition to enhancing the protein content, locust protein hydrolysates performed well as bio-preservatives because of their potent antibacterial and antioxidant properties. The preservation potential of locust protein hydrolysates is increased by pretreatments with microwaves or ultrasonication. They increase antioxidant activity and post-digestion health advantages, and they improve the quality of refrigerated storage for up to three months. For more sustainability and security, future studies should examine their effects when cooked as well as their uses in other foods.

To sum up, when insect protein is recognized for its potential to lower environmental impacts, it can serve as an alternative ingredient in food products, particularly in bakery and meat analogs, providing enhanced protein and micronutrient content. Studies (Table 1) have shown that adding insect protein—such as cricket, locust, or mealworm powder—can boost essential amino acids, fatty acids, and the overall nutritional value of products like bread and pasta. However, sensory and textural challenges, such as changes in bread hardness or dough behavior, often accompany insect protein additions, suggesting the need for careful formulation adjustments. Despite its nutritional benefits, insect protein faces consumer resistance, especially in the Western world, primarily due to unfamiliarity and cultural biases. Acceptance rates generally improve with education about its environmental and health advantages. Sensory evaluations also indicate that lower substitution levels, around 5% to 10%, maintain favorable texture and taste, especially in bakery products. Moreover, using insect protein in meat analogs shows promise for partial substitution, enhancing protein content and offering texture profiles comparable to other plant-based proteins. For broader consumption, further innovations in formulation and consumer education will be essential, aiming to balance sensory properties with sustainability and nutrition benefits.

4.2. Applications of Proteins Derived from Plants and Mushrooms

4.2.1. Mushrooms as APs: Food Applications and Consumer Acceptance

Mushrooms are regarded as a healthy food product because they offer excellent nutritional qualities and are rich in high-quality proteins, dietary fibers, vitamins, minerals, and phenolic compounds [59].

Mushrooms provide 20–30% of crude protein, including all essential amino acids, making them suitable for meeting the nutritional requirements of both humans and animals. The protein digestibility of mushrooms typically ranges from 72% to 83%. They offer a complete amino acid profile, containing the eight essential amino acids required by humans [60,61]. Mushrooms are rich in dietary fiber and classified as "high fiber" by the European Commission [62]. They are cholesterol-free and high in polyunsaturated fatty acids and contain essential fatty acids such as linoleic and linolenic acid [63]. Carbohydrates make up about 60% of their dry weight. Mushrooms also provide vital micronutrients, including B complex vitamins (B1, B2, B3, and B5), and essential minerals such as potassium, phosphorus, sodium, calcium, and magnesium. They also offer essential trace elements like copper, zinc, iron, molybdenum, and selenium [28,64]. Mushroom extracts have found their application in a variety of food products, especially in different meat products. Among the analyzed studies, meat analogs were suggested as the most common application (4), while the less common applications were in bread (1) and sponge cake (1) (Table 2).

Table 2. Mushrooms as AP sources and their impact on food properties.

No.	Protein Source	Type	Enriched Product	Form of Protein	Findings	Substitution Level	Reference
1.	*Fusarium venenatum*	Mushroom	Silver carp (*Hypophthalmichthys molitrix*) sausage	Mycoprotein	↑Nutritional value of sausages	1 and 3%	[65]
2.	*Agaricus bisporus*	Mushroom	Beef burgers	Cooked mushroom	10% and 15% substitution had a good overall liking and the highest sensory acceptability.	0, 5, 10, and 15%	[66]
3.	Enoki (*Flammulina velutipes*)	Mushroom	Goat meat nuggets	Mushroom stem waste	4% substitution was best for improving the nutritional profile, physicochemical properties, and shelf life	2, 4, and 6%	[67]
4.	*Lentinus edodes, Pleurotus ostreatus*, and *Coprinus comatus*	Mushroom	Sausages	Powder	15% addition and water content of 35% exhibited textural profiles similar to real beef	15 to 100%	[68]

Table 2. *Cont.*

No.	Protein Source	Type	Enriched Product	Form of Protein	Findings	Substitution Level	Reference
5.	*Pleurotus ostreatus*	Mushroom	Bread	Flour	↑Protein; 5–15–80% and 10–10–80% m shroom–cassava–wheat composite flour recommended for better bakery results	2.5 to 10%	[69]
6.	*Pleurotus sajor-caju*	Mushroom	Sponge cake	Powder	Incorporating 10% mushroom powder enhanced nutritional quality, maintaining acceptable sensory attributes	0, 5, 7, 10, and 12%	[70]

↑—increasing value. AP—alternative proteins.

Bahmani et al. [65] added mushroom extracts to sausages to examine the enrichment of silver carp sausage with *F. venenatum* mycoprotein at 1% and 3%. The addition of mycoprotein significantly increased the sausages' protein content and other nutritional values. However, it also led to higher thiobarbituric acid (TBA) and volatile nitrogen base (TVB-N) levels, indicating potential spoilage. Sensory evaluation showed that mycoprotein-enriched sausages were less durable and desirable compared to the control sample. Despite these drawbacks, *F. venenatum* mycoprotein is considered valuable for its nutritional benefits and cost-effectiveness. Patinho et al.'s [66] research demonstrated that replacing animal fat with *A. bisporus* (AB) mushrooms in beef burgers increased moisture and yield, reduced cooking losses, and improved oxidative stability, especially at higher AB concentrations. AB 10% and AB 15% burgers were well-liked for their juiciness, tenderness, and flavor. This approach shows promise for creating healthier low-fat burgers. Banerjee et al. [67] investigated the impact of different amounts (2%, 4%, and 6%) of enoki (*F. velutipes*) mushroom stem waste (MSW) powder on the physicochemical qualities (color and textural), oxidative stability, sensory attributes, and shelf life of goat meat nuggets. They concluded that incorporating MSW in meat nuggets enhances emulsion stability, water-holding capacity, and oxidative stability, extending their shelf life without affecting color or sensory attributes. Using 4% MSW can improve nutritional value and create healthier meat products. Yuan et al. [68] investigated the effect of water content (35%, 70%, and 100%), using three types of edible mushrooms, in a mixture of equal proportions, as well as their amounts (from 15% to 100%) on the physicochemical and structural profiles. The study demonstrated that a mushroom-based meat analog made from *C. comatus* (15%) and SPI (35% water) closely mimics the texture of real beef. Flavor profiling identified 64 volatile compounds, with dried processing yielding more aromatic substances than steam processing. This research presents a viable method to create high-quality non-animal meat products with appealing sensory properties.

In addition to being introduced into meat products, several authors [69,70] used mushroom protein sources as protein replacements for different bakery products. Ngirabakunzi Irakiza et al. [69] conducted a series of experiments by substituting wheat flour with cassava flour at proportions of 10–25% to find the optimal combination and with the aim of identifying the optimal wheat–cassava–mushroom combination, improving the nutritional value of breads while keeping their physicochemical and organoleptic properties. The authors recommend using composite flour blends of 5–15–80% and 10–10–80% mushroom–cassava–wheat for better bakery outcomes, consistency, and higher protein and energy content, being mainly aimed at enhancing the nutritional status of populations in tropical non-wheat producing regions. The study also showed that improving the taste and color of mushroom-fortified bread is essential to boost its market acceptance and competitiveness. Das et al. [70] aimed to enhance the nutritional quality of sponge cakes with oyster mushrooms (*P. sajor-caju*). The study evaluated the addition of mushroom powder in cakes at levels of 0% (control), 5%, 7%, 10%, and 12%. Both hot air-dried (HD) and freeze-dried (FD) mushroom powders increased the protein, fat, ash, and fiber content in the cakes, while carbohydrate and moisture content decreased. Notably, FD mushroom powder at 10% was highly acceptable sensorially.

In summary, research shows that using mushrooms in various food products, such as sausages, burgers, and baked goods, enhances their nutritional profile and sensory attributes. Incorporating mushroom powders or extracts can improve the quality and health benefits of these foods, making them viable alternatives to traditional meat and wheat-based products.

4.2.2. Plants as APs: Food Applications and Consumer Acceptance

There is an increasing interest in using plant-based materials as functional ingredients in food products due to their high content of dietary fiber and other bioactive compounds, such as vitamins, minerals, and polyphenols [47,48]. These fibers, combined with phenolic compounds, create antioxidant dietary fibers [71], which can enhance gastrointestinal health when used as supplements and can extend the shelf life of foods by preventing lipid oxidation [72,73]. Among the studies analyzed, all considered using plant proteins as meat analogs, such as beef patties (3) or sausages (1) (Table 3).

Table 3. Plants as AP sources and their impact on food properties.

No.	Protein Source	Type	Enriched Product	Form of Protein	Findings	Substitution Level	Reference
1.	Soybean	Plant	Beef patties	Soy-based textured vegetable protein	Substitution of 10–20% was more similar to the control patty	10 to 40%	[74]
2.	Soy protein and wheat gluten	Plant	Burger patties	-	Protein at 50% and 130 °C die temperature showed the most potential for producing meatless patties with desirable qualities	50 and 60% of soy protein, wheat gluten, and starch at a ratio of 5:4:1	[75]
3.	Pea (*Pisum sativum*) protein	Plant	Beef patties	Powder	Improved cooking yield in beef patties, with better juiciness, flavor, and texture	2.5 and 5%	[76]
4.	Grape seed flour	Plant	Vegetarian Sausages	Powder	↑Antioxidant capacity and polyphenol content	0, 1, 3, 7, 10, and 20%	[77]

↑—increasing value. AP—alternative proteins.

Tremlova et al. [77] examined adding grape flour to vegan sausages to reduce processed meat intake and boost healthy plant-based nutrients. Six sausage batches with various grape flour levels (0%, 1%, 3%, 7%, 10%, and 20%) were analyzed. The results showed that grape flour increased antioxidant capacity and polyphenol content. Sausages with 1% and 3% grape flour were most acceptable to panelists. Although protein content slightly decreased, the overall nutritional benefits and use of sustainable byproducts from wine production were significant. Plant protein sources are often used as protein replacements for different meat products to enhance cooking performance, physical and textural qualities, and sensory appeal. Shen et al. [76] investigated how adding pea protein, modified via sequential deamidation and conjugation (PGG), affects the cookability, physical and texture properties, and sensory attributes of beef patties in comparison with regular patties. The study found that beef patties containing PGG, especially at 5%, showed improved fat and moisture retention, reduced cooking losses, and a tender texture, making them potentially preferable for certain consumers, such as the elderly. Also, Bakhsh et al. [74] investigated how the incorporation of soy-based vegetable protein (TVP) into beef patties impacted various characteristics. Their results showed that TVP significantly reduced moisture and fat content while increasing crude fiber. Patties with TVP had higher pH, lighter color, and different texture properties. Sensory attributes varied, with higher sourness, astringency, umami, and saltiness at higher TVP levels. The study suggests that TVP is a promising ingredient for enhancing beef patties' nutritional profile and physical qualities. Samard et al. [75] compared the cooking and textural properties of meatless patties and commercial meat patties. They also evaluated the physicochemical properties, cooking characteristics, and textural features of the resulting textured vegetable proteins (TVPs) in plant-based burger patties. The study showed that while the cooking and textural characteristics of the tested vegetable-protein-based meatless burger patties did not fully match those of commercial meat patties, the patty made with 50% moisture content and 130 °C die temperature showed the most promise for further research. They recommend investigating other plant proteins, binding agents, formulas, and extrusion conditions to develop plant-based patties that more closely resemble meat patties.

4.3. Applications of Proteins Derived from Microalgae

Microalgae have gained significant interest as a novel protein source due to their numerous technological and commercial benefits, including rapid cell growth, low cultivation costs, and no competition with traditional food production areas [78]. Microalgae are rich in valuable compounds such as proteins, fatty acids, pigments, vitamins, and minerals, making them increasingly popular in the food industry. Their biomass can be used in the production of ready-to-eat products and for direct consumption. Therefore, many studies have been conducted on how adding different types of microalgae affects the nutritional and sensory properties of various bakery and confectionery products (Table 4).

Table 4. Microalgae as AP sources and their impact on food properties.

No.	Protein Source	Type	Enriched Product	Form of Protein	Findings	Substitution Level	Reference
1.	*Chlorella sorokiniana*	Microalgae	Gluten-free bread	Biomass powder	↑Protein content and lutein content, ↑omega-3 content in fatty acids, and 2.5 g addition to bread had the highest acceptance rate	2.5 g/100 g and 5.0 g/100 g	[79]
2.	*Arthrospira platensis* (spirulina), *Chlorella vulgaris*, *Tetraselmis chui*, and *Nannochloropsis oceanica*	Microalgae	Vegetable creams	Dried single-cell ingredients of microalgae	↑Protein content and nutritional quality	1.5 and 3.0%	[80]
3.	*Arthrospira platensis*	Microalgae	White chocolate	Powder	Adding 4% biomass significantly enhanced the nutritional profile without negatively impacting sensory qualities	0.5, 1, 2, and 4%	[81]
4.	*Spirulina platensis*	Microalgae	Ayran	Powder	↑Growth of probiotics and enhanced the nutritional value	0%, 0.25, 0.5, and 1%	[82]
5.	*Arthrospira platensis*	Microalgae	Marshmallow	Powder	Best results observed when 25% of gelatin was replaced at 80 °C for hydration and at 40 °C for aeration	25, 50, 75, and 100%	[83]
6.	*Spirulina maxima*	Microalgae	Vegan biscuits	Powder	↑Nutritional profile without compromising sensory acceptance	5, 10, 15, and 20%	[84]

↑—increasing value. AP—alternative proteins.

Paula da Silva et al. [84] aimed to develop nutritionally enriched and sensorially well-accepted biscuits by incorporating spray-dried microencapsulated *S. maxima* biomass. The study showed that Spirulina biomass, rich in high-quality proteins, iron, and essential fatty acids, can be effectively incorporated into biscuits up to a level of 20% without affecting sensory acceptance while improving their nutritional profile, thus showing great market potential. Çelekli et al. [83] investigated the effect of adding *A. platensis* on the physicochemical, texture, and microstructure properties of marshmallows. They found that the incorporation of *A. platensis* at various concentrations, hydration levels, and aeration temperatures significantly affected marshmallow density, with 25% gelatin replacement at 80 °C and aeration at 40 °C yielding the best results. This approach shows great potential for novel marshmallow production methods. Özbal et al. [81] evaluated the effect of adding

A. platensis on the nutritional and sensory attributes of white chocolate. They found that adding up to 4% *A. platensis* significantly improved the nutritional content, including protein, amino acids, lipids, and minerals, without altering sensory acceptance. These findings suggest that *A. platensis* is a promising ingredient for enhancing the nutritional profile of white chocolate. Also, Prandi et al. [80] investigated the quality and digestibility of proteins in vegetable creams reformulated with the addition of microalgae. The study found that microalgae ingredients differed in protein composition but had similar digestibility across species. Adding microalgae to vegetable creams significantly increased protein and amino acid content without reducing protein digestibility, suggesting a potential for enhancing nutritional quality. Further research on higher levels of microalgae addition is recommended to explore their full impact.

In addition to looking at applications in bakery and confectionery products, Çelekliet al. [82] investigated the possibilities of their application in milk-based products and their protein-boosting and probiotic effects. The study investigated the effects of *S. platensis* and whey protein hydrolysates (WPHs) on the growth of probiotics in Ayran, a fermented milk product. The results showed that adding *S. platensis* and WPHs significantly boosted the growth of several probiotic cultures and improved Ayran's nutritional value. The highest total solid level and protein content were observed with a 1% addition of *S. platensis* and WPHs, highlighting their potential to enhance probiotic growth and the nutritional profile of Ayran. Among the analyzed studies, microalgae showed the highest variety of applications as technological enhancers and substitutes in the confectionery industry (3), milk industry (1), bread and cereal industry (1), and vegetable processing industry (1).

In summary, microalgae have gained significant interest as a novel protein source due to their high nutritional value and technological benefits. Studies have shown that incorporating microalgae into food products, such as biscuits, marshmallows, white chocolate, vegetable creams, and Ayran, improves their nutritional and sensory properties without compromising their quality. This suggests great potential for microalgae in enhancing various food products.

4.4. Alternative Proteins in Review: Practical Applications, Nutritional Impact, and Replacement Potential

APs increase opportunities for enhancing food products, each offering unique benefits suitable for various applications. Insect protein, derived from sources like crickets, locusts, and mealworms, has gained recognition for its potential to lower the environmental impact of food production. It serves as a viable ingredient in bakery products such as bread and pasta, as well as in meat analogs. Insect protein has moderate substitution flexibility, with effective incorporation levels typically around 5–10%. While it enhances the nutritional content significantly by providing essential amino acids and micronutrients, it can also alter the texture, impacting product properties like bread hardness and dough behavior. These changes necessitate careful formulation adjustments. Despite its nutritional advantages, insect protein faces consumer resistance, particularly in Western markets, largely due to cultural biases. However, consumer acceptance tends to improve after being educated about its environmental and health benefits.

Mycoprotein, specifically from *F. venenatum*, stands out as another promising alternative, especially in applications like sausages and other meat products. It has been shown to be a valuable nutritional enhancer, with notable benefits at substitution levels of 1–3%. However, while increasing the protein content, mycoprotein can lead to spoilage issues, indicated by higher thiobarbituric acid (TBA) and volatile nitrogen base (TVB-N) levels. Sensory evaluations show that mycoprotein-enriched sausages may be less durable and desirable compared to controls. Its cost-effectiveness and nutritional profile position it as a potential alternative, despite challenges related to consumer acceptability due to textural and sensory drawbacks.

Mushroom protein, derived from species like *A. bisporus* and *F. velutipes*, also demonstrates significant potential for food substitution, particularly in beef burgers and various bakery products. With high substitution flexibility, mushroom protein can be effectively used in ratios of 10–15%, enhancing moisture, flavor, and overall sensory attributes. At these levels, it not only improves the nutritional content but also tends to reduce fat levels, making it appealing to health-conscious consumers. However, ensuring that improvements in flavor and texture translate into market acceptance remains a challenge.

Grape flour has emerged as an innovative alternative addition to vegan sausages and processed meats. Effective at low substitution levels of 1–3%, grape flour enhances antioxidant capacity and polyphenol content. Although its incorporation slightly reduces the protein content, the overall nutritional benefits and the use of sustainable byproducts from wine production are significant advantages. Nevertheless, the search for additional protein sources may be necessary to complement its lower protein levels.

Soy-based vegetable protein, particularly textured vegetable protein (TVP), has been widely studied for its potential use in beef patties and other meat alternatives. TVP showcases high substitution flexibility, with notable effects on moisture and fat content while increasing dietary fiber. Although sensory attributes can change, particularly with higher concentrations, its ability to enhance nutritional profiles while reducing fat content makes it a promising option. Further research into formulations may be essential to optimize taste and texture.

Lastly, microalgae, such as Spirulina (*A. platensis*), display remarkable versatility across various applications, from bakery products like biscuits and marshmallows to dairy products like Ayran. This AP source can be incorporated at levels of up to 20% without sacrificing sensory quality, with optimal effects noted between 1 and 20% depending on the product. Microalgae significantly boost protein, iron, and essential fatty acid content, representing a highly nutritious option. However, consumer familiarity remains a barrier to their widespread acceptance, signaling the need for further research to enhance sensory appeal.

In summary, each AP source shows unique potential for substitution in food products, with varying impacts on nutritional enhancement and sensory characteristics. The choice of the most suitable source will largely depend on the specific food application and the desired product attributes. As the market for APs continues to expand, ongoing research and consumer education will be vital for overcoming challenges related to sensory acceptance and optimizing formulation strategies.

4.5. Safety Concerns of Alternative Proteins

When discussing the safety concerns associated with consuming APs, it is essential to address several key factors: allergens, pesticide residues, mycotoxins, and potential side effects. APs, such as those derived from legumes, insects, microalgae, and fungal sources, can pose significant allergenic risks. For instance, legumes like soybeans contain allergens that can cause IgE-mediated immunological reactions, manifesting in skin, gastrointestinal, cardiovascular, and respiratory symptoms [85,86]. Similarly, edible insects like mealworms and grasshoppers have allergens such as arginine kinase and tropomyosin, which can cross-react with allergens in crustaceans and house dust mites [87]. Microalgae and fungal proteins have also been reported to cause allergic reactions, especially in individuals with mold allergies [88,89].

Genetically modified (GM) crops used in plant-based proteins, such as GM soybeans, might contain pesticide residues like glyphosate. Although there are limited data on glyphosate accumulation in plant-based meats, there are concerns about the potential adverse health effects. Continuous monitoring and strict quality controls are necessary to mitigate these risks [90,91].

Mycotoxins, toxic compounds produced by certain fungi, are another concern. Insects can metabolize some mycotoxins, but this ability varies by species. Fungal proteins are produced under conditions that typically prevent mycotoxin formation, but alternative carbon sources in the production process can alter this risk [92]. Similarly, microalgae might accumulate mycotoxins when grown in contaminated environments.

APs also carry various potential side effects. Plant-based proteins contain anti-nutrients like protease inhibitors, tannins, and phytic acid, which can impair nutrient absorption and enzyme activity. These effects can be mitigated through proper processing. Thermal processing of APs can lead to the formation of carcinogenic compounds like polycyclic aromatic hydrocarbons and nitrosamines [93]. Edible insects and microalgae can accumulate heavy metals depending on their growing conditions, potentially exceeding safe consumption levels. Both plant-based and insect proteins can harbor pathogenic bacteria and fungi [94]. Proper thermal processing and quality controls are vital to reduce these risks.

In conclusion, while APs offer nutritional and environmental benefits, addressing the safety concerns related to allergens, pesticide residues, mycotoxins, and potential side effects is crucial. Rigorous research, quality control, and continuous monitoring are essential to ensure their safe consumption.

5. Conclusions

Proteins from alternative sources such as insects, mushrooms, plants, and microalgae offer promising alternatives for enhancing the nutritional profile and sensory attributes of various food products. Insect protein can boost amino acids and nutritional value in bakery items and meat analogs, although careful formulation is needed to address sensory challenges. Mushrooms improve the quality and health benefits of products like sausages, burgers, and baked goods. Plant-based materials can significantly enhance nutritional and sensory qualities. Grape flour boosts antioxidant capacity and polyphenol content in vegan sausages, while pea protein and soy-based vegetable protein improve the nutritional profile and texture of meat alternatives. However, careful formulation is needed to address sensory challenges, making these ingredients promising for healthier and more sustainable food products. Microalgae enrich food products such as biscuits, marshmallows, and dairy alternatives, providing significant nutritional advantages. Together, these AP sources present opportunities for innovation in creating healthier and more sustainable food products.

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