AHFES

A **QUADRUPLE HELIX** ATLANTIC AREA **HEALTHY FOOD** ECOSYSTEM FOR **GROWTH OF SMES**

Alternative Proteins Report

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Content

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Abbreviations and Acronyms

EC	European Commission
EU	European Union
FAO/WHO	Food and Agriculture Organization of the United Nations & World Health Organization
GMO	Genetically Modified Organism

Executive summary

This report presents the new trends in alternative proteins for the healthy food and beverage market in the coming years.

Throughout the scientific research, it was observed that new plant proteins appear as alternatives to existing ones. New, unexpected products appear in innovation and are currently taking the first steps in creation.

Of the new proteins, it should be noted that the pea is the one with the most applications in the food sector. It may have greater acceptance by the consumer, as processes are already being developed to overcome the characteristic flavour of this plant.

On the other hand, insects are the next alternative protein to emerge in European markets. In addition to being a new protein, studies are still needed to understand how its nutritional profile varies according to the species, the animal's diet and the cooking process. There are several existing research projects in this area to assess the benefits of this product.

Finally, there is the laboratory meat that we would have thought would be in the distant future until a long time ago. There are already products launched outside Europe, but they still need more research to improve the product visually and tastefully. However, several companies have chosen to advance in this area due to the accelerating lack of water, deforestation, and climate change.

1 Introduction

Over the generations, agriculture and the food industry have created safe, tasty, convenient, and affordable food that has helped reduce hunger and malnutrition. However, it has been remarkable that the current food production system has become detrimental to people and the planet. With the growth of the global population, the pressure on the food production sector translates into increased consumption of foods rich in protein and the search for viable protein alternatives compared to the traditional ones. In addition, there has been a shift towards diets with more processed or even ultra-processed foods[1]. Since the world population stands at 7.9 billion and is expected to reach 9.5 billion by 2050, the population makes a sustainable food challenge, as, in addition, the expansion of agricultural land is associated with many environmental pressures, such as soil degradation, pesticide and nutrient leaching, loss of biodiversity, emissions of carbon and more. This leads to a decrease in the nutritional quality of the food produced today, which may jeopardize our population's health and environmental sustainability due to the pressure caused by the increase in exploitation[2].

The food industry, especially in Western culture, has been one sector that has taken more significant advantage of the growing demand for plant-based food and food products. With a growth in the market for alternative products to animal protein, as well as options and variety of this type of products available in large commercial stores, which is mainly due to the gradual urbanization, environmental concerns, increase in the vegetarian and vegan community, ethics and animal welfare and consumer trends[3,4,5]. This industrialization leads to increased processing of these foods with the resulting nutritional consequences. As a consequence of the high energy density and nutritional composition of this type of food or food products, the consumption of ultra-processed foods seems to be positively associated with various health outcomes such as increased weight and obesity, increased risk of cardiovascular disease, hypertension, type 2 diabetes mellitus and cancer[6]. These non-communicable diseases kill 41 million people, equivalent to 71% of all deaths globally. Some may be associated with unhealthy eating habits, such as cardiovascular disease and diabetes. The first is associated with 17.9 million people annually, followed by cancer (9.3 million), respiratory diseases (4.1 million) and lastly, diabetes (1.5 million)[7].

With the issues mentioned above, it is necessary to find healthy alternatives that are nutritionally comparable with the original products, specifically for meat analogues. But also, environmentally sustainable options at all levels and the following ethical trends regarding changes in dietary patterns. With the increase in world population and the unavailability of food resources, we have to find alternatives to the consumption of animal protein. As examples of emerging foods to combat these current problems that tend to worsen, we have alternative plant-based proteins, cellular agriculture products, macroalgae and edible insects. There has been an increase in the consumption of plant-based products due to the scientific rise in their benefits.

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[1] McClements DJ, Grossmann L. The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. Compr Rev Food Sci Food Saf. 2021;20(4):4049–100

[2] Duro JA, Lauk C, Kastner T, Erb KH, Haberl H. Global inequalities in food consumption, cropland demand and land-use efficiency: A decomposition analysis. Glob Environ Chang [Internet]. 2020;64(July):102124.

[3] European Commission. Europe's plant-based food industry shows record-level growth | News | CORDIS | European Commission [Internet]. 2021

[4] EUVEPRO. THE USE OF PLANT-BASED PROTEINS IN FOOD AND BEVERAGES IN THE EU A 10-year review of New Product Launches Containing Plant-Based Proteins across EU 28 [Internet]. Protein Trends and Technologies Seminar. Brussels; 2019.

[5] https://www.meticulousresearch.com/product/meat-substitute-market-4969

[6] Chen X, Zhang Z, Yang H, Qiu P, Wang H, Wang F, et al. Consumption of ultra-processed foods and health outcomes: A systematic review of epidemiological studies. Nutr J. 10 2020;19(1):1–10.

[7] World Health Organization (OMS). Non-communicable diseases. Public Health: An action guide to improving health. 2021.

2 Alternative Protein Sources

In recent years, there has been a growing demand for alternative dietary protein sources that can replace animal proteins. This dietary change is generally related to sustainability, health and ethical reasons. Therefore, it is crucial to know how alternative sources of proteins could impact human health.

In this chapter, it is intended to report the alternative sources of proteins.

2.1 Established Protein Sources

Several vegetable proteins have been applied in human nutrition over the last few years. These proteins stand out from this group, soy, peas, lupine, and potatoes.

2.1.1 Soybean

Soy protein is an economical and reliable source of protein that can be used as an alternative to meat and milk proteins. Soy contains approximately 36% protein, 30% carbohydrates and 18% oil (figure 1). This protein source is of high quality and provides adequate amounts of essential amino acids, following FAO/WHO guidelines. However, the nutritional characteristics of soy may vary depending on the variety, geographic location, and climate [8].

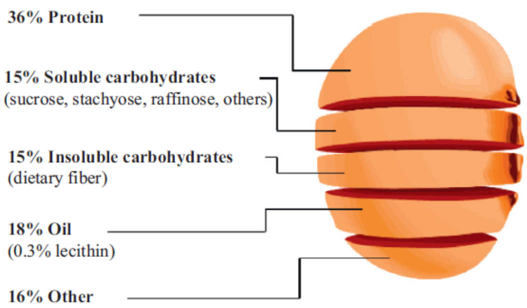

Figure 1. Soybean composition[8]

Today it is possible to create new varieties with high yields by applying different agronomic practices and seed development. Soy as an ingredient is a versatile protein, allowing it to perform several functions in foods: solubility, gelling, viscosity, emulsification, foam and flavour/aroma. On this property, soy can be applied in meat products, infant formulas, dairy products, bread, and pasta-based. Functionally, soy protein can replace animal protein (meat, dairy, egg) while reducing costs without altering taste and quality[8].

However, soy has some factors that limit the development of soy-based alternatives, such as the allergenicity and smell of soybeans. Another factor is the juiciness that affects the flavour of the soybean and is strongly related to the water retention capacity of the product. Thus, this is one of the critical points to investigate so that new compounds and aroma precursors can be explored.

[8] M. Thrane, P.V. Paulsen, M.W. Orcutt, T.M. Krieger, Chapter 2 - Soy Protein: Impacts, Production, and Applications, Editor(s): Sudarshan R. Nadathur, Janitha P.D. Wanasundara, Laurie Scanlin, Sustainable Protein Sources, Academic Press, 2017

On the other hand, soy protein is deficient in vitamin D, E and B12, requiring fortification to compensate for the decreased intake of the respective vitamins. Furthermore, it should be noted that some soy products contain undesirable anti-nutrients such as phytic acid and trypsin inhibitors that reduce the bioavailability of nutritional and functional components.

To overcome these obstacles, lipoxygenase-free soybean varieties are being developed. After germination, the seeds have shown promising results in nutritional improvement and elimination of the soybean flavour. In addition, there are also approaches such as enzymatic hydrolysis and the fermentation process that improve nutritious and sensory qualities and improve physiological functions.

Bueno et al. (2020) demonstrated that the germination process of soybeans improves the nutritional and nutraceutical values of soy milk. However, it will still be necessary to carry out a sensory evaluation of the new product developed so that conclusions can be drawn about the potential of this innovative product on the market[9].

Finally, in meat products such as hamburgers and nuggets, it is normal for reformulations of these products to replace 30 to 40% of the meat with soy protein. However, it is necessary to consider that this protein does not have much flavour. Still, they are excellent absorbers of artificial and natural flavours and can also be coloured with colours so that the final product is appealing to consumers.

2.1.2 Pea

Pea protein is attracting more and more attention in alternative proteins. Pea is an excellent season crop, and its nutritional composition depends on the seed crop (figure 2). In addition to its nutritional composition, pea protein has attracted a lot of attention as a substitute for animal and soy protein due to its low allergenicity, having a non-GMO status and achieving sustainable crop availability. According to WHO/FAO recommendations, pea protein is considered a high-quality protein because of its balanced proportion of amino acids[10].

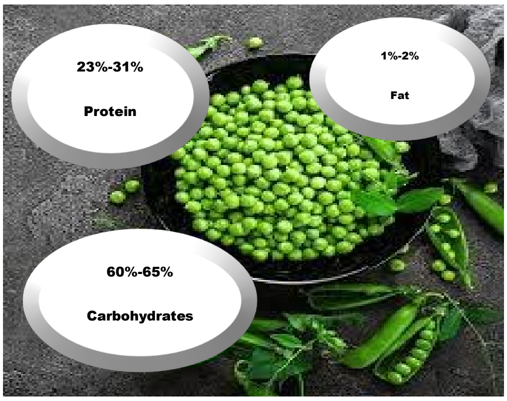

Figure 2. Pea nutritional composition

[9] Bueno, D.B.; da Silva Júnior, S.I.; Seriani Chiarotto, A.B.; Cardoso, T.M.; Neto, J.A.; Lopes dos Reis, G.C.; Glória, M.B.A.; Tavano, O.L. The germination of soybeans increases the water-soluble components and could generate innovations in soy-based foods. LWT 2020, 117, 108599.
[10] Fatma Boukid, Cristina M. Rosell, Massimo Castellari. Pea protein ingredients: A mainstream ingredient to (re)formulate innovative foods and beverages. Trends in Food Science & Technology. Volume 110. 2021

In the market, this protein is available in flours, concentrates or isolates. Despite the great interest in these products, it appears that the inclusion of pea protein in foods and beverages is still a challenge for the food industry, mainly in terms of flavour and impact on functional and technological properties. One of the processes that have been investigated to improve the taste of this protein is the fermentation process. During fermentation, biochemical changes occur, such as degradation and formation of organic substances, developing a more intense aromatic profile. Several studies investigate the effect of this process on the nutritional and functional properties of pea proteins. It was concluded that it is necessary to consider that fermentation affects protein solubility, emulsification, and foaming capacity. These properties are essential for food products such as alternatives to vegetable milk, ice cream and mayonnaise. Therefore, it is crucial to control and select appropriate microorganisms for later application of pea protein fermented products in these cases. Another way to overcome the taste of pea protein is by modifying the processes and methods of extracting pea protein.

Considering the beneficial properties of pea protein and the evolution of extraction processes, the protein market is projected to grow by 7.6% annually between 2020 and 2027. Nowadays, several commercial applications in the food area are already available, such as flours, concentrates and isolates. However, it has been verified that although it is in the industry's interest to include this new protein in the market, this is still a challenging task due to the taste and impact on functional and technological properties. There have been several studies on different processing and extracting pea protein to obtain a good flavour for the consumer. Garcia et al. 2021 noted that one of the ways to improve sensory properties is through fermentation, during which biochemical changes (degradation and formation of substances) occur that can give rise to different aromatic profiles. The author found that 48-hour lactic acid fermentation of pea protein extracts improves the aroma profile and reduces off-flavours in the final product. However, Garcia et al. 2021 highlight that it is necessary to consider that the fermentation process affects the solubility, foaming capacity and emulsifying capacity of pea protein. Thus, when this process is used, it is necessary to control and select appropriate microorganisms for later application of the fermented products of pea protein[11].

[11] García Arteaga, V.Demand, V.; Kern, K.; Strube, A. Szardenings, M.; Muranyi, I.; Eisner, P. Schweiggert-Weisz, U. Enzymatic Hydrolysis and Fermentation of Pea Protein Isolate and Its Effects on Antigenic Proteins, Functional Properties, and Sensory Profile. Foods 2022, 11, 118

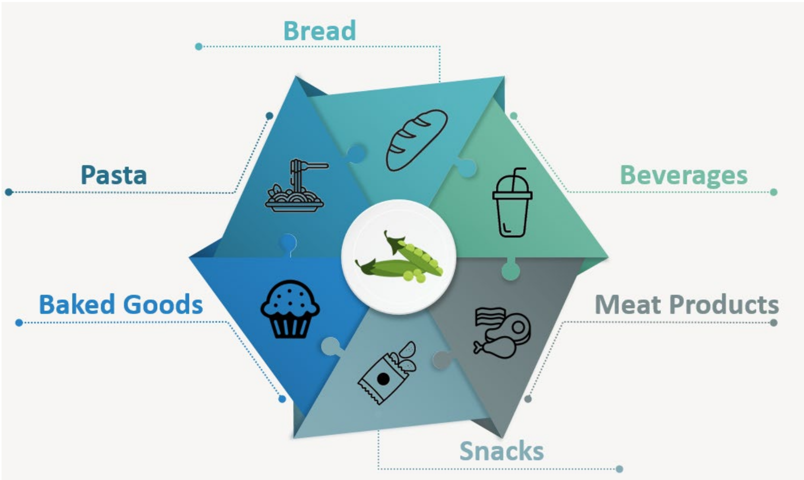

Figure 3. Pea protein applications

One of the food applications of pea protein is in the most consumed food in Portugal, bread. Studies in the literature conclude that the application of this protein in bread with gluten makes it possible to increase the quantity and quality of the protein of the final product, also managing to improve the amino acid profile. However, the application of protein in bread does not replace the function of gluten. Studies have shown that replacing just 15% of the wheat flour with pea protein isolates (85% protein) results in a bread with less volume, having a compact crumb structure with a hard texture. On the other hand, in the case of gluten-free bread, they benefit from adding protein ingredients favouring the nutritional composition of gluten-free bread. It is observed that the addition of 5% pea protein results in a bread with volume and thickness similar to the control gluten-free bread (bread without modification in the formulation) (figure 4). These results can be explained based on pea protein's high water absorption capacity, which allows much less moisture loss during the bread baking phase[12].

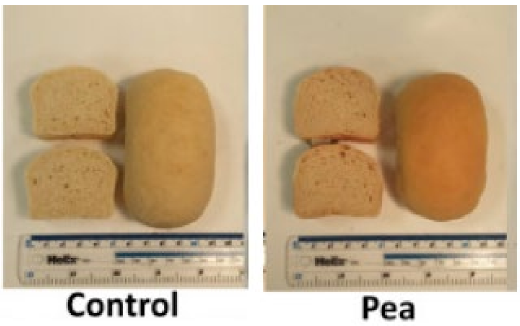

Figure 4. Appearance of free glúten bread with addition of pea protein

In the case of pasta, there has been a tendency to add pea protein to enrich the final pasta product nutritionally. Furthermore, the addition of protein may contribute to health, as it modulates the release of glucose during digestion. This effect was observed when 7.5% heat-denatured pea

[12] Andrea Hoehnel, Claudia Axel, Jürgen Bez, Elke K. Arendt, Emanuele Zannini. Comparative analysis of plant-based high-protein ingredients and their impact on quality of high-protein bread. Journal of Cereal Science. Volume 89. 2019

protein was added. On the other hand, it was concluded that the addition of denatured protein does not affect the texture and sensory properties of the dough[10].

2.1.3 Lupine

Lupine is a food that has been increasingly sought after by the food industry. It is a rich source of dietary fibre with low fat and carbohydrate content, and the protein concentration is similar to that of soy. However, the amino acid composition is soft like other legume proteins, containing cysteine and methionine and high in lysine[13].

As previously mentioned, lupine has a low starch content (gluten-free). Still, it is rich in insoluble fibre (30% to 40%) and protein (30% to 42%), and its nutritional profile varies according to the lupine species. Considering the nutritional composition, lupine has been increasingly investigated due to its health benefits targeting bakery products and plant-based dairy products.

Several studies have concluded that lupine proteins isolated by selective fractionation or salt extraction can be applied for foaming, emulsification and gelling. Thus, the resulting extracts are more suitable for additives in meat processing, powdered ingredients or formulas. Lupine can replace egg proteins and butter thoroughly, considering the foaming and emulsifying properties. On the other hand, this protein can also be used as a substitute for mayonnaise and salad dressings, achieving physical properties similar to those of the commercialized product. On the other hand, lupine proteins produced through alkaline extraction-isoelectric precipitation or ultrafiltration show higher yields but more structural damage than salt extraction. In this way, these proteins resulting from the alkaline extraction process are more suitable for creating analogous meat products by cooking at high temperatures.

The protein can also be applied in the formulation of cookies with and without gluten; however, it is necessary to ensure that the structure and the sensory properties are not affected until the inclusion of 10% of lupine protein. Adding protein to the formulation improves the nutritional value and quality of the cookies.

It is also worth mentioning that lupine protein can be added to pasta, cakes, and even fried and sweet snacks. This addition improves the texture, nutritional value, taste and shelf life of the final product, which turns out to be helpful, for example, for individual's intolerant to egg whites or even individuals on a low-cholesterol diet.

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[13] Mota, Joana & Lima, Ana & Ferreira, Ricardo & Raymundo, Anabela. (2020). Lupin Seed Protein Extract Can Efficiently Enrich the Physical Properties of Cookies Prepared with Alternative Flours. Foods. 9. 1064. 10.3390/foods9081064.

2.2 Emerging Alternative Proteins

According to scientific research, it is observed that a set of ingredients that are less used in the food market are slowly being adopted by the alternative protein market and are in the development and testing phase. This chapter mentions new emerging alternative proteins: beans and lentils.

2.2.1 Fava Bean

Fava beans (Vicia Faba L.) is a legume grown in winter and is one of the cheapest protein sources in most countries. It is a sustainable agronomic protein source with great nutritional potential and functional properties to be applied in food products.

This legume has in its constitution a percentage of protein between 31% and 34%, depending on the species of bean we grow. The carbohydrate content varies between 44% and 47%, and the dietary fibre is approximately 8%. Compared to other legumes, fava stands out nutritionally due to the high proportion of protein and the profile of essential amino acids for humans. This bean has anti-diabetic and anti-inflammatory properties and has recently been studied for having an essential role in preventing Parkinson's disease. On the other hand, it is crucial to bear in mind that this legume has anti-nutrients in its constitution, which reduce the bioavailability of protein and minerals. The presence of lectins, saponins, alkaloids that interfere with the digestion and absorption of nutrients can favour the development of specific pathologies such as food allergies stands out[14].

In line with the general nutritional profile of the broad bean and the health benefits, it has been introduced in the food industry as an alternative protein. However, the main form that exists more on the market is in dry form. So far, few studies have been carried out with this protein, but the development of bread, cake and snack formulations stand out. Atef et al. noted that raw and sprouted fava bean flour could be mixed with wheat flour, supporting the high protein source blends and increasing amino acid levels. Furthermore, it was concluded that the protein composition of the samples tested plays a crucial role in the rheological, technological and sensory properties of bakery products.

[14] Multari, Salvatore & Stewart, Derek & Russell, Wendy. (2015). Potential of Fava Bean as Future Protein Supply to Partially Replace Meat Intake in the Human Diet. Comprehensive Reviews in Food Science and Food Safety. 14. 10.1111/1541-4337.12146.

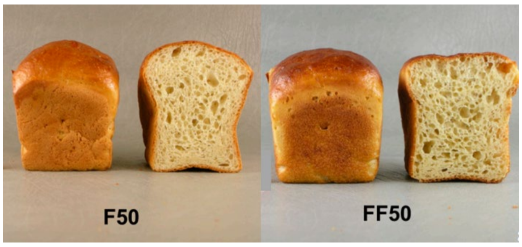

Figure 5. Bread with fava bean flour[15]

On the other hand, Sozer et al. demonstrated that applying lactic fermentation to fava bean flour improves the nutritional profile of gluten-free bread in terms of amino acid content and biological value (figure 5).

In conclusion, this protein is still slowly appearing on the market. However, several factors are needed to study the best process for its nutritional, functional and organoleptic profile to be accepted and beneficial to the consumer. It is crucial to find a balance between dietary benefits and sensory profile so that the food industry can increase this new alternative protein in consumer food.

2.2.2 Lentil

Since ancient times, the lentil has been considered a staple food, a highly nutritious legume with ample amounts of carbohydrates (40g) and a good amount of protein (18g), minerals, vitamins, phytochemicals, and fibre (16g). It stands out for its low-fat content (1g) and high fibre content[16].

However, its use has been limited mainly due to the lower digestibility of proteins, the presence of anti-nutritional factors, flatulence and poor culinary qualities. Aryee et al. analyzed several approaches to overcome these obstacles, such as dry milling and cooking. According to the study carried out, it was shown that there is an improvement in the digestibility of lentil protein using the methods that the author addresses in the article. In the case of trypsin inhibitors, these can be reduced by applying the techniques of immersion, cooking and germination of lentil seeds[17].

On the other hand, according to its nutritional profile, this alternative protein is an excellent source of several dietary factors. Its consumption is associated with positive effects on human health. However, it is crucial to bear in mind that in the case of lentils, there is the flavour factor, which many consumers refer to as “bean flavour”, which should be considered when developing the product. Several studies have developed strategies to reduce the bean flavour factor. Applying organic solvents (acetone, ethanol and isopropanol) or infrared heating stands out.

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[15] Sozer N, Melama L, Silbir S, Rizzello CG, Flander L, Poutanen K. Lactic Acid Fermentation as a Pre-Treatment Process for Faba Bean Flour and Its Effect on Textural, Structural and Nutritional Properties of Protein-Enriched Gluten-Free Faba Bean Breads. Foods. 2019 Sep 21

[16] Khazaei, Hamid, Maya Subedi, Michael T. Nickerson, Cristina Martínez-Villaluenga, Juana Frías and Albert Vandenberg. “Seed Protein of Lentils: Current Status, Progress, and Food Applications.” Foods 8 (2019): n. pag.

[17] Alberta N.A. Aryee & Joyce I. Boye (2016): Improving the Digestibility of Lentil Flours and Protein Isolate and Characterization of their Enzymatically Prepared Hydrolysates, International Journal of Food Properties

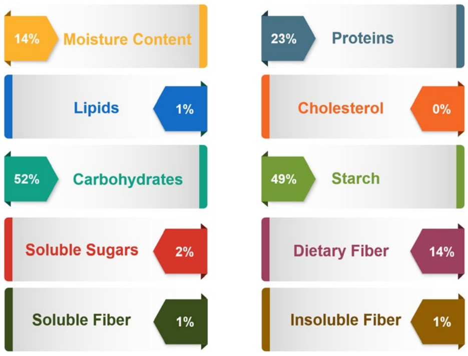

Figure 6. Lentil flour composition

There are several possible applications for this protein, and it usually appears on the market in the form of flour, with a balanced nutritional profile (figure 6). Based on the nutritional profile and functional properties, lentil flour can be applying to several products as shown in figure 7.

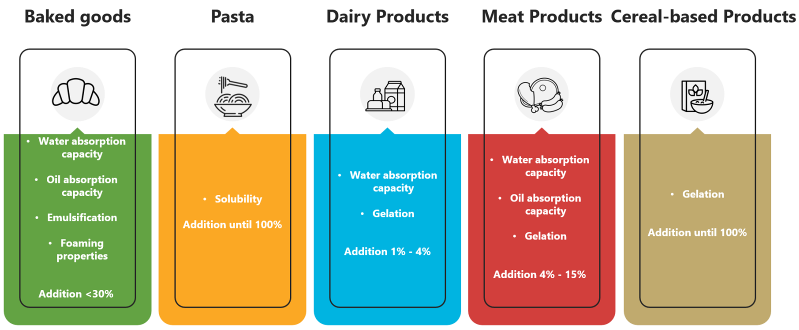

Figure 7. Functional properties

The application of this protein in cereal-based products or combined with other cereal proteins stands out since lentils have a high lysine content. On the other hand, there are already some products on the market that use lentil protein to replace eggs in doughnuts and muffins. In the case of hamburgers, it can be used as a binding agent to make hamburgers. Finally, Han et al. developed a gluten-free cracker snack, which consisted of 100% lentils. Consumers rated the product highly for its acceptability but said it had a strong bean flavour.

In this way, lentil protein still has some research and innovation path ahead, with the obstacles of taste and consumer acceptability as one of the main focuses for tremendous success in the future market.

2.3 Upcoming Protein Sources

This chapter describes the future alternative proteins that are currently starting on the market or in the development process and still need to be reviewed in terms of regulation and consumer acceptance.

2.3.1 Micro-algae

Algae are the most common organism on earth, with a wide geographic distribution, and can grow in either fresh or saltwater. It is estimated that the number of known species of algae varies between 30,000 and 1 million. Some information about some species can be consulted in this AlgaeBase internet database (http://www.algaebase.org), which describes more than 150,000 species. In this particular case, the macroalgae consist of marine algae[18].

Its consumption is also since there is an increase in the growing awareness of consumers about organic and environmentally sustainable products. As a result, according to the Seafood Source report, the global market is expected to grow to USD 22.1 billion by 2024. This growth trend has been verified between 2011 and 2015, which has seen an increase in consumption in the European market by 147 %.

Currently, among the three types of macroalgae (green, red and brown), brown algae are the most consumed species (66.5%), followed by red algae (33%) and green (5%)[19]. Globally, the production of macroalgae corresponds to 85% of food for human consumption.

This type of food has shown potential interest due to its broad spectrum of secondary metabolites and essential bioactive substances, including proteins, carbohydrates, lipids, polyunsaturated fatty acids (PUFA), including omega-3 fatty acids, polysaccharides, polyphenols, sterols and pigments (chlorophyll, carotenoids,, phycobilins). In addition, algae are a good source of dietary fibre and contain vitamins: A, B1, B12, C, D and E, riboflavin, niacin, pantothenic acid and folic acid[18].

The main polysaccharides obtained from seaweed are alginate, agar and carrageenan. These hydrocolloids are commonly used in foods and can be used to formulate substitutes for protein sources, namely through the formulation of gelling agents and thickeners, presenting themselves as a technological innovation in the manufacture of vegetarian products. As an example, agar is used as high-fibre vegetarian gelatine. Algae-based flours and powdered lipids are currently much discussed, as they are an element of modern cuisine, also appreciated in the vegan market, where they are used in place of eggs[18,20].

Algae has excellent potential in the food industry as fat substitutes or improving novel foods' shelf life. However, the uses of these compounds for food applications are not visible enough due to the intensive pre-treatments (e.g., several hours, high temperatures) that are essential for conventional extraction methods. These conventional extraction protocols also employ large amounts of chemicals (i.e., sodium hydroxide or hydrochloric acid) and longer extraction times, resulting in safety issues, degradation of extracted compounds, and therefore increased processing cost.

The main application of macroalgal polysaccharides is often related to their hydrocolloid properties. Its thickening and gelling properties allow hydrocolloids to form a film/coating for food or pharmaceutical products or be used as packaging materials.

Macroalgal carbohydrate polymers such as cellulose, xylan, sulfated galactans and alginic acid have been widely used to improve functional properties in novel food formulations, mainly playing

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[18] Ścieszka S, Klewicka E. Algae in food: a general review. Crit Rev Food Sci Nutr [Internet]. 2019;59(21):3538–47.
[19] Afonso NC, Catarino MD, Silva AMS, Cardoso SM. Brown macroalgae as valuable food ingredients. Antioxidants. 2019;8(9)
[20] McClements DJ, Grossmann L. The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. Compr Rev Food Sci Food Saf. 2021;20(4):4049–100

an essential role in food formulation. In addition, macroalgal carbohydrates can play a role as techno-functional ingredients acting as emulsifying agents texture modifiers, helping as fat substitutes and improving the water absorption capacity of foods. In this regard, a strong influence on the flavour, texture and consumer acceptance of food products formulated with macroalgae carbohydrates.

The challenge we can find in this type of food is its exploration, harvested manually, for example, in the case of the brown algae Ascophyllum nodosum and the red calcified algae. However, the future implementation of mechanical harvesting methods, which are not applicable in all producing countries, will likely require a review of existing legislation to maintain appropriate access rights and sustainable harvest yields.

Mechanical harvesting of Laminaria spp. is already present in several European countries, such as France and Norway, providing a valuable resource for making such revisions[21].

2.3.2 Insects

Edible insects have been part of our diet since ancient times, being part of the diet of many countries, but there is still some resistance to their consumption in some regions of the globe.

We are considering the current global situation in terms of population growth and environmental sustainability. There is a need to introduce edible insects in our diet due to an increase in consumer demand, reduced availability of arable land, the lack of alternative sources of protein, and reduced animal production, which has a significant impact on climate change.

In nutritional terms, most insects contain the necessary nutrients for a human being's diet, including fat, protein, fibre, vitamins and minerals. We can consider that many insects can be valued and safe food ingredients[22],[23].

These insects can be consumed as eggs, larvae, pupas, or as adults. The protein value of this type of food ranges from 20% to 70% in dry matter, with an average digestibility of 76%-98%. Furthermore, its fat content ranges from 10% to 50%.

However, there is a variation between insect species determined by some factors: environmental factors (life stage, food and sex) and their creation. These contain all the essential amino acids but are characterized by having low methionine and cysteine values but are rich in lysine, tryptophan and threonine[24].

We can use these insect foods and transform them into by-products, such as insect flours and protein powders or insect oil extraction. An approach to obtain nutritional and sustainable benefits from insect consumption is to grind insects into powder form or utilize ingredients derived from insects such as protein, lipids and chitin. We can produce flour by dehydrating or roasting whole insects, then grinding them into a fine powder form.

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[21] Gomez LP, Alvarez C, Zhao M, Tiwari U, Curtin J, Garcia-Vaquero M, et al. Innovative processing strategies and technologies to obtain hydrocolloids from macroalgae for food applications. Carbohydr Polym [Internet]. 2020;248(July):116784

[22] Skotnicka M, Karwowska K, Kłobukowski F, Borkowska A, Pieszko M. Possibilities of the development of edible insect-based foods in europe. Foods. 2021;10(4).

[23] Nowakowski AC, Miller AC, Miller ME, Xiao H, Wu X. Potential health benefits of edible insects. Crit Rev Food Sci Nutr [Internet]. 2020;0(0):1–10

[24] Orkusz A. Edible insects versus meat—nutritional comparison: Knowledge of their composition is the key to good health. Nutrients. 2021;13(4).

But first, we need to Insect oil extraction is critical to obtain a high yield of quality food/feed oil and facilitate subsequent protein isolation, to produce a better protein profile product and be more healthy[25].

The main limitation of this type of food is the acceptance by the consumer. However, its consumption is beneficial in terms of health and, as an alternative source of protein, sustainable for the environment. However, there is still a tendency to resist its consumption in Western countries. Another apparent limitation is food safety, divided into three categories: Allergens, Biological Hazards and Chemical Hazards.

Some authors point to some proteins in edible insects, including arginine kinase, as a potential allergen source. Other common allergens are α-amylase and tropomyosin, which is also present in shellfish, and there may be a risk of cross-reaction.

Another limitation inherent in consuming this type of food is that some insects contain a layer of chitin, which gives the insect some resistance. Nevertheless, this layer has to be degraded by an enzyme called chitinase. However, although present in all human beings in their gastric juice, this enzyme is particularly inactive in the European population[22,23,24].

The concept of an edible insect as a food product did not exist in European legal terms until 2018. However, its consumption was not banned by European legislation, leaving its use to the country's discretion. The production of edible insects for consumption through Regulation (EC) No. 178/2002 on food law principles and general rules. It is necessary to identify potential hazards by applying the precautionary principle.

As this was new food, it was possible to introduce edible insects through Regulation (EC) No. 258/97. Nowadays, the introduction of these products is subject to Regulation (EU) 2015/2283, concerning the introduction of novel foods. However, products placed on the market before 2018 under the rules previously applied must be reported to the European Commission with "new food" or "traditional food from countries outside the European Union[25,26,27].

In terms of legislation, there is little matter in referring to these products. However, all edible macroalgae species are not authorized as food or food supplements. The consumption history of these species affects their regulatory status. The entry of macroalgal species and extracts on the market is regulated by the Novel Food Regulation, which states that the species have not been used as food to a significant degree in any of the EU member countries. In particular, regulations (EC) No 258/97) and (EC) 2015/2283.

2.3.3 Cellular Meat

More and more consumers consider that the health of the planet is essential, and that is why the development of laboratory meat has been increasing. This trend has several advantages for the earth's sustainability (figure 8). This market has developed since 2013 when the first cultured beef hamburger was produced. Several companies are developing laboratory meat for the production of chicken, beef and pork. It should be noted that although there are several advantages in this

[25] Liceaga AM. Processing insects for use in the food and feed industry. Curr Opin Insect Sci [Internet]. 2021 Aug;1–12. Available from: https://doi.org/10.1016/j.carbpol.2020.117314
[26] Parlamento Europeu e Conselho da União Europeia. Regulamento (UE) 2015/2283, de 25 de novembro de 2015. J Of da União Eur. 2015;L 327/1:1–22.
[27] Parlamento Europeu, Conselho da União Europeia. Regulamento (CE) N.o 258/97 do Parlamento Europeu e do Conselho de 27 de Janeiro de 1997. J Of das Comunidades Eur. 1997;40(43):1–6.

process, there are still some difficulties in terms of nutritional value, texture and flavour that need to have more researched to be successful in the future market[28].

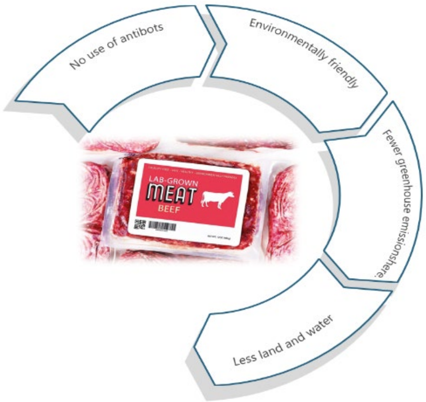

Figure 8. Advantages Meat lab

These developed products are created from cell cultures of live animals using tissue engineering and synthetic biology. Overall, two different sets of technology, processes and methods can be used to produce cell-based material: fermentation-based methods and tissue engineering-based methods. The first method is based on common industrial biotechnology to produce milk, egg whites and other meatless animal products. It usually involves the genetic modification of bacteria, or yeast, that are fermented to produce proteins and fats. The second method is based on biotechnologies applied to animal cells to create muscle tissue similar to animal flesh. This method combines tissue engineering with biofabrication technologies, such as bioprinting, which allows the formation of skeletal muscle tissue for human consumption.

This type of product can also be considered as "personalized nutrition, in which we can analyze the genetics of each consumer. Many startups are already working on commercialising these products; however, the field is still very early and has two significant challenges to overcome: the high costs for small production and consumer acceptance.

It is important to note that laboratory meat is considered a "new product" and requires a regulatory framework. Furthermore, regulation is not only crucial for consumer food safety but at the same time allows the consumer to gain confidence and accept this new product. This new food was revised in the EU and came into force in 2018 and was followed by a series of implementing acts and guidelines from the European Food Safety Authority (EFSA).

The mind map (figure 9) highlights the five critical areas for the advancement of the laboratory meat industry and which will be the following steps: cell lines, culture media, scaffolding and structuring, bioreactors, and supply chain distribution[29].

[28] Stephens, D.N., Dunsford, I., Lucy Di Silvio, Ellis, D.M., Glencross, A., Sexton, D.A., Bringing cultured meat to market: Technical, socio-political, and regulatory challenges in Cellular Agriculture, Trends in Food Science & Technology (2018), doi: 10.1016/j.tifs.2018.04.010
[29] Cassiday, Laura. (2018). Clean meat. INFORM International News on Fats, Oils, and Related Materials. 29. 6-14. 10.21748/inform.02.2018.06.

Figure 9. Mind map for laboratory meat

2.4 Protein extraction Methods

The need for protein has increased in recent years because of the increased population and the difficulty of keeping up with this growth in food production. To keep up with this trend, we often lead to over-exploitation of soils, making the food produced over crops nutritionally impoverished[30]. Because of these consequences of modern times, we need to find alternative protein sources, and we can resort to extracting protein from food so that protein needs are met and are of nutritional quality.

The protein extraction processes we have can consider more classic or innovative. In classical methods, we have the case of extrusion, and it is a common and widely used practice to convert 50-70% of herbal materials containing proteins into fibrous products. Several raw vegetable proteins are used as ingredients for extrusion, such as defatted soy meal, soy protein concentrate and isolates, wheat gluten, pea protein concentrate and isolate, and peanut protein.

We can divide innovative methods into the following categories: dry protein extraction, wet protein extraction, and cell breakdown.

There are two types of extrusion processes based on the amount of water added during the process; low moisture extrusion (20-40% added moisture) and high moisture extrusion (40-80% added moisture). Textured proteins with low moisture normally must be rehydrated before use, usually combined with other ingredients. Extracted products with high moisture content may not require further processing before use. (Ismail.2020)

In wet extraction methods, Protein extraction generally starts with the solubilization of the rich protein source in a medium with a pH far from the isoelectric point, followed by its precipitation in a medium with a pH close to the isoelectric end of the solubilized proteins. The other approach is to achieve protein solubilization using saline solutions, followed by protein pre-precipitation caused by salt removal by ultrafiltration and diafiltration. The protein thus produced has a micellar structure before being dried, with a preserved native state.

Finally, cell disruption is classically performed by mechanical methods (e.g. crushing, grinding) or thermal and chemical treatments. However, due to the high sensitivity of proteins to heat or solvent use, new processing technologies have emerged and have been used for cell disruption, demonstrating more efficient yield, extraction time, costs and environmental impact (e.g., extraction assisted by microwave, ultrasound-assisted extraction, supercritical fluid extraction, pressurized liquid extraction, pulsed electric field) Although several new technologies in the food industry have emerged, ultrasound and microwaves have been identified as the most convenient from an economic point of view, processing and energy efficiency.

A group of researchers introduced shear cell technology at Wageningen University, the Netherlands, around 2005. It is another technique where a combination of shear and heat is used to form meat analogues with layered fibrous structures, which resemble the mouthfeel and texture of an authentic beef steak. The cutting device used in this technology is called a shear cell, where intensive shear can be applied. There are two types of shear cells: conical cells based on conical plate rheometer and cylindrical-shaped Couette cell, which was developed for a magnification process.

In addition to their advantages, these protein extraction processes also have some disadvantages. These are as follows: 1) it is an extensive process, which requires large amounts

[30] Pam Ismail B, Senaratne-Lenagala L, Stube A, Brackenridge A. Protein demand: Review of plant and animal proteins used in alternative protein product development and production. Anim Front. 2020;10(4):53–63

of water and energy, 2) the protein extraction yield decreases when extracting extracts from proteins with high purity and 3) the process can change the structure of the native protein. The last disadvantage can considerably reduce the interface stabilizing properties of plant proteins, especially globulins[31].

In short, we can achieve different product qualities in the protein extraction process by reducing or enhancing some characteristics. By extracting the protein content on food products, we can produce products with thickened starchy texture, fibre mouthfeel and anti-nutritional components. And to improve protein gel strength (less network disruption), formulation flexibility, protein nutrient density and digestive tolerance.

With this evidence, we can conclude those protein extraction methods can make a difference in our lives these days, supporting the lack of good protein sources to support the escalating global population growth and lowering extended agricultural effects on the soil. But like many innovative products, have some disadvantages, and we need to weigh in the consequences of these methods to the traditional agriculture process

[31] Yang J, Sagis LMC. Interfacial behavior of plant proteins — novel sources and extraction methods. Curr Opin Colloid Interface Sci [Internet]. 2021;56:101499

3 Conclusion

Several new alternative proteins will stand out in the coming years, from plant-based and religion-based proteins to laboratory meat. Regarding plant-based proteins, it is essential to remember that there are still several obstacles to overcome for the products to succeed after being launched on the market. Consumer acceptance of the final product is crucial; however, some proteins still need to be improved for flavour. In the case of insects and laboratory meat, these markets already existed in Asia; however, due to all the climate changes that we have observed, new markets are beginning to develop focused on insect protein and laboratory meat. In this case, there are several studies on which insects we can eat and what the nutritional profile of each one is. However, we would like to point out that it is always important to carry out a risk-benefit assessment of each new alternative protein. In conclusion, for the future it is necessary to understand the complex interactions between proteins and other ingredients in formulations and during food processing.