UNIVERSITÀ DEGLI STUDI DI PADOVA
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Meat vs. meat alternatives: weighing up the pros and cons based on their sustainability and human health

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Summary

Abstract

Feeding the growing global population while facing the current threat of climate change represents the greatest challenge that *Homo sapiens* has ever faced. The recent allegations of environmental, social, and ethical impact raised against the production of conventional meat have provided the opportunity for food industries to create the so-called meat alternative products. These represent an emerging group of foods aimed to provide the consumer with the same organoleptic characteristics of conventional meat but with a lower environmental impact and without killing any animal. To date, meat alternatives are produced starting from various protein-rich edible materials, among which plants, algae, fungi, insects, and cell cultures are the most studied ones. These protein sources are then processed, through a series of industrial processes, into whole muscle-based or (coarse or fine) particle-based alternatives. Despite the recent market growth, the development of these meat alternatives is still strongly slowed down by various technological, economic, and societal factors. Among these, the most important are by far the scant knowledge about the raw materials and the technologies used; the presence of substances potentially hazardous for human health; and the negative perception that many consumers have towards these products. Furthermore, the lack of epidemiological studies on their long-term consumption generates a serious gap that prevents the assessment of their potential sustainability. Therefore, the food industries should focus their financial resources towards further research intended to cover these knowledge gaps, which are essential to ensure the successful development of these meat alternatives.

Riassunto

Nutrire la crescente popolazione globale mentre si combatte l'attuale minaccia dei cambiamenti climatici rappresenta la più grande sfida che *Homo sapiens* abbia mai dovuto affrontare. Le recenti accuse di impatto ambientale, sociale ed etico sollevate verso la produzione di carne convenzionale hanno fornito l'opportunità alle industrie alimentari di creare i cosiddetti prodotti alternativi alla carne. Questi rappresentano un gruppo emergente di alimenti volti a fornire al consumatore caratteristiche organolettiche simili a quelle della carne convenzionale, ma con un minore impatto ambientale e senza uccidere alcun animale. Ad oggi, i prodotti alternativi alla carne vengono ottenuti partendo da materie prime edibili ricche in proteine, come piante, alghe, funghi, insetti e colture cellulari. Queste fonti proteiche vengono poi elaborate, attraverso una serie di processi industriali, nell'intento di ottenere prodotti simili a pezzi di carne da animali o formulazioni a base di particelle (grossolane o fini). Nonostante la recente crescita di mercato, lo sviluppo di questi prodotti alternativi alla carne è ancora fortemente rallentato da diversi fattori tecnologici, economici e sociali. Tra questi, i più importanti sono di gran lunga la scarsa conoscenza delle materie prime e delle tecnologie impiegate, la presenza di sostanze potenzialmente pericolose per la salute umana e la percezione negativa che molti consumatori hanno verso questi prodotti. Inoltre, la mancanza di studi epidemiologici sul loro consumo a lungo termine genera una grave lacuna che impedisce di determinarne la potenziale sostenibilità. Pertanto, le imprese alimentari coinvolte nella produzione di prodotti alternativi alla carne dovrebbero focalizzare le loro risorse finanziarie verso ulteriori ricerche atte a colmare queste lacune.

Introduction

The 10 billion of humans estimated for the 2050 represent a prominent challenge for the current food system (Theurl et al., 2020). The food system includes all the actors and activities carried out along the supply chain of food products, including the related economic, societal, and natural environments as well. A food system is considered sustainable when is able to ensure nutritious and safe food to everyone without negatively impacting its economic, societal, and environmental bases. Therefore, the system relies on three different forms of sustainability: 1) economic sustainability: it must be profitable; 2) social sustainability: it must benefit the society; 3) environmental sustainability: it must have a positive or neutral impact on the environment (Nguyen, 2018). The food system currently adopted in most countries, however, causes detrimental effects on the environment, animal welfare, and human health as it heavily depends on industrial animal agriculture (Bryant, 2022). In particular, meat is usually the one accused of such effects albeit these problems are not related to meat consumption itself, but rather to its overconsumption, and the consequent overproduction (Smetana et al., 2023). Accordingly, consumers have become more concerned about their diet-related consequence, and thus started to reduce their meat intake by shifting their diets from animal-based towards plant-based diets. The popularity growth of both vegetarian and flexitarian diets, together with the related increase in plant-based foods demand, have led to a remarkable acceleration in the development and production of a variety of meat-free protein-rich foods, with particular relief towards meat alternatives (Starowicz et al., 2022).

Meat alternatives are emerging products, also called meat substitutes or alternative proteins, that attempt to mimic conventional meat in terms of nutritional values and sensory properties such as flavour (the combination of aroma and taste), tenderness, juiciness and appearance (Starowicz et al., 2022). They have been created mainly to attract consumers that decided to lower their meat-intake frequency after becoming more cognisant about nutrition and health, but also towards common meat-eaters that seek more variety in the market of protein-rich foods (Xiong, 2023). These meat-free products are developed from a range of novel protein-rich edible materials, including plants (mainly pulses and cereals seeds), fungi, algae, insects, and *in vitro* cultured cells (Van der Weele et al., 2019). Most of the meat alternatives currently available in the global market are derived from vegetable raw materials and are generally sold as coarse particle-based products, like burgers, nuggets, and sausages. The other protein-sources, instead, have limited availability due to recurring barriers in their development. Nevertheless, the increasing capital invested in these alternatives are boosting the scientific research to find new production methods that are more resource-efficient and less costly (Xiong, 2023). During the past few years, the market of meat alternatives has seen remarkable acceleration regarding the release of new products worldwide, whereas consumers increasingly consider meat alternatives as a promising avenue for a better and healthier future (Bryant, 2022).

Despite years of scientific research and efforts, however, the scientific communities still encounter several technical hurdles and impediments in the recreation of whole muscle-like analogues. Particularly, the major challenge consists in the recreation of the extremely complex and hierarchical structure, which renders the muscle such a unique product. Indeed, there has not been a single meat-like construct considered a total success till now (Xiong, 2023).

Besides the technological impedances, the market development of meat alternatives is also hampered by the consumers' perception towards these products. The increasing variety of edible protein-sources, together with the novel technologies used for product structuring, have led to an increased occurrence of food neophobia and food technology neophobia, respectively, among consumers. The former refers to a form of disgust towards certain foods and/or to conservative behaviour towards traditional foods. Food technology neophobia represents the consumer concern towards emerging industrial technology which elicit different uncertainties about food safety and sustainability (Siddiqui et al., 2022a). Therefore, although meat alternatives are touted as the most promising solution for the future protein landscape, the current knowledge gap about technology, sustainability and safety needs to be covered through further investigations. Till then, the role of meat alternatives in the future and complex food system network will remain unclear.

The aim of this thesis is to bring a systematic overview of the most important protein-rich materials exploited so far encompassing themes such as market trends, production methods, and consumer perception. Furthermore, meat alternatives are compared with conventional meat based on their social sustainability, and thus with particular regard towards their nutritional compositions and health-related effects. To conclude, a SWOT analysis, summarizing strengths, weaknesses, opportunities, and threats of the assessed meat alternatives, will be used in the attempt to make a clear point about their future trends based on the current available knowledge.

Chap. 1 – The striated muscle

The muscle is an essential organ formed by a collection of different and specialized tissues. The most important are muscular, connective, and adipose tissues, in order of presence; other tissues include nerves and blood vessels. The muscular and connective tissues are distributed in order to create five hierarchical levels: whole muscle, bundles of fibres, fibres, myofibrils, and lastly myofilaments (Xiong, 2023). Among the first three levels, a thin sheet of connective tissue envelops the structure conferring the organ a unique structure.

The lowest hierarchical level is represented by the myofilament: protein fibres elongated in a filamentous structure present as thin (actin) and thick (myosin) filaments interposed with each other to form repeating units called sarcomeres (Brainkart, 2023). The sarcomere represents the smallest contractile unit of the muscle. It presents a highly organized structure (Figure 1.1) which is composed of myosin filaments surrounded by actin filaments and delimited by two Z disks on both sides (Ertbjerg & Puolanne, 2017). The Z disk is a network of protein fibres arranged perpendicular to both actin and myosin filaments and thus has a stabilization function. The Z disk also provides the anchoring points for the actin filaments, with which form a complex called I-band. The central portion of the sarcomere, where myosin and actin filaments overlap with each other, represents the A-band (Brainkart, 2023).

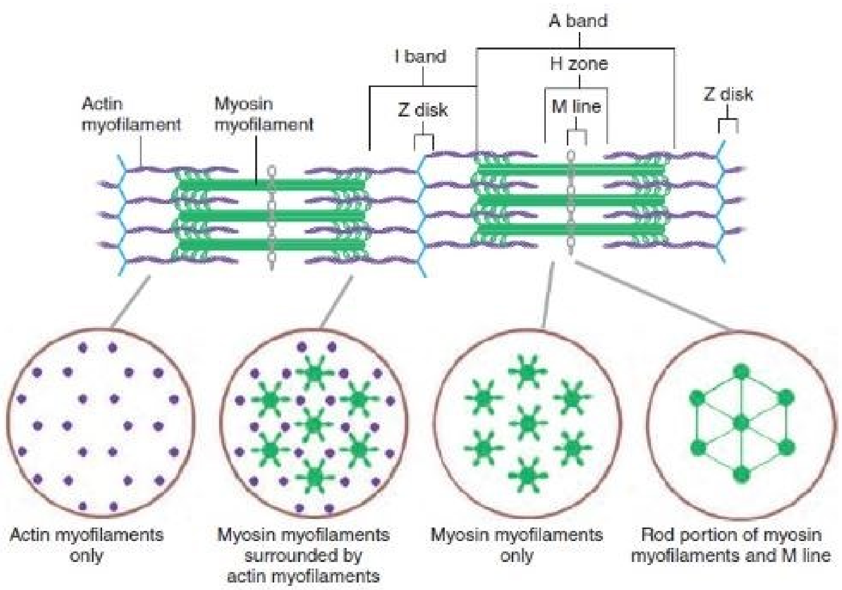

Figure 1.1 – The highly organized structure of two adjacent sarcomeres with transversal organization of the two myofilaments, namely actin and myosin (Brainkart, 2023).

During muscle contraction, the myosin heads attach iteratively to the actin binding sites causing the sarcomere shortening. This process is elicited by a nervous impulse, transmitted through the neuromuscular junction; whereas, the sarcomere shortening is ATP-driven, which is break-down by myosin heads producing energy and heat (Ertbjerg & Puolanne, 2017). The sarcomere length has a profound, effect also in the animal post-mortem phase for the muscle-to-meat conversion process and thus on the final sensory properties of meat, in particular tenderness and water-holding capacity (Ertbjerg & Puolanne, 2017). Finally, the sarcomeres feature the repetitive unit of the myofibril: a threadlike structure composed of segments (sarcomeres) and organized in bundles to form the muscle fibre (Brainkart, 2023). The muscle fibre represents the cell of the muscle since it possesses a cell membrane (the sarcolemma) along which runs the blood vessels together with several nuclei. The single fibre is stabilized by a thin sheet of connective tissue called endomysium (Brainkart, 2023). Several muscle fibres compose a fibre bundle, which is also enveloped with connective tissue (perimysium), and finally clusters of fibre bundles made up the whole muscle. Even the muscle is surrounded by a layer of connective tissue called epimysium (Xiong, 2023). The whole muscle structure is depicted in Figure 1.2.

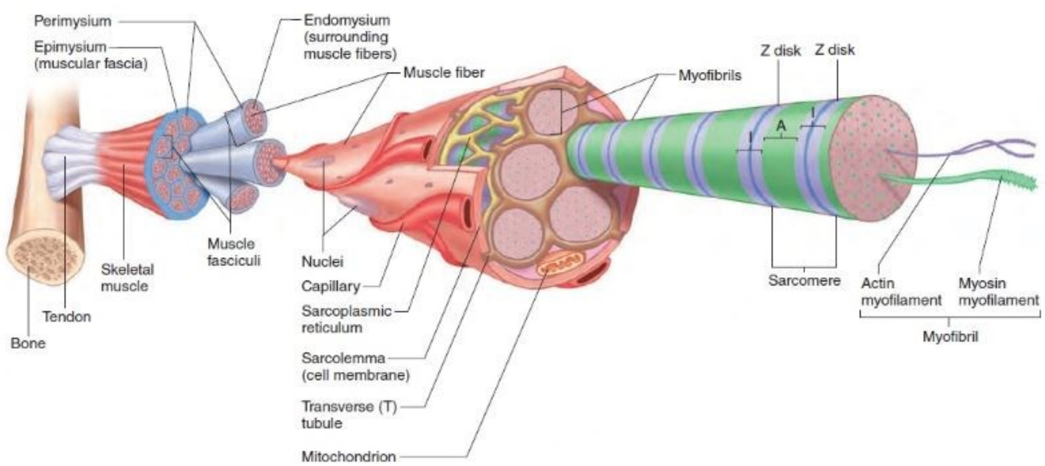

Figure 1.2 – Hierarchical structure of the striated muscle (Brainkart, 2023).

Besides the staple hierarchical muscle structure, it is important to also consider the different types of muscle fibres. Muscle fibres can be widely classified based on their structural or functional features, such as colour (white and red fibres), contraction speed (slow and fast fibres) and metabolism (oxidative, glycolytic, and oxido-glycolytic fibre). Another approach was carried out by Brooke & Kaiser (1970) which classified muscle fibres in I, IIA and IIB based on the histochemical myofibrillar ATP-ase. Classifying the muscle fibres is important because their metabolic and contractile activity affects the muscle chemical composition, thus the meat sensory properties during muscle-to-meat conversion. In Table 1.1., white muscles (mostly IIB fibres) are characterized by a fast contraction speed and a predominant glycolytic metabolism. Hence, they generate ATP mostly from muscular glycogen and phosphocreatine which stores are relevant. On the other hand, red muscles (mostly I fibres) feature a slow contraction speed and a predominant oxidative metabolism, thus the ATP is mostly produced through the aerobic catabolism of both glucose and fats. Indeed, red muscles present high content of lipids but less of glycogen and phosphocreatine compared to white muscles (Bottinelli & Reggiani, 2000).

Table 1.1 – Muscle fibre types based on structural and metabolic features.
Where ↑↑: high presence; ↓: low presence (Brooke and Kaiser, 1970; Bottinelli and Reggiani, 2000).

	MUSCLE FIBRE TYPES		
Brooke and Kaiser classification	I	IIA	IIB
Structural features			
Colour	red	red	white
Myoglobin content	↑↑	↑↑	↓
Contraction speed	slow-twitch	fast-twitch	fast-twitch
Metabolic features			
Predominant metabolism	oxidative	oxido-glycolytic	glycolytic
Glycogen	↓	↑↑	↑↑
Phosphocreatine	↓	↑↑	↑↑
Lipids	↑↑	↑↑	↓
Myofibrillar ATP-ase	↓	↑↑	↑↑

The striated muscle is also composed of other two important tissues: the connective and the adipose tissue. The former is mainly composed of collagen: the main structural protein found in the extracellular space of various connective tissues, such as tendons, cartilage, bones, skin, etc. Collagen provides structure and strength to the muscle, with also effects on some meat properties such as tenderness and texture. Meat toughness, indeed, strictly depends on the amount of mature, heat-stable cross-links set between the collagen molecules that make up epimysium, perimysium, and endomysium. The proportion of these cross-links increases with the animal age, but it is also affected by genetics and other external factors such as nutrition, rearing conditions and growth rate (Weston et al., 2002). The adipose tissue can be found in the muscle as adipocytes and intramuscular lipids. In the adipocytes, lipids are stored mainly as triglycerides (> 90%), whereas a marked portion of intramuscular lipids consists of phospholipids since they are the main structural components of cell membranes. Other lipids that can be found in the muscle are glycolipids and steroids: the former is an important constituent of nerve cell membranes; the latter, instead, is commonly found as cholesterol which can be found in both cell membrane and lipoprotein particles. The muscular fatty acid profile is highly variable among species due to the different metabolism and diets; however, it can change also among different breeds (Wood et al., 2008).

This simplified explanation about the structure of the striated muscle serves to give an idea of what these emerging meat alternatives are trying to emulate. Considering the extremely complex hierarchical structure, is not surprising that its recreation remains the most important technical challenge encountered so far despite the endeavours of the food industries that want to produce whole muscle-like products (Xiong, 2023).

Chap. 2 – Muscle-to-meat conversion

According to European Regulation No. 853/2004, meat is a food product derived from a part (muscle or edible tissue) of a living animal that undergoes gradual degradative processes which involves metabolic, physical, and structural changes along the animal *post-mortem* phase (Wood et al., 2023). All the conversion steps, together with the processing conditions, are essential for meat production since they deeply affect its most important sensory properties. Indeed, abnormal conversion and processing conditions could lead to undesired changes in meat compromising the fresh consumption and processing processes.

The muscle *post-mortem* changes begin with the removal of blood from the stunned animal through bleeding. Suddenly, the muscle no more receives both the energy sources and oxygen essential for its natural functions, while the metabolism products start to accumulate because can no longer leave the muscle through the blood flow (Meurant, 1986). The anoxia condition established by bleeding, firstly, stops the cell aerobic metabolism by blocking Kreb's cycle and the oxidative phosphorylation. As a response, the cell attempts to replenish the ATP deficiency by choosing Creatine Phosphate (CP) as substrate. CP is a high-energy compound that accumulated in muscles, which stores however last only a few minutes. After that, the new substrate becomes glycogen which is catabolized through anaerobic glycolysis. The anaerobic metabolism leads to an accumulation of lactic acid that lowers the intracellular pH, which drop, coupled with the carcass high temperatures, deeply affects the meat primary quality traits, especially colour, texture and Water-Holding Capacity (WHC) (Przybylski & Hopkins, 2015). Figure 2.1 summarizes the *post-mortem* trends of the energy substrates used in the muscular glycolysis and the related pH drop.

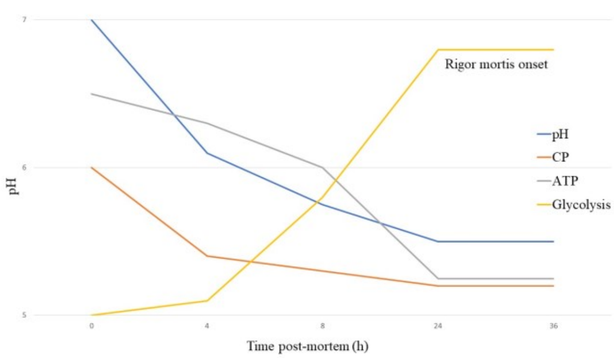

Figure 2.1 – Trends of the muscular glycolysis, the substrates used for muscle contraction reaction and the consequent pH drop.

As the level of glycolytic ATP decline, permanent cross-bridges between actin and myosin increase, reaching the maximum when ATP is depleted. The result is an irreversible muscle contraction called *rigor mortis*. Microstructurally, during the *rigor mortis* development the muscle presents the same structure as alive. During the resolution phase, however, the enzymatic degradation of the myofibrils leads to essential changes in muscle integrity. Therefore, *post-mortem* proteolysis rate is highly correlated with final meat tenderness, where degradative processes are carried out by the calpain/calpastatin system found in the sarcoplasm. Aside from the muscular tissue, the *post-mortem* changes affect also both connective and adipose tissue. Collagen undergoes a minimal proteolytic degradation, while cross-links are not affected. The lipid fraction, instead, is converted into precursors of both lipophilic and volatile aromatic compounds through several chemical reactions including hydrolysis and oxidation. The physio-chemical *post-mortem* changes also influence meat colour. The colour is given by pigments, mainly composed of hemoglobulin and myoglobulin, and is essentially defined by the oxygen presence. When is absent, like during the early *post-mortem* period, the muscle is purple red because consists mostly of deoxymyoglobulin. After fresh meat is cut, the exposure to air oxygenates the myoglobulin located on the surface and thus obtain a bright red colour. Finally, if meat is stored in presence of oxygen, myoglobulin can oxidate resulting in a brown colour due to a metmyoglobulin prevalence on the other pigments (Przybylski & Hopkins, 2015).

The muscle-to-meat conversion represents a sequence of complex physio-chemical changes that result essential to obtain such a unique product which is meat. Furthermore, there are still processes that are unknown but still have important effects in meat quality traits (Przybylski & Hopkins, 2015). Accordingly, after seeing how many complicated processes the muscle must encounter to be converted into meat, is obvious to ask how the food industries can faithfully replicate these processes (and the unknown ones) to produce their meat alternatives. The knowledge gap about these conversion processes remains a prominent challenge for the successful replication of meat sensory properties.

Chap. 3 – Meat alternatives

Before assessing meat alternatives, it is necessary to clarify the terms used to describe the substitutes for meat-base products. Firstly, "meat alternative" is a general term which refers to a different protein source that can be consumed to replace meat. The aim of these food products is supplying alternative protein to the consumer without necessarily mimicking the nutritional and sensory properties of meat. Conversely, "meat analogue" precisely refers only to foods able to mimic and thus convey the sensory properties of conventional meat (Smetana et al., 2023). Structurally, meat analogues can be classified into two groups: 1) whole muscle-like products; and 2) particle-based restructured products. Furthermore, the second group consists of other two subcategories: coarse particle-based and fine particle-based products. Whole muscle-like products might include all the foods aimed to structurally resemble the animal muscle. Coarse particle-based analogues mimic the texture of ground meat and are used to create products such as burgers, nuggets, and sausages. This group includes the most sold meat analogue types. Lastly, Fine particle-based analogues consist of gelled emulsion that replicate the matrix of conventional frankfurters (Xiong, 2023).

The meat alternatives produced so far derive from various alternative proteins including plants (mainly legumes and cereals), algae, fungi, insects and cultivated animal stem cells.

3.1 – Plant-based alternatives

Plant-Based Meat Alternatives (PBMAs) are protein-rich foods basically produced from vegetable proteins to provide consumers a wider range of meat-free choices. The market of PBMAs has recently diverted towards the strategy of faithfully mimicking conventional meat in terms of flavour, texture, appearance, and nutritional value through specific products called "Plant-Based Meat Analogues" (Andreani et al., 2023).

Although in Asian countries PBMAs like tofu, tempeh and seitan are traditionally consumed for a long time, these products still represent a novelty for most of the countries worldwide. However, the meat shortages and the consequent price fluctuations caused by the recent COVID-19 pandemic prompted the shift towards these plant-based products. Indeed, the PBMAs market has undergone a marked acceleration in product launches, with the highest spike during the COVID-19 pandemic. Despite this rapid growth, the market shares of PBMAs are still significantly lower compared to those of meat sector. Furthermore, meat market shares are forecasted to remain similar to the current ones, without any declining trends in the near future. PBMAs are still a weak analogue for meat products, indeed they are seen most likely as an alternative rather than a replacer. However, the wide availability of PBMAs in the supermarkets, coupled with their increasing demand, reflects an active and profitable market, worthy of further exploration (Andreani et al., 2023).

PBMAs are produced starting from plant proteins which include a huge variety of heterogenous proteins differing in structure and functionality (Xiong, 2023). The most used plant proteins in PBMAs are legume and cereal proteins. From the legumes' seeds (pulses) are extracted the globular storage proteins, mainly from soybean, peas, lentils, and kidney beans. From cereals like wheat, corn, oat, rice, and barley are extracted proteins in the form of fibrous polypeptides, therefore called filamentous proteins (Lee et al., 2023). Other types of vegetable proteins are extracted from potatoes, pseudocereals (like amaranth and quinoa), oilseeds (like canola, sunflower and flaxseeds), cauliflower, and jackfruits (Kumar et al., 2022a). Soybean provides the most used vegetable proteins for PBMAs production due to their functional properties that are water- and fat-absorbing, emulsifying, and gelling activities. Besides that, soy is widely used also due to its availability, low cost, organoleptic features, and nutritive value (Kumar et al., 2022a). However, other pulse proteins (like those of beans, peas, lentils, and chickpeas) are gaining importance mainly because of concerns regarding soy cultivation, such as biodiversity valorisation and GMOs perception, besides the absence of allergenic compounds (Andreani et al., 2023). Soy and pea proteins are currently the most used ingredients to recreate meat-like texture, whereas other plant proteins contained in wheat, potato, mung bean, and rice proteins serve for structural and nutritional purposes (Safdar et al., 2022). From cereals, the most important protein studied and used so far is wheat gluten. The common use is essentially explained by its availability (a by-product of the starch industry) and its functional properties including structuring and cross-linking capacity. These properties, in addition to its unique 3D network form, are exploited by the food industries to provide the desired meat-like consistency to meat alternatives (Kumar et al., 2022a). However, wheat gluten presents some limitations such as the increased cases of gluten allergy and celiac disease, as well as its water insolubility (Lee et al., 2023).

The most important steps of the PBMA production are four, namely raw materials selection, protein isolation, formulation, and processing, which are depicted in the Figure 3.1.1.

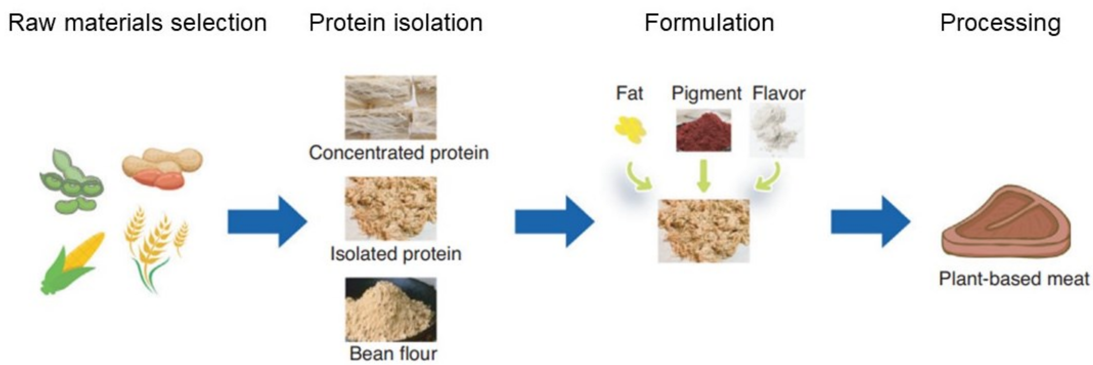

Figure 3.1.1 – Schematic diagram of the most important phases in the PBMA production chain (Zhang et al., 2022).

Regardless of the vegetable source, the most used raw materials in PBMAs production are protein isolates, with a protein content between 75 – 90%. However, their use is currently diminishing due to the used production techniques that are often considered inefficient and unsustainable. Conversely, besides the lower protein content (up to 50 – 65%), protein concentrates are acquiring notoriety in the PBMA production because considered more sustainable than protein isolates. Indeed, they are produced through dry separation rather than wet separation techniques, used instead in protein isolates, thus requiring less water and energy. Furthermore, dry separation processes allow to better preserve the protein technological functionality. The range of formulation ingredients rapidly expand when considering the number of sensory properties that must be recreated. Indeed, several plant-based ingredients are needed in order to replicate meat colour, flavour, and juiciness. The former is reproduced by primarily using soy leghemoglobin and red beet pigments, whereas flavour profile, which consists of both volatile and non-volatile compounds, is produced by using herbs and spices, useful also for covering the likely presence of beany off-flavours. Lastly, vegetable oils (such as sunflower, canola, and coconut) are added to mimic meat juiciness and other sensory attributes. However, fats has increasingly been replaced by binding agents, like starches, hydrocolloids or oleogels, because high-fat contents are correlated to a worsen in protein degradation, an essential process to obtain a high-quality meat-like structure (Andreani et al., 2023). Finally, when the necessary ingredients are gathered, the most suitable structuring technique is selected. The meat-like texture is achieved essentially through the conversion of pulse globular proteins into fibrous structure, that are elongated and ordered using different technologies, namely extrusion, wet or electro-spinning, freeze structuring and 3D printing (Andreani et al., 2023).

Extrusion technologies rearrange protein structures by using water, high temperatures, and pressure, which can be changed based on the desired final products. Extrusion processing consists of three steps: 1) mixing raw materials and water while heating; 2) compressing and 3) mixing the ingredient dough inside an extrusion chamber. The chamber consists of a metal barrel containing one or more co-rotating screws that mix and push the dough against the walls which are heated by a steam-jacket surrounding the entire chamber. While the dough is conveyed along the barrel by the screw, proteins are denatured by both heating and pressure. At the end of the barrel there is a die with different size and form based on the desired shape for the final product (Lee et al., 2023). The extrusion processes can be divided based on the used moisture level into low-moisture and high-moisture extrusion. The first processing method is performed at a low moisture level (30% or less) and used to produce Texturized Vegetable Proteins (TVPs). When extruded, TVPs are similar to ground beef, but hydration and another cooking are needed to obtain the typical meat-like texture. High-moisture extrusion (Figure 3.1.2), instead, works with high moisture levels (more than 50%) coupled with a cooling die to prevent dough expansion. This permits the fibres to align and stabilize creating the typical meat-like structure (Andreani et al., 2023).

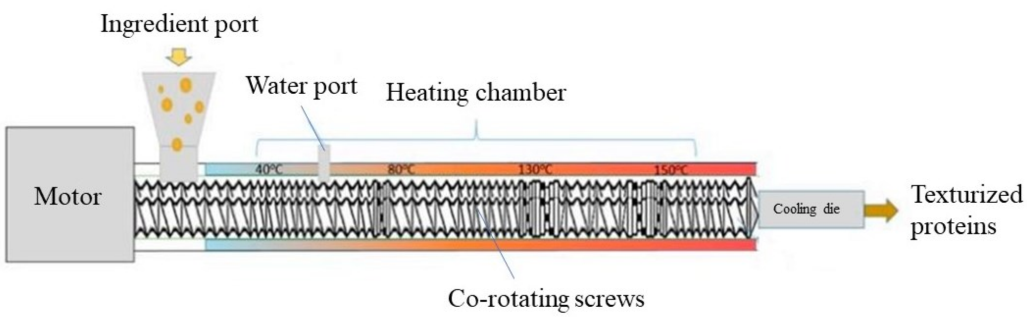

Figure 3.1.2. – Illustration of the schematic processing of high moisture extrusion (Zahari et al., 2022).

Extrusion is still the most widely used technology in PBMA production due to its high production rates and energy efficiency, in addition to the versality with other vegetable proteins. The high temperatures of the extrusion barrel result in the denaturation of the present anti-nutritional factors, however they are also responsible to trigger several Maillard reactions, which represent the main disadvantage of this technology since they might worsen the final product through colour changes, protein hydrolysis and pigment degradation (Boukid, 2021). To address the limitation of high temperatures, new technologies such as supercritical fluid extrusion (SCF) can be used. A SCF is a compressed gas with a high penetration capacity and low viscosity of a gas phase, whereas possesses the solvent power and decreased surface tension of a liquid phase. Particularly, in extrusion processing is used the supercritical carbon dioxide (SC-CO_2) which presents a low critical temperature (31 °C) that allows the preservation of heat-labile nutrients thus improving the product nutritional value (Kumar et al., 2022a). The spinning technologies includes wet spinning and electrospinning methods, both produce ultrathin protein fibres starting from a stable solution of polymers. In the first spinning method, an alkaline polymer solution is immersed in an acid bath by passing through a spinneret where the polymers precipitate and then solidify, thus obtaining thin filaments that can be elongated and rearranged to create defined fibrous protein products. In the electrospinning processing, instead, the polymer solution is subjected to an electric charge while passing through a spinneret, thus allowing instant evaporation of the solvent obtaining ultrathin dry protein fibres that are rearranged with spinning movements (Figure 3.1.3).

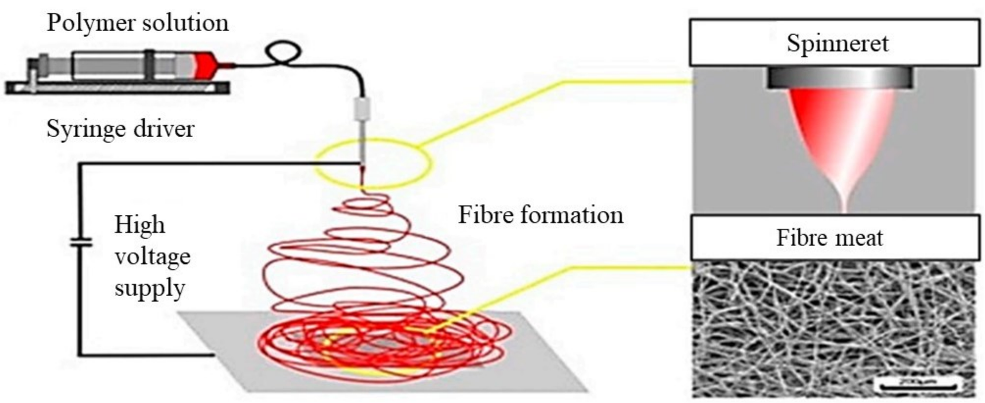

Figure 3.1.3 – Schematic picture of electrospinning processing (Zahari et al., 2022).

Electrospinning is increasingly replacing wet spinning because more cost-effective and scalable. Furthermore, the large amount of wastes, in addition to the need of a solution with chemical additives, high salt concentration and low pH, make wet spinning less sustainable and feasible for the industrial production of PBMAs (Boukid, 2021). Freeze structuring/alignment enable the formation of a meat-like texture that is achieved by freezing and subsequently drying an emulsion of vegetable proteins. During the freezing phase, the creation of ice crystal layers develops a unique porous microstructure of proteins that resemble the muscle. Protein alignment, and thus their textural properties, can be tailored by the proper freezing conditions and the direction of heat removal. The high temperatures applied during drying ensure the stabilization of the protein fibrous texture (Kumar et al., 2022a). Besides the scale-up potential possessed by this technology, there are several freezing conditions that still need proper control and optimization to ensure an adequate supply of PBMAs for an enlarged market (Boukid, 2021). Finally, the 3D printing technology is another novel technology used by food industries, based on additive manufacturing that allows to produce vegetable proteins with tailored structures and shapes resembling muscle fibres. Furthermore, this method ensures extreme flexibility in the development of product shape, texture, nutritional content, and flavour. Currently, 3D printing is considered highly sustainable and energy efficient as it leads to an efficient use of the ingredients while allowing a wider control on the compositional and nutritional aspects of the products. However, since it is still a novel technology, limitations such as the high production costs and the knowledge required for the printing procedures represent the major hurdles for its industrial utilization (Kumar et al., 2022a).

Despite the current technological innovation, another prominent barrier for the future spread of PBMAs is related to consumer perception. Identify and understand the main drivers and barriers affecting consumer acceptance is essential for the future reformulation of meat alternatives. Both drivers and barriers include a large variety of attributes that goes from the more standard ones such as taste, price, and convenience, to those more indirect including health, sustainability, animal welfare, and familiarity. Furthermore, these attributes are extremely subjective, having a different importance depending on geographical regions, income status, habits, and attitudinal factors. This is only a briefly explain of the extreme complexity behind consumer behaviour and how difficult is for the food companies to understand it (Boukid, 2021). To assess consumer perception towards PBMAs, past studies have relied on surveys distributed among consumers. According to the surveys, the main drivers for PBMA purchasing might include health and environmental sustainability. Although a lower price could also be a strong motivator to consume such products, a competitive price will unlikely be achieved in the near future. PBMAs have been created to achieve acceptance also by meat eaters. However, the ideal of recreating the unique and complex meat properties has also affected consumer perception towards these new products, which have increasingly been depicted as ultra-processed foods. Indeed, the large amount of food additives used especially in the recreation of meat colour and flavour is likely to convey a sense of unnatural as well as unhealthy among consumers. Besides, the long list of unfamiliar ingredients, consumer perception is negatively affected also by the high degree of processing that these products require. Indeed, food innovation is known to be related to both food and food technology neophobia (Andreani et al., 2023). Finally, considering that PBMAs are essentially emerging products, the low familiarity of consumers towards them could affect their market expansion as long as appropriate education interventions focused on both preparation and cooking methods are deployed (Siddiqui, 2022a).

In the last years, PBMA market have seen a rapid increase worldwide; however, to keep this growth boosted, further research is required to improve technological processing and efficiency which in turn can help consumers to improve their perception and make rational choices.

3.2 – Algae-based alternatives

Algae are a highly-diverse group of marine organisms that have been consumed and traded so far in many countries of Asia, South-America and Africa, but represent a novelty for most Western countries, especially when presented as a protein-rich product (Van der Heijden et al., 2023). Algae are commonly divided based on size as macroalgae, pluricellular organisms also known as seaweeds; and microalgae, which are unicellular. The latter group includes the two dominant algae species consumed in human nutrition which are spirulina (*Arthrospira platensis*) and chlorella (*Chlorella vulgaris*) (Xiong, 2023). Both macro and microalgae have recently been identified as a potential source of alternative proteins due to their high protein content (up to 47 – 70% of dry matter) (Mellor et al., 2022), coupled with the huge biomass production and the attractive Essential Amino Acids (EAA) profile which, in some species, is comparable to some animal-derived proteins (Xiong, 2023).

Currently, algae are cultivated for different purposes: spirulina and chlorella are commonly consumed as dietary supplements whereas seaweeds are used to produce hydrocolloids including agar-agar and alginate (Geada et al., 2021). Besides human supplementation, microalgae species are cultivated also for food fortification to enhance the nutritional profile with proteins, fibres, micronutrients, and Essential Fatty Acids (EFAs). Despite the great number of microalgae species discovered (more than 200,000), only a few of them can be sold and thus allowed for human consumption. The Food and Drug Administration (FDA) has provided till now the GRAS

(Generally Recognized As Safe) status only to six species (Gohara-Beirigo et al., 2022), while the European Food Safety Authority (EFSA) have authorized the sale of eleven species, including spirulina and chlorella. Unlike microalgae, the number of macroalgae authorized by both FDA and EFSA is significantly higher due to their presence in the human diet for centuries (Geada et al., 2021). Microalgae have become part of the human diet only in recent times mainly due to their nutritional qualities which helped them to rapidly gain popularity among the food industries. Consequently, considering the current increase in revenue and sales of microalgae supplements and fortified products, the future market shares are expected to rapidly increase due to their potential use as food additives (Gohara-Beirigo et al., 2022). On the other hand, macroalgae are commonly subjected to industrial processing to produce hydrocolloids which are compounds presenting typic gelling and stabilizing activities, thus used in the formulation of desserts, canned foods, and meat cold cuts. Macroalgae are also consumed as whole food and used in recipe such as ramen and sushi (Geada et al., 2021). To date, algae-based meat alternatives have not been developed yet, plus the functional properties of algal proteins have been subject to few trials therefore presenting limited knowledge. However, some microalgae proteins featured excellent emulsifying and surface activity which renders them a potential ingredient in the formulation of fine-particle meat alternatives (Xiong, 2023).

The algae cultivation is divided based on the cultivation system (Figure 3.2.1), namely open and closed pond systems.

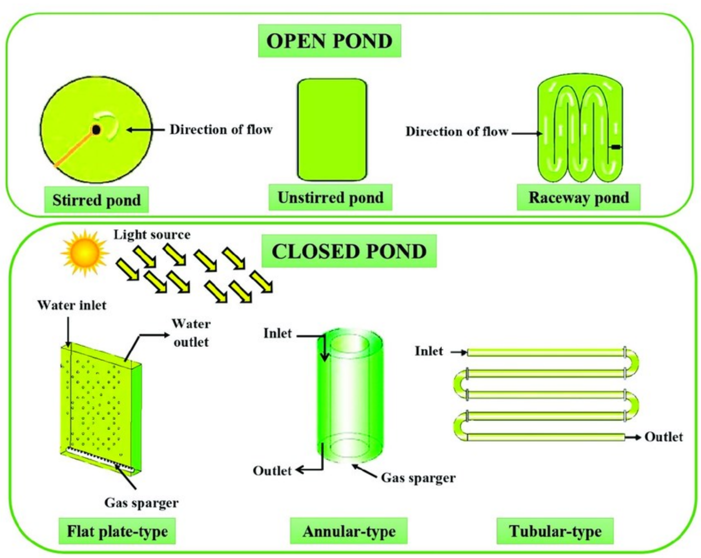

Figure 3.2.1 – Schematic illustration of open and close pond systems utilized in the cultivation of both macro- and microalgae.

The open systems represent the standard cultivation method for macroalgae, but it is commonly used also for microalgae due to the easier management and lower costs. However, the high risk of contamination and weather-dependence have boosted the diffusion of more controllable closed systems (Van der Heijden et al., 2023). These systems are emerging photo-bioreactors designed for microalgae cultivation that are increasingly replacing open system despite the numerous drawbacks including low production rates and high harvesting costs (Geada et al., 2021). Future technology innovations could assist the reduction of the high initial costs and energy requirements of close systems, allowing their scale-up for the industrial algae cultivation (Van der Heijden et al., 2023). After cultivation, algae biomass undergoes harvesting and dewatering processes where the latter consists in concentrating algal solid contents while requiring important amounts of energy. Once dewatered, the concentrated biomass must be dried to obtain a paste. Dehydration is the most critical process as drying conditions deeply affect algal sensory properties (including colour and odour), in addition to be the most energy-intensive step of the production chain. Drying is not essential for protein extraction, but it is useful to improve cell porosity and ultimately protein recovery. Indeed, algal proteins can be also extracted from wet biomass, however with additional pre-treatments, multiple extraction processes and a lower efficiency. The sustainability of the algae production chain mainly depends on dewatering and dehydration, therefore future research focused on improving these two critical steps will help to spread the industrial algae utilization as well as lower the environmental footprint of this alternative protein (Kumar et al., 2022b). With dehydration, algae biomass is ready to be commercialized as a whole food. However, the presence of a robust cell walls (particularly for microalgae) and the common high viscosity interfere with the bioavailability of the intracellular nutrients, thus lowering algal protein digestibility. Industrial treatments for protein recovery, in fact, represents a good alternative to obviate the poor algae digestibility. The available protein extraction methods firstly include the cell wall mechanical disruption, followed by a non-mechanical extraction. The first step consists in subject algal cells to high stress using pressure, shearing and electric fields, resulting in an improved solvent penetration, namely a quick access to the internal constituents. Some mechanical technologies include Bead Milling (BM), microwaves, Pulsed Electric Fields (PEF) and ultrasounds. The non-mechanical extraction instead exploits biological or chemical agents to further damage the cell walls as well as extracting the intracellular components. The most common mechanisms operate by using osmotic shock, enzymatic hydrolysis, or chemical extraction. Algae proteins are then separated into soluble and insoluble fractions through wet fractionation, which includes centrifugation or membrane technologies such as UltraFiltration (UF). While the insoluble fractions are excluded, the proteins in the soluble fraction undergo purification and final drying processes, thus obtaining protein concentrates (Geada et al., 2021). The production chain of algal protein concentrates is summarized in Figure 3.2.2.

Figure 3.2.2 – Principal steps used in the production of algal protein concentrates (Kumar et al., 2022b).

Currently, the extracted algal proteins are mainly used by food industries in the form of additives with a great potential application even in the formulation of some meat alternatives. However, the research gap regarding the structural and physiochemical properties (Xiong, 2023), exacerbated by an unfavourable pigmentation and fishy smell, represents one of the main limitations encountered in the industrial production of algae-base meat alternatives (Zhang et al., 2022). Since algae are novel foods in most developed countries, the biomass production still faces major challenges for large-scale cultivation, particularly regarding the production costs. Therefore, algal biomass production is still considered economically and energetically expensive for an industrial scale-up due to the strict environmental conditions required during algal growth and the numerous high energy-requiring processes essential for the recovery of algal proteins (Gohara-Beirigo et al., 2022).

As the market of algal meat alternative is developing, the knowledge regarding consumer preferences is still limited and poorly documented because can be based only on the consumer perception. Indeed, the assessment of food specific features, such as taste and appearance, represents an important gap for food manufacturers. A study conducted in three European countries (Germany, France, and the Netherlands) based on the perception of a large number of consumers showed the two main factors affecting consumer choices are the degree of meat consumption and health benefit communication. Consumers with high levels of meat consumption were included in the hard-to-convince target group, whereas consumer that perceive meat as unhealthy had positive perceptions and higher willingness to pay for meat alternatives. The other important factor regards a proper food labelling coupled with a good communication strategy. In the study, positive claims such as "local", "organic", and "environmental-friendly" showed a great potential to increase consumer preference and therefore market demand.

Despite being the newest raw material implemented in the production of meat alternatives, algae are yet a promising food innovation for several reasons. However, further research in food production, processing and marketing is necessary to ultimately develop a meat alternative based on algae and fully understand consumer perception towards these emerging food products (Weinrich & Elshiewy, 2019).

3.3 – Fungi-based alternatives

The fungi kingdom is characterized by a huge variety of species ranging from macrofungi (or edible mushrooms) to microfungal species including moulds and yeasts. Some species of this wide kingdom, such as mushrooms and truffles, have been consumed by many cultures for centuries therefore are currently strongly accepted thanks to their quality traits. However, these well-known species are not considered in the formulation of meat alternatives due to their low protein contents and growth rates. Conversely, filamentous fungi are considered valuable meat substitutes thanks to the high protein content and growth rates. Some species of filamentous fungi are not a novelty for some communities as they have been exploited for many years in the production of fermented foods to enhance their nutritional and functional properties. A classic example is represented by the species *Penicillium roquefortii* is used as an ingredient in the production of blue cheeses like Gorgonzola and Roquefort. Some species of filamentous fungi have recently been exploited by several food industries to produce great amount of protein-rich biomass (called mycoprotein) from the fermentation of vegetative mycelia. Mycoprotein is a whole food designated as Generally Recognized As Safe (GRAS) by the FDA since 2002 (Souza Filho et al., 2019). The most successful commercial source of mycoprotein is by far the fungal species *Fusarium venenatum* (Xiong, 2023). Besides *F. venenatum*, there are other five species presenting a high potential in the industrial cultivation of mycoproteins, namely *Neurospora intermedia*, *Pleurotus eryngii*, *Agaricus bisporus*, *Pleurotus sajor-caju*, and *Lentinula edodes*. These species are well-known foods, widely produced and available worldwide, therefore characterized by higher acceptance levels among consumers (Kee et al., 2022).

Currently, mycoprotein is commercially available and sold in the form of food ingredients mostly found in coarse particle-based products like burgers, patties, and sausages. The high protein content, coupled with the balance Essential Amino Acid (EAA) profile (Van der Heijden et al., 2023) make mycoprotein the best potential ingredient in the production of meat analogues. This is further enhanced by its quite unique fibrous texture (Kee et al., 2022) and sensory attributes, including a meaty flavour provided by its high content of sulphur-containing amino acids (Zhang et al., 2022). The characteristic fibrous texture, which derives from its high fibre content, renders mycoprotein appropriate to mimic meat consistency. The successful use of mycoprotein as a meat alternative building block can be explained by Quorn: a British brand commercialized since 1985 and sold in 15 countries worldwide (Hashempour-Baltork et al., 2020). Quorn is produced by combining filamentous fermented proteins (obtained by the fungus *F. venenatum*) with egg albumen and then sold as burgers, sausages, and nuggets (Figure 3.3.1).

Figure 3.3.1 – Representation of one of several products sold as Quorn.

The product does not require an industrial process like extrusion to recreate a meat-like texture due to the ability of mycoprotein to mimic muscle fibres. Therefore, Quorn represents the perfect example of a successful meat alternative, however further research is needed to transform it into a meat analogue (Xiong, 2023). In the last years, filamentous fungi have reached high market acceptance levels due to the related safety and health benefits. The mycoprotein global market has been growing since the Quorn launch, with market shares of 2019 expected to double by 2027 (Zhang et al., 2022).

The industrial production of mycoprotein starts from a fungal inoculum on a synthetic media: a sterilized solution containing glucose, ammonium, salts, and trace elements like biotin (vitamin B_7). The fungus grows naturally inside a bioreactor (Figure 3.3.2) where the environmental conditions are strictly controlled and constantly adjusted to maximize its growth rate.

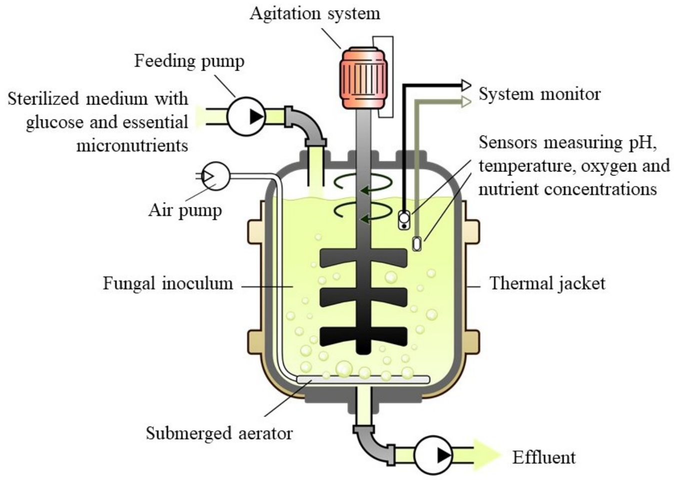

Figure 3.3.2 - Schematic representation of a bioreactor used in the production of fungal biomass (Hashempour-Baltork et al., 2020).

After the fermentation processes, the resulted broth is extracted and subjected to separation methods, including centrifugation or filtering, to obtain a biomass composed of fermented filamentous proteins. In the case of Quorn, mycoprotein biomass is mixed with powdered egg albumen (an emulsifier), water, and natural colourant and flavours. The broth is then transferred in a die and moulded together using pressure and steam (which promote protein denaturation). Before packaging, the biomass is subjected to a freeze structuring process which allows the formation of ice crystal layers, thus achieving a favourable meat-like texture. The total production costs largely depend on the substrate type. Indeed, using by-products of the agro-industrial sector, like the ones derived from pea processing or molasses, can lower the total costs while mitigating industrial wastes (Hashempour-Baltork et al., 2020). Despite Quorn market popularity, mycoprotein processing still requires further research to ensure large-scale production and to formulate fungi-based meat analogues. Currently, the most important limitations include low production efficiency, obsolete processing technologies, and a characteristic fungal odour (Zhang et al., 2022). However, the structure-building capacity of mycoprotein, provided by its functional properties, is one of the main reasons for promoting future research on mycoprotein-based products (Xiong, 2023).

Regarding market acceptance, mycoproteins have an advantage over other meat alternatives since their safety and health benefits have already been recognized by consumers. Indeed, the high protein and fibre contents are well-established features of mycoprotein. Furthermore, the comparability with the conventional edible fungi plays an important role in consumer choices

(Zhang et al., 2022). However, even mycoproteins are not safe from some factors that negatively affect the overall consumer perception. A study conducted by Chezan et al. (2022) to assess the acceptance of fungi-based meat alternatives among European participants showed that the perceived benefits, retrieved from surveys and interviews, established high scores regarding claims like environmental impact, ethics, and animal welfare. However, consumer purchase intention was limited by other attributes regarding primarily taste and naturalness. Taste ended up being the greatest barrier in the acceptance of mycoprotein-based products although most of the results were based on perceptions. In fact, about 60% of the participants had not tasted any products and therefore their ratings were affected by personal perceptions, knowledge, and beliefs. Besides taste, another barrier was naturalness due to the use of industrial processing techniques (food technology neophobia) plus the addition of unfamiliar ingredients such as several colorants and flavours. These processing methods were negatively perceived by the interviewees since they recognized a product with a high processing degree, directly related to a low sustainability level. Product naturalness proved to be an important driver to accept meat alternatives; however, fungal meat alternatives heavily rely on these processing methods because they are still essential in the recreation of meat sensory properties. This demonstrates that promoting further research on food processing could help to obtain less processed food products. Finally, the association of fungi-based products with moulds was found to notably affect interviewee acceptance, therefore acting as an elicitor for uncomfortable and disgusting feelings. The study results indicate that sensory properties are a key point in consumer acceptance. Hence, future studies aimed to enhance these crucial attributes are needed to enhance consumer perception towards fungi-based meat alternatives (Chezan et al., 2022).

3.4 – Insect-based alternatives

Insect is an animal class that is currently being reintroduced as part of human diet. Humans have probably always eaten insects; however, until now they have been traded and consumed as traditional food only in local markets of many developing countries, whereas representing a novelty for many Western countries (Van der Weele et al., 2019). Today, insects are mostly diffused in Africa, Asia and Latin America, especially those countries located near the tropics, because their environmental conditions provide higher insect biodiversity as well as allowing their cultivation in open environments (Baiano, 2020). Over 2,000 insect species have been considered edible for humans among which the most commonly consumed are depicted in the Figure 3.4.1.

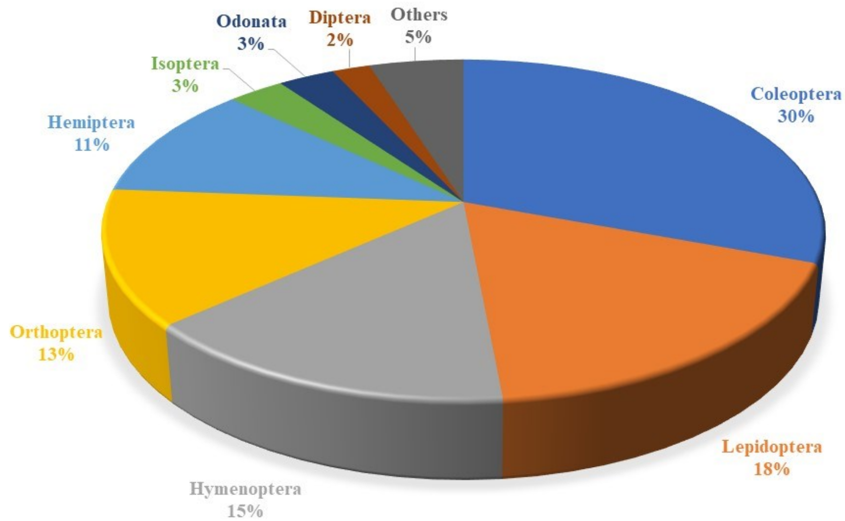

Figure 3.4.1 – Visual representation of the insect species most commonly consumed worldwide (Van der Heijden et al., 2023).

Up to 92% of the insect consumed worldwide are naturally harvested whereas only a small percentage is grown in artificial conditions. However, modern insect farming technologies has rapidly become a standard production method in developed countries in order to overcome unfavourable environmental conditions as well as guarantee higher quality and safety levels compared to the insects found in nature (Baiano, 2020).

In Western countries, insects are generally processed into powder to overcome food neophobia. From this powder protein concentrates and flour can be produced, which can be directly sold or used as an ingredient in the formulation of other industrial products such as burgers, cookies, protein bars, and pastes (Van der Heijden et al., 2023). Whole insects are predominantly found in developing countries rather than in Western countries, where the familiarity towards these products is deeply rooted. Regarding the legal aspect of insects used as food, since 2018 both insects and insect-based products are considered "novel foods", thus included in the Novel Food Regulation (NFR, Reg. 2283/2015) established by the European Commission (EC). According to this Regulation, a food based on insects can be placed on the EU market whether authorized by the EFSA through safety assessments that must last 17 months at least (Baiano, 2020). Currently, the EFSA is evaluating the safety of nine insect species, however, in 2022, three species have already been assessed, thus representing the only insect species that can be placed on the EU market as novel food. These three species include mealworm larvae (*Tenebrio molitor*), migratory locust adult (*Locusta migratoria*) and house cricket adult (*Acheta domesticus*) (Liguori et al., 2022). Despite the legal and psychological limitations, insects and insect-based products are gaining importance also in Western countries. Indeed, more than 250 insect-based products were launched on the global market in 2019. Since that year, insect-related market shares have continued, and are expected to continue, to grow (Lumanlan et al., 2022). The increasing interest in using insects in the production of meat alternatives derives from several drivers including the generally high protein content (Xiong, 2023), a low Feed Conversion Ratio (FCR), a rapid growth and, thus, an increased efficiency in food production (Baiano, 2020). Moreover, the opportunity to use organic wastes as insect feed permits to improve their environmental and social sustainability (Zhang et al., 2022).

Insect processing (Figure 3.4.2) is an essential part of their sale since, right after slaughter, insects must undergo several operations to minimize microbial hazards as well as enhancing their shelf-life. The processing-related technologies are different based on the sold product, which can be divided into whole insects, powder insects, and insect extracted products such as proteins, oils, and chitin. Drying processing is the first processing step needed for all the insect-derived products and is obtained through sun drying, smoke drying, and roasting for whole insects (Lee et al., 2023), whereas through oven-drying and freeze-drying for insect flours and powders (Baiano, 2020). Drying is essential during insect processing because it reduces the water activity leading to the inactivation of pathogens, spoilages, and gut microorganisms (since insects are not eviscerated). For whole insects, drying is the only one process needed before packaging (Lee et al., 2023). To produce powders, insects undergo comminution and grinding processes which reduce them into ground and paste form. Insect proteins, oils and chitin are obtained by using traditional or novel extraction technologies. The first category includes extraction using water, organic solvents, and enzymes (Baiano, 2020), whereas non-conventional technologies include extraction using supercritical CO_2 and ultrasound (Lee et al., 2023).

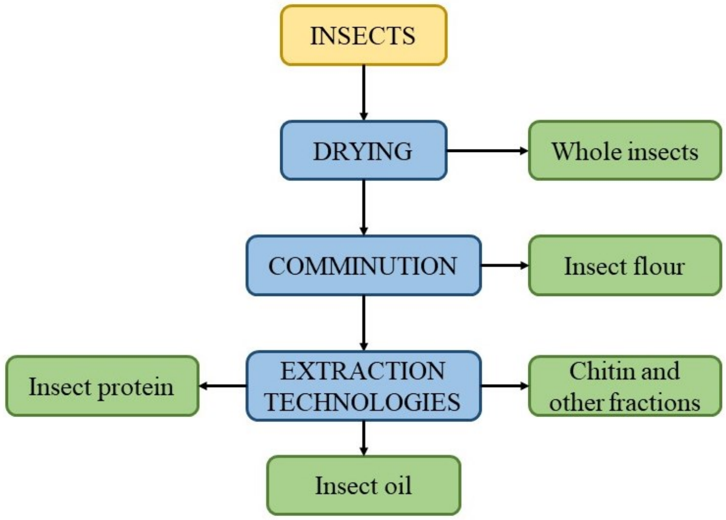

Figure 3.4.2 – Schematic diagram representing the principal steps faced during insect processing.

The processed insect-derived products present an attractive nutritional composition that makes them promising ingredients to add to the formulation of staple foods, in particular insect proteins. The attractive functional properties provided by these proteins, namely the formation and stability of emulsion and gel, and the water- and oil-retention capacity, allow their use in a wide variety of food products (Borges et al., 2022). To date, insect-derived ingredients have been incorporated primarily in meat and bakery products. Regarding the reformulation of meat products, insects have been added as flour or protein concentrates essentially to replace meat proteins with insect proteins, which have been shown to improve the stability of various meat emulsions and decrease their cooking losses, in addition to improve the product sustainability. However, in most studies, insect incorporation has not exceeded 10% of the total formulation to avoid unfavourable changes in colour and texture of the meat products. Numerous studies, in fact, established that increasing the replacement of meat protein over a certain threshold could result in a worsening of the textural properties, including decreased firmness and chewiness. Therefore, food reformulation using insect proteins requires further research to reduce the adverse effects related to their incorporation. Insect proteins presents promising technological properties that could be helpful also in the production of meat alternatives. However, a meat alternative fully based on insects has not been produced yet, essentially due to texture-related challenges. To date, insect-based meat alternatives with desirable quality traits have been achieved by mixing insect with vegetable proteins (Borges et al., 2022). In summary, the potential use of insects as an ingredient in meat product reformulation, food fortification and meat alternatives production still present important hurdles that can be overcome through further studies aimed to improve the technological knowledge of insect incorporation.

The insect consumption trends are still held back essentially by deeply rooted negative attributes that include food neophobia, ethical beliefs, and insect perceptions (Siddiqui, 2022a). Edible insects are responsible for a well-known food neophobia able to elicit sense of fear and disgust among consumers. A solution could be processing insects into unrecognisable forms (such as flour and powder) and add them to other food products to mislead consumer perception. The negative insect perception is also exacerbated by the possible risks related to entomophagy, mostly regarding allergens and harmful microorganisms. The allergen risk perception can be mitigated only through the correct product labelling, whereas the product microbiological safety must be insured by the correct industrial processing of the raw materials (Baiano, 2020). Another factor that plays a key role in the acceptance of edible insects regards the sensory properties related to their incorporation into staple foods. A sensory trial submitted 90 participants to compared insect-based burgers with conventional ones in 3 different tests: blind tasting, informed tasting and expected experience. During both tasting trials, insect burgers received low scoring for aroma and flavour. In particular, the latter was recognized as “nutty”, therefore high scores were given to the product off-flavour. However, the participants of the informed tasting showed higher acceptance and healthiness perception than participants of the blind tasting (Starowicz et al., 2022). Accordingly, strategies aimed to improve consumer perception towards insects must be based on providing enhanced information, health and sustainability claims, better nutritional knowledge, and awareness to the targeted consumers (Siddiqui, 2022a). The results of another sensory trial showed that taste and appearance were recognized above neutral. This confirms that appearance does not represent a barrier in consumer acceptance when insects are minced or powdered and therefore incorporated into familiar food products. Nevertheless, the provided information during the trial about entomophagy has once again confirmed the importance of improving consumer knowledge and familiarity towards these novel food products (Megido et al., 2016).

In the near future, the food industries will need to understand if insects are a protein source worthy of further research and capital investments, with special focus in the development of meat alternatives primarily based on insects. Moreover, gaining a more in-depth understanding of consumer perception towards insect and insect-based foods is recommended to positively influence the targeted consumers and thus increase the insect market shares.

3.5 – Cell cultured-based alternatives

The cultivation of isolated animal cells is an emerging technology consisting in the isolation of muscle cells and their cultivation in specific bioreactors to produce cell biomass. The appropriate term for this fermented mass is still under discussion by the scientific communities, however most of the consensus seems related to "cultivated/cultured meat", whereas others impose on "clean meat", "synthetic meat" and "artificial meat" (Luneau, 2021). However, all these terms remain quite broad since they refer to different hybrid products partially composed of animal cells. The rest is represented by organic ingredients, such as plants, algae, and fungi derivatives, and inorganic ingredients, including emulsifiers, flavourings, and colourants (Thorrez & Olenic, 2023). As established by the EFSA, due to the absence of a legal definition, all the foods obtained through the propagation of animal or plant cells, and assisted by engineering technologies, must be recognized as "cell cultured-derived foods of animal or plant origin" (EFSA, 2023). Furthermore, the Regulation 1169/2011 of Food Information to Consumer established by the European Parliament lays down that food products obtained through cell cultivation do not fall into the official meat definition. Accordingly to the aforementioned legislation, specific labels containing information regarding food type and used production methods are essential for the sale of cultivated food to avoid misleading the consumers' choices (Chodkowska et al., 2022).

The goal of these novel products is to recreate conventional meat but in a way that reduces the negative impact of the conventional farming, in particular, the related environmental footprint and ethical issues. The intensive development occurred in the last years for the cultivation of animal cells originated by the need to successfully produce meat during space travels. In fact, the NASA was the first industry to strongly invest in this kind of technologies and, even now, it provides an important share of financial investments. Today, the number of food companies and startups related to cell cultivation increased to hundreds around the globe, where US and EU represent the leaders of this innovation (Chodkowska et al., 2022). What is interesting, is that the rapid rise of this meat analogue was possible by the many billionaires and firms that invested huge amounts of money in these technologies in exchange of future profits (using the so-called “effective altruism”) (Luneau, 2021). Considering the recent marketing data, the dynamic development of cultivated cells predicts that these products will probably occupy an important share of the food market, together with other meat alternatives. However, cell-derived products are currently allowed in the market of only two countries, which are Singapore (from 2020) and USA (from 2022). Despite being one of the leading forces of this sector, the EU does not allow any cultivated foods on its market yet. Both animal and plant cell cultured-derived foods are considered novel food, thus falling into the Novel Food Regulation of the EC. The EFSA has not provided any risk assessment for these products yet; however, the EC has already provided a directive which establishes that each member state has to control its own market of cultured foods, including production and sales (Chodkowska et al., 2022). Technical and legal barriers appear to be the main reasons why these products are still not available worldwide. Despite these limitations, several market research show that the future trends of cell cultured derived foods are expected to double during the period 2025 – 2032 (Chodkowska et al., 2022). At the moment, the *in vitro* market presents only one product authorized for the sale which is the chicken nugget produced by Good Meat. The recreation of a cultivated whole muscle is still unfeasible and hypothetical with the available technologies (Thorrez & Olenic, 2023). Aleph Farms (an Israeli company specialized in the production of cell-derived red meat) was the first and only one that in 2018 successfully recreated a whole muscle of red meat. However, the obtained piece was only of few millimetres in height, therefore unmarketable (Luneau, 2021). In the near future, the technology innovation in cell cultivation probably will lead to the successful recreation of whole animal muscle, resulting in a further increment of the market shares of cell-derived foods (Chodkowska et al., 2022).

The whole production process of cell-based foods consists of essentially four principal phases, namely cell selection, production, harvesting and food processing (Figure 3.5.1), which are carried out with specific manufacturing processes that vary based on the cell sources (cattle, pig, poultry, or fish) and the desired final product (FAO & WHO, 2023). After the selection of the desired cell type, the first phase starts with a biopsy procedure made on a living or recently slaughtered animal to extract different types of stem cells (satellite and mesenchymal cells). Each type can differentiate in specific cell lineages: satellite cells differentiate into skeletal muscle cells, whereas Mesenchymal Stem Cells (MSCs) are the progenitors of a variety of cell types that includes osteoblasts, myocytes, adipocytes, and chondrocytes (cells of cartilage). To replicate the meat sensory properties, various cell lineages such as adipocytes and chondrocytes must be co-cultured with myocytes. After their collection, animal cells undergo purification and sterilization processes in order to obtain an isolated and sterile sample ready for being stored (FAO & WHO, 2023).

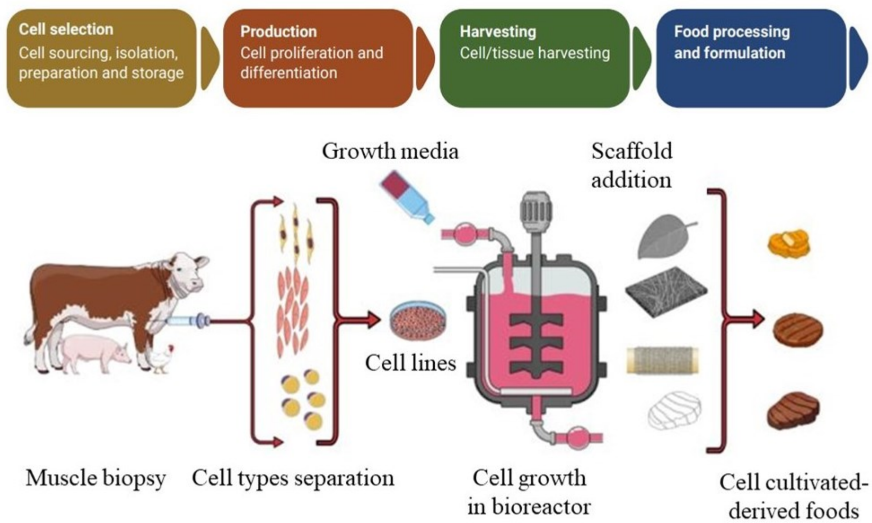

Figure 3.5.1 – Schematic representation of the production of cell grown food products divided in four principal steps (FAO & WHO, 2023).

For the second phase (i.e., cell proliferation) a culture media is required to supply cells with the proper nutrients to speed up the process. Culture media represents the major costs of the whole process, ranging from 55 up to 95 % of the total production costs (Chodkowska et al., 2022). Generally, the growth media used for cell proliferation contains a certain percentage (up to 10 – 30%) of Fetal Bovine Serum (FBS) which is increasingly being replaced by in lab-created media mainly due to ethical reasons (Thorrez & Olenic, 2023). Artificial media must have specific characteristics to reply as closely as possible to the complex environment where cells live and growth, thus promoting their proliferation and differentiation into various lineages. The serum must be chemically well-defined, with specific amounts of macro- and micronutrients, hormones, and antibiotics to prevent the proliferation of microorganisms. Furthermore, the media should not contain any impurities that could interfere with the cell growth. The production cost of culture media represents the major barrier for the scale up of cell cultivation. Several solutions are currently under study by the food industries, including media recycling and the replacement of media growth factors (Chodkowska et al., 2022). However, the development of serum-free media has led to limited success despite the considerable efforts made in the last years. Moreover, the use of this kind of growth media could further hamper the economic viability of cell-based foods due to its higher costs (Thorrez & Olenic, 2023). Cell cultured-derived foods can be produced also with the use of a third ingredient, which is the scaffold: artificial structure used to resemble the 3D-microenviroment of muscular tissue whereas providing the site for cell attachment, proliferation, differentiation and finally maturation. The scaffold can be organic or synthetic, edible or removable, whereas the structure varies based on the desired product (Rao et al., 2023). Regarding raw materials, scaffolds are often produced using natural polymers such as plant proteins, collagen, polysaccharides, and hyaluronic acid, since are more biocompatible with animal cells than artificial scaffolds (Seah et al., 2022). A promising scaffold formulation was recently studied by Kim et al. (2023) which produced scaffolds by combining soy protein with agarose, which resulted in a high cell adhesion and a well-porous microstructure. The structures, instead, are obtained with different processing technologies (like freeze drying, 3D-printing, electrospinning and gas foaming) based on the desired final product (Seah et al., 2022). Figure 3.5.2 illustrates the most used scaffolding structures that are produced from natural polymers.

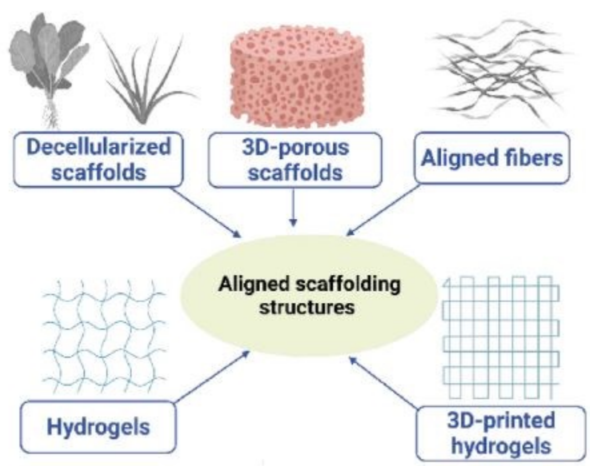

Figure 3.5.2 – Representation of the most used scaffolding structures obtained with different biomaterials (Rao et al., 2023).

However, the scaffolding technology is still obsolete, since most of the scaffolds used so far need to be removed prior to consumption because not edible, thus damaging the final product. Future scaffold technologies should include edible or biodegradable polymers that degrade without negatively affect the product sensory qualities. During the production of cultured foods, scaffolding is not always needed, indeed cells can develop a structured biomass by growing in a self-organizing way (Chodkowska et al., 2022). When the ingredients are collected, they are placed in the bioreactors, inside which the various cell types are co-cultured in high densities and in a well-controlled environment, which mimics as much as possible the internal conditions of a living animal. The environment is automatically maintained and regulated to maximize the grow rate and yield of the cultured cell, but also to avoid changes in the sensory qualities of the final product (e.g., hypoxia condition during cell growth can help to obtain biomass with higher myoglobin content and therefore a redder colour). Furthermore, automatization is essential for industrial production because ensures high levels of reproducibility and repeatability (Chodkowska et al., 2022). Cell production phase is divided into two sub-phases, namely cell growth and differentiation. Once reached the maximum density, animal cells start differentiating due to a change in the medium constituents (i.e., addition of growth factors, micronutrients, and amino acids), which reduces the growth rate while promoting cell differentiation (called "differentiation medium"). When fully differentiated, the cultivated cells are harvested from bioreactors using different technologies such as filtration or centrifugation. Furthermore, the cells must be dissociated from the scaffold prior to processing whether the used materials are not edible or biodegradable (FAO & WHO, 2023). Finally, the harvested biomass undergoes several processing steps to recreate the meat-like sensory properties. Firstly, the cell biomass commonly undergoes extrusion or 3D printing technologies to obtain the desired structure and texture (FAO & WHO, 2023). The other properties are then achieved with the addition of several ingredients and compounds, including proteolytic enzymes to soften the muscle, preservatives, flavour precursors (like amino acids and lactic acid), several vitamins (B_1, D_3, E, and B_{12}) and minerals (Lee et al., 2022). Despite these corrections using food engineering techniques, the quality traits of cultivated foods are still not comparable to the ones of conventional meat. Indeed, the simple addition of necessary components can perhaps change their bioavailability as well as affecting the overall product sustainability (Wood et al., 2023).

The perception of cell cultured-derived foods is very complicated. Cell cultivation is currently fostered by advanced food industries, start-ups, and activist groups which believe that this technology has the highest potential to replace conventional farming when compared to other meat alternatives. Most of the supporters are also firmly convinced that these lab-derived products will definitively replace their conventional counterparts soon (Siddiqui et al., 2022b). However, livestock farming is deeply involved in a variety of societal roles, often obliterated by cell cultivation supporters, that might impede cultured foods to fully replacing conventional animal products which, instead, will cover a percentage of the future global market (Wood et al., 2023). Environmental and welfare concerns are the two main motives that drive this increasing trend. For these reasons, cell-derived muscle is also touted as “clean meat” because considered a suffering-free and environmental-friendly product, in addition to representing a label with a high purchasing potential (Siddiqui et al., 2022b). However, this terminology was found being inappropriate by the scientific community which decided to replace it with other truthful labels such as cell culture-derived foods (Luneau, 2021). The current data on cell cultivation, particularly related to safety and healthiness, environmental impact, economic, and consumer acceptability are still limited or uncertain because based on a product that does not exists yet (Wood et al., 2023). Regarding safety and healthiness, cultured foods are strongly associated with the perception of unnaturalness, thus representing one of the major hurdles to overcome together with the related food and food technology neophobia. These two adverse feelings result from the perception of food grown in a laboratory, thus considered unsuitable for consumption as well as creating a sense of disgust and fear among consumers. Several surveys revealed the frequent correlation of cultured products with cancer due to the possible growth of malignant cells during replication. However, this fear has already been debunked since malignant cells are inactivated during stomach digestion (Siddiqui et al., 2022b). Long-term research on human health is, in fact, still developing thus providing limited data on the cultured food health implications (Wood et al., 2023). Conversely, some survey participants also expressed health benefits related to cultivated foods including the possibility of adjusting their nutritional composition through 3D printing techniques; the absence of hazard related microorganisms and zoonotic diseases (like the Bovine Spongiform Encephalopathy/BSE); and the absence of antibiotic residues (Siddiqui et al., 2022b). Despite the environmental footprint seems to be one of the main strengths of this technology, uncertainties regarding an energy efficient large-scale production still exist. Due to current knowledge gaps, the environmental impact of large-scale cell cultivation relies only on estimates and assumptions based on the data available from laboratory experiments. From the current evidence has been envisioned that cell cultivation will have a lower environmental impact when compared to conventional meat, especially beef. Nevertheless, this goal will be achieved only if cell cultivation reaches the worldwide market in a cost-efficient manner and by using low-emissions energy sources, all terms that cannot be achieved in the near future (Smetana et al., 2023). Besides reducing the environmental impact, cell cultivation technology must be improved also to achieve a reachable price. Indeed, the obsolete technology currently used still leads to the production of expensive food which therefore is not widely affordable in most countries (Wood et al., 2023). These potentially high costs have been highlighted in US surveys as a major barrier to culture food development (Siddiqui et al., 2022b). Finally, the societal acceptability (obtained via surveys) of cultivated foods has seen inconsistent responses by consumers since their answers were based only on expectations. In addition, the overall acceptability of surveys has resulted often been overestimated because based on the consumer willingness to "try the product" which is different from the willingness to consume it regularly (Wood et al., 2023).

This rough picture of cell cultivation allows to highlight how much is the uncertainty behind this technology. To date, a positive perception towards this meat alternative can be found only in certain category of people including investors and activist groups. Conversely, most the consumers are driven by sceptical and fearful feelings, mainly due to high levels of uncertainty and untrust of science (Wood et al., 2023). Furthermore, a crucial issue remains the price, particularly whether future technologies will be able to lower the current high price of cell-derived products and make them affordable for most consumers (Siegrist & Hartmann, 2023). In future, the technology innovation cultured products will be boosted by further research as well as their market shares. However, the latter will be defined by the ability of these foods to ensure both consumer healthiness and acceptability whereas lowering the environmental impact and promoting product sustainability.

Chap. 4 – The sustainability gap

As the worldwide population continues to grow the demand for meat rise as well, especially in developing countries, where the incidence of malnourishment is high. The World Health Organization (WHO, 2023) refers to malnutrition as "deficiencies or excesses in nutrient intake, imbalance in essential nutrients or impaired nutrient utilization". However, malnutrition represents a growing problem also in many developed countries, where food overconsumption and a sedentary lifestyle are becoming common practices. The Double Burden of Malnutrition (DBM) is a recent term coined by the WHO indicating "the coexistence of undernutrition along with overweight, obesity, or diet-related Non-Communicable Diseases (NCDs), within individuals, households and populations" (Davis et al., 2020). Meat is firmly considered a high-quality nutrient containing a highly valuable amino acid profile and providing many essential micronutrients, all factors that render meat a perfect tool to face the DBM, since undernutrition commonly manifests as micronutrients deficiencies. As an example, the recent study published by Beal et al. (2023) showed that the diet proposed by the EAT-Lancet planetary health lacks in nutrients, particularly essential micronutrients. This diet suggests to consume more plant-based foods at the expense of animal-based ones, thus resulting in potential micronutrient deficiencies essentially due to the significant presence of anti-nutritional factors. This study highlighted the positive role of meat in a healthy and sustainable diet since it represents a nutrient-dense food essential to meet many of the recommended micronutrient dietary intakes without relying on fortification or supplementation. However, meat and related products are typically recognized as an environmentally costly solution, expressed in terms of Green House Gasses (GHGs) emissions, land and water requirements, biodiversity loss, and pollution; not to mention that are obtained by killing animals (Van der Weele et al., 2019). The environmental footprint is further exacerbated when considering the alarming rates of meat overconsumption and the consequent industrialization of animal farming, representing an essential adaptation to meet the demand trends of developed countries. Besides the environmental footprint, meat overconsumption has also been strongly associated with several metabolic diseases such as obesity, thus contributing to the high rates of DBM in developed countries (Smetana et al., 2023). For these countless reasons, more consumers have started to follow different meat-reduction strategies (from flexitarianism to veganism), raising the demand for alternative protein sources (Siddiqui et al., 2022a). This market gap has opened the doors to the development of meat alternatives, which essentially try to provide the same nutritional benefits and sensory properties of conventional meat while, at the same time, reducing its environmental and ethical issues. The current gap in scientific knowledge, however, does not yet allow to establish whether these meat alternatives are a more sustainable solution than conventional meat, particularly their potential in lowering the DBM at individual, household, and country levels. Furthermore, there are increasing uncertainties concerning the naturalness of the various meat alternatives, as well as their labelling, namely whether they must be labelled as "Ultra-Processed Foods" (UPFs). According to the NOVA classification system (Figure 4.1), the UPF group includes all formulations of ingredients deriving from industrial techniques and processes such as comminution, chemical modifications, extrusion, moulding, frying, use of culinary ingredients and classes of food additives (Monteiro et al., 2019); hence, meat alternatives fall into this category. Considering the label, new doubts emerge regarding their healthiness which is directly related to the degree of processing required for their production.

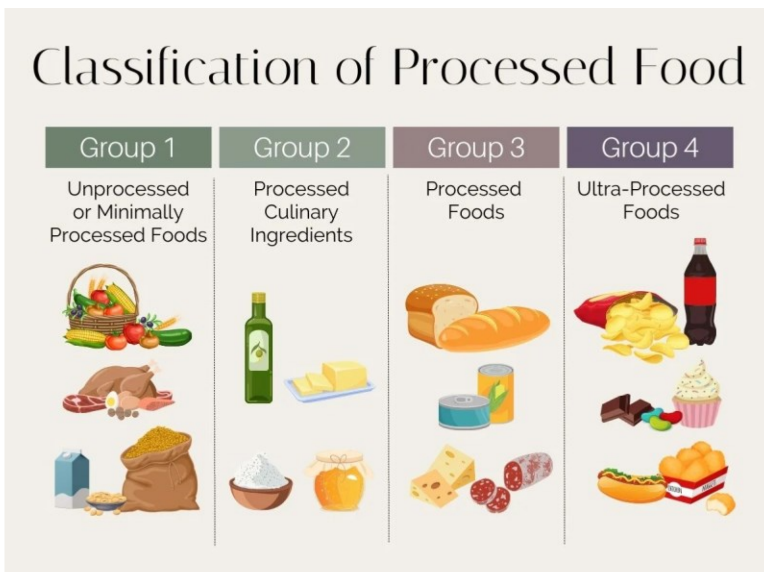

Figure 4.1 – The NOVA classification system

According to the NOVA system, ultra-processed foods are generally unhealthy since characterized by high energy density, palatability and glycaemic index, and a low satiety potential (Monteiro et al., 2019). However, this definition cannot be fully applied to PBMAs since they provide high levels of fibres and proteins, therefore high satiety potential and low energy density. The lack of clinical studies related to these emerging products represents a significant issue when determining the overall product healthiness. Therefore, further research is needed to have a clear picture of the health-related effects deriving from the integration of meat alternatives in the human diet.

In this chapter, each meat alternative will be assessed with a focus on its nutritional qualities while comparing them with those of meat. At the end of the chapter, the overall sustainability of meat alternatives will be assessed through a SWOT analysis, including strengths, weaknesses, opportunities, and threats conveyed to the society. The drawn conclusions perhaps will permit to have a clearer image of the future sustainable protein that will help to reduce the DBM around the globe whereas facing the current threat of climate change.

4.1 – Plant-based alternatives

PBMAs are the most studied and assessed alternatives since plant-based raw materials have been exploited for longer times than other protein sources. Accordingly, PBMAs have undergone more studies relating to their sustainability, however, a straightforward comparison with conventional meat and meat products remains not statistically representative since PBMAs are still not able to perfectly replicate them.

Despite the recurring differences towards conventional meat, to assess the PBMAs' impact on human health is important to start by analysing the nutritional composition of these products. Considering a large number of plant-based products commercially available around the globe, large nutritional variability can be found also among PBMAs, often with contrasting results. The comparison is made by simply retrieving and then confronting the data reported on the respective food labels. Analytical studies mainly conducted in US and EU markets found that PBMAs generally present higher contents of salt and total carbohydrates, including both fibres and sugars, and lower levels of energy, total and saturated fats (Bryant, 2022). Despite the inconsistent results among food products, the total protein levels were found to be quite similar between plant- and meat-based products, though large variations were seen when comparing the amino acid profiles (Andreani et al., 2023). Plant-derived proteins have generally a less favourable total and essential amino acid profiles than animal proteins, indeed most of the plant-based sources present at least a deficiency in one of the nine EAAs. As indicated in the Table 4.1.1, pulses are deficient in methionine whereas cereal proteins in lysine (however when combined they form a complementary protein with a completed EAA profile). Therefore, meet the protein

Recommended Dietary Allowance (RDA) with plant proteins could result in an extra caloric intake as higher amounts of plant-based foods are needed (Van der Heijden et al., 2023).

Table 4.1.1 - Essential Amino Acid composition (expressed in % of total proteins) found in different plant-based protein sources compared to the WHO/FAO recommendations (Van der Heijden et al., 2023). The red values indicate a deficiency of a certain EAA.

Protein source	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl alanine	Threonine	Trypto phan	Valine	EAAs
Beef	3.5	4.6	8.3	9.0	2.7	4.0	4.4	1.0	5.0	42.5
Chicken	3.2	5.0	8.0	8.9	2.7	4.0	4.4	1.2	5.1	42.6
Soybean	2.6	4.6	7.7	6.3	1.3	4.9	4.1	1.4	4.7	37.5
Peas	2.7	4.5	7.8	8.2	0.9	5.3	3.8	0.7	4.8	38.7
Wheat (gluten)	2.4	3.9	6.8	2.2	1.6	5.0	2.7	1.3	4.3	30.2
Kidney bean	2.9	4.7	8.4	7.2	1.6	5.7	4.4	1.2	5.5	41.8
Lentil	3.1	4.7	7.9	7.6	0.9	5.4	3.9	1.0	5.4	39.8
WHO/FAO essential amino acids recommendations				**4.5**	**1.6**					**27.7**

Besides the amino acid profile, protein quality is determined also by the digestibility, which is generally expressed as the Protein Digestibility Corrected Amino Acid Score (PDCAAS) and the Digestible Indispensable Amino Acid Score (DIAAS). The first metric determines protein digestibility by correcting the Amino Acid Score (AAS) of a particular protein with its faecal digestibility. However, PDCAAS present some limitations, in particular the overestimation of protein digestion. Accordingly, the FAO recommends to use the DIAAS, which estimates protein quality relying on ileac rather than faecal digestibility, thus minimizing the estimation errors. The protein digestibility and amino acid bioavailability of plant-derived sources are known to be lower than animal-derived ones. Despite being lower, the digestibility of plant proteins can vary greatly depending on the used raw materials and processing technologies. As a matter of fact, the DIAAS of the various plant proteins ranges from 0.29 to 1.00, with soybean and peas presenting the highest values (0.99 and 1.00, respectively). In general, cereals present lower DIAAS values than legumes, therefore in the formulation of PBMAs is better to prefer legumes while not exceeding with cereals to not negatively affect the final product digestibility (Van der Heijden et al., 2023). The fat content of PBMAs varies largely depending on their formulation. Most of the statistical analysis supports that PBMAs present lower saturated fats compared to meat whereas providing higher contents of unsaturated fats. However, the fatty acid profile can be tailored by the addition of vegetable oils: for example, the oleic and linoleic acid contents are enhanced with the addition of sunflower or coconut oils. Lastly, cholesterol is naturally absent in plant-based foods (Bryant, 2022). The data related to micronutrient content result rather inconsistent among the available PBMAs. Regarding minerals, both calcium and sodium are generally contained in higher concentrations in PBMAs rather than animal-based foods. Likewise, the iron and zinc contents are also higher, although they are less bioavailable than in conventional meat, since their absorption is negatively affected by phytates, mineral antagonism and various fibrous compounds (Swing et al., 2021). About the vitamin profile, all B-vitamins, except for niacin (B_3), pantothenic acid (B_5) and cobalamin (B_{12}), appear to be higher in PBMAs, although their bioavailability might be negatively affected whether undergo Maillard reactions and antagonism from minerals. PBMAs also present higher contents of vitamin E, whereas fat soluble vitamins such as A and D are generally under the detection limit when compared to animal-based products (Swing et al., 2021). The available literature establishes that the nutritional composition of PBMAs only reaches a similar level with respect to animal-based products. In fact, compounds including linolenic acid and niacin can be found only in meat and meat products, whereas vitamin C, phytosterols, and some antioxidants are only in PBMAs. Many comparison studies suggests that PBMAs could improve by lowering the salt and sugar contents while also considering fortification processes with iron and vitamin B_{12} (Bryant, 2022). The systematic comparison PBMAs with their animal counterparts raise questions about the PBMA overall sustainability as meat products do not need a fortification process of iron and any B-complex vitamins (Lima et al., 2023); further animal-based foods represent a more bioavailable source of proteins and many micronutrients such as iron and vitamin B_{12} (Bryant, 2022). To date, PBMAs are not yet able to provide meat's nutritional composition, thus remaining alternatives to conventional meat and not analogues. Future research must consider in-depth investigations focused on the raw material properties and processing technologies that could improve PBMAs at the nutritional level.

To date, there is a lack of long-term controlled studies regarding the PBMAs health benefits and potential risks related to their nutritional composition. However, from the limited available studies, the integration of PBMAs in the diet appears to provide several health benefits including lowering total cholesterol and LDL-cholesterol concentrations as well as improving gut microbiota by providing prebiotic compounds (such as beta glucans). Furthermore, plant-based products contain lower levels of purines when compared to animal- and fungi-derived foods, thus representing a beneficial choice for those who suffer from hyperuricemia (Bryant, 2022). The effects of PBMAs on appetite instead show contrasting results. The high protein and fibre contents ensure a higher satiating potential than meat, which is favourable for consumers suffering from overweight or obesity. However, this satiating capacity can be negatively affected to an unknown extent by the processing methods required by these food products (Flint et al., 2023). On the other hand, the analytical studies carried out so far highlighted also many potential hazards related to the PBMA consumption.

The majority of PBMAs available on the market are obtained through extrusion processing, which submits the products to extreme conditions including temperature, moisture, and pressure. These conditions are yet essential for PBMA development since they provide a meat-like texture and product safety by reducing the activity of several microorganisms, allergens and antinutrients (Hadi & Brightwell, 2021). However, extreme processing conditions are also strongly associated with different degradation and oxidation processes that negatively affect the final product. Firstly, they tend to degrade some of the product's sensory properties (mainly colour and flavour) that must be re-established in post-processing with several food additives, some of which might lead to detrimental effects during the digestion processes including epithelial inflammation and microbiota disorder. In addition, the degradative processes can also affect different nutrients that must be added in post-processing to restore the initial nutritional composition. Secondly, the processing can be responsible for the formation of thermally induced toxic compounds derived from lipid peroxidation and caramelization (Xiong, 2023). The latter processes are responsible for the formation of the Advanced Glycation End products (AGEs), which are compounds strongly associated with many degenerative diseases (Nie et al., 2022). Plant-based foods are well-known to naturally include antinutritional compounds such as phytates, trypsin inhibitors, tannins and alpha-galactosides, which are involved in a hindered digestibility and systemic bioavailability of proteins and micronutrients. Phytates are the most recurring antinutritional factors in the plant world, responsible to inhibit the absorption at gut level of several micronutrients, including iron, zinc, and calcium. All the antinutritional factors can be minimized through various processing including extrusion, blanching, and roasting (Van der Heijden et al., 2023). Despite being reduced by the processing conditions, allergen residuals may represent a potential hazard to susceptible consumers. Therefore, clinical studies in post-processing and proper labelling regulations are essential measures to significantly reduce the incidence of allergic reactions. Furthermore, since the increasing occurrence of soy and gluten allergies, the use of different raw materials for the PBMA formulation (such as peas) may be a viable solution (Hadi & Brightwell, 2021). Another hazard related to PBMA consumption is represented by the salt content which is commonly high in the available food products. Since high salt consumption is strongly associated with an increased occurrence of certain cardiovascular diseases, its content need be reduced in the marketed products. Considering the listed potential hazards, PBMAs must be reformulated through processing methods aimed to improve healthiness while mitigating hazards. Fortification could be a promising strategy to augment the food total content of vitamins and bioactive compounds. Promising results were also seen with the incorporation of spirulina in some PBMAs, which improved their nutritional composition through the addition of beneficial compounds like phenols, flavonoids, antioxidants, and some vitamins. On the other hand, hazard minimization strategies might include lowering salt, allergens and antinutritional factors contents, in addition to choose another processing technology beyond extrusion to avoid product alteration and detrimental by-products (Bryant, 2022). However, the product improvement through further processing might result in a two-edge blade and turn PBMAs into UPFs, thus arising negative perceptions towards the product's naturalness and healthiness. To date, the available long-term studies assessing the behaviour of PBMAs during digestion are still limited to experimental animals. A study conducted by Sánchez-Terrón et al. (2023) on Wistar rats showed that the consumption of ultra-processed PBMAs had negatively changed the microbiota composition, promoting the growth of proteolytic microbes involved in different intestinal diseases. Indeed, PBMAs provoked impaired digestion resulting in the presence of different undigested proteins in the colon from which were produced detrimental metabolites by the proteolytic microbes. In conclusion, the long-term consumption of PBMAs was associated with a likely impaired health, resulted from the dysregulation of different digestive processes and the related dysbiosis. Consequentially, future studies are needed to discover valuable solutions able to limit the potential hazards related to these products, as well as improve their nutritional composition whereas diminishing the degree of processing at which are currently subjected. Furthermore, the sustainability of plants as an alternative protein source must be carefully assessed in the future in order to better understand their role in the fight against the global DBM and therefore if they are worthy of further research and funding.

4.2 – Algae-based alternatives

The current knowledge regarding the production of algae-based meat alternatives is still limited, therefore assessing their overall sustainability is quite complicated. To date, the available sustainability studies have been focused on the production of alternative proteins from both macro and microalgae. Therefore, the evaluations of nutritional composition, effects on human health and sustainability are still limited to the raw materials, with particular emphasis on microalgae as they are considered a more promising protein source than macroalgae (Geada et al., 2021).

While macro- and microalgae are cultivated for different purposes, both have recently found an application as a food additive. The incorporation of algae biomass or protein isolates serves to enhance the nutritional value and/or the functional properties of the final product (Gohara-Beirigo et al., 2022). Considering the nutritional composition, proteins are part of the major components of algae. In microalgae, proteins range from 23 – 63% of the Dry Weight (DW), thus exceeding soybean protein content (36,5 % DW) in certain species. However, these percentages in protein content are often overestimated because based on the total nitrogen content which is obtained by using the universal nitrogen-to-protein conversion factor (N × 6,25). Indeed, this formula accounts also the Non-Proteinaceous Nitrogen (NPN) content, which is present as nucleic acids, cell wall components, and intracellular compounds, thus reaching up to 29 – 54% of the total nitrogen in certain species (Kumar et al., 2022b). Regarding macroalgae, the protein content varies based on the species, ranging from 3 – 15% DW in brown macroalgae, 9 – 22% DW in green macroalgae and up to 47% in some red seaweed species (Geada et al., 2021). Although macroalgae present lower protein content than microalgae, they feature a higher protein bioavailability (Kumar et al., 2022b). Besides their content, another important aspect of algal proteins is their quality which depends on the EAA profile and their digestibility. Regarding the EAA composition, both macro and microalgae have a better EAA profile than vegetable sources, which lack some EAAs, and similar to some animal-based sources (Figure 4.2.1). However, both algae presented lower content of sulphur amino acids and histidine, whereas tryptophan has not been yet quantified due to degradation processes that occur during protein extraction (Geada et al., 2021). Considering the WHO's EAA requirements, both algae have the potential to meet these recommendations, indeed the AAS of some species is higher than 1,00 (Kumar et al., 2022b).

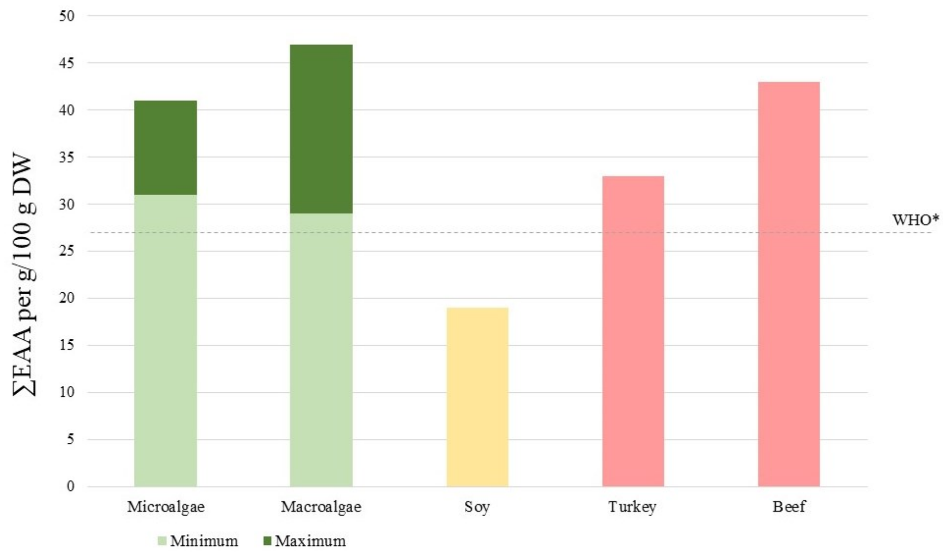

*Figure 4.2.1 – General EAA profile in macro and microalgae species compared to both plant- (soy) and animal-based protein sources. The content of EAA is expressed in g/100 g DW. *EAA basal requirements recommended by the WHO expressed in g/100 g DW (Geada et al., 2021).*

Despite the promising nutritional profile, both algae often present low digestibility when consumed as a whole food, similar to insects (Geada et al., 2021). Although the PDCAAS scores of both algae are lower when compared to reference proteins (namely egg, casein, and soy proteins), macroalgae present higher digestibility compared to microalgae, which scores remain higher than wheat and peanut. The poorest digestibility of microalgae is attributed to their characteristic thick cell wall which also affects the nutrient bioavailability. Mechanical processing technologies such as mechanical rupturing can be used to improve microalgae digestibility and nutrient bioavailability. To date, the digestibility data of algal proteins are still limited because obtained through animal bioassay whereas no reports based on human digestibility and on DIAAS values have been published (Kumar et al., 2022b). For these reasons, evaluating algae-based sources based only on their nutritional composition is extremely limiting when looking for a sustainable solution that can be suitable for most of the consumers. Algal proteins, besides being an alternative source of protein, also provide several bioactive peptides (BAPs). These protein by-products are small peptides deriving from the action of gastrointestinal proteases or fermentation processes (Geada et al., 2021). When absorbed from the gastrointestinal lumen, BAPs enter directly into the bloodstream, providing a range of bioactive properties where the antioxidant activity is the central one. Other properties related to these peptides are anti-inflammatory, anti-hypertensive, anti-coagulant, anti-microbial, anti-atherosclerotic, and anticancerous activity. Currently, consistent efforts are addressed to industrial technologies able to isolate these BAPs in order to better comprehend their therapeutic properties and perhaps use them as food additives in the near future (Kumar et al., 2022b).

Besides proteins, algae also contain polysaccharides, lipids, fibres, vitamins, minerals, and pigments. Another major component is carbohydrates, mainly found as polysaccharides with energy-storage and structural roles. Most of these polysaccharides are recognized as dietary fibre. Lipids represent the third main component composed of an attracting fatty acid profile. Indeed, most of the lipids are ω-3 PUFAs, in particular EPA (EicosaPentaenoic Acid) and DHA (DocosaHexaenoic Acid): bioactive compounds with well-known health-benefit properties. Despite their attractive profile, lipids represent a scant percentage of algae nutritional composition; hence, considerable amounts of algae-based supplements or fortified products must be consumed to obtain notable health benefits. Besides macronutrients, also the algal micronutrient profile presents important features, including pigments, vitamins, and minerals, all found in highly variable percentages depending on species and growth conditions. Regarding pigments, the most prevalent are carotenoids, sometimes found higher than in plants. Secondary pigments include phycocyanin and chlorophyll, photosynthetic pigments presenting important bioactive properties. Considering vitamin profile, besides the high content of β-carotene (precursor of vitamin A), algae mostly contain antioxidant vitamins such as tocopherol and ascorbic acid (vitamin E and C, respectively), but also vitamin D and some vitamins of the B-complex including vitamin B_2 and B_7. Finally, algae are also a source of important minerals like potassium, sodium, zinc, iron, calcium, magnesium, etc. (Gohara-Beirigo et al., 2022). Another interesting feature of algae is the possibility to obtain a tailored nutritional composition by simply altering the growth environmental conditions. When subjected to changes in growth conditions indeed algae defend themselves by accumulating specific metabolites, particularly in case of unfavourable changes. For example, increasing the medium nitrogen or phosphorus levels results in higher protein contents of biomass of some species (Kumar et al., 2022b). In conclusion, the attractive nutritional profile, and its plasticity during algal growth, makes algae a valuable tool for the industrial formulation of staple foods as well as meat alternatives. Indeed, spirulina has been already used in the reformulation of some PBMAs with promising results regarding the improvement of their nutritional composition (Bryant, 2022).

Despite the several advantages obtained by adding algae to staple foods, food industries are still facing many challenges regarding the incorporation of algal biomass. To date, the main challenge regards various side effects due to algae poor palatability. An excessive algae inclusion level indeed can result in a worsening of both physical and sensory properties of the final product caused by the typical algal fishy aroma and intense green colour. Researchers have pointed out that algal incorporation should not be greater than 10% of the total formulation, because passed this threshold the food product would result altered in taste and appearance as well as undergo increased hardness. The established optimal inclusion level currently ranges between 3 – 4% of the total formulation. Besides physical and sensory properties, the algae biomass utilization can worsen the product also through their nucleic acid and heavy metal content, toxin productivity and thick cell wall. The occurrence of toxins is mainly associated with the cultivation of toxin-producing algal strains or their presence in standard biomass. These strains are commonly found in the microalgae groups of dinoflagellates and cyanobacteria (the latter also includes the marketed species *A. platensis*). The use of polluted water for algae growth can also be a source of toxins which can then be found in the final product. In addition to toxins, the presence of heavy metals in the water sources is another important problem. Indeed, algae can absorb and bioaccumulate heavy metals which are consequentially contained in the marketed product. Another challenge in algae utilization is associated with the high presence of nucleic acids which represent most of the algal NPN content. Likewise, for bacteria and yeasts, regular consumption of algae-derived nucleic acids is detrimental to human health because associated with diseases including gout and kidney stones. The highest contents of nucleic acids are found in some microalgae genera characterized by faster multiplication rates such as *Arthrospira* sp. and *Chlorella* sp., both widely available on the market. The already-mentioned thick cell wall characterizing microalgae is another factor strongly limiting the industrial processing of microalgae biomass. This cell wall consists of several complex components responsible to lower the overall product digestibility because resistant to human digestive enzymes. Industrial pre-treatments aimed at disrupting the cell and enhancing the recovery of intracellular compounds are necessary for most of the microalgae species. Indeed, some species of the *Chlorella* sp. genus present the toughest cell wall (Kumar et al., 2022b).

Algae have been recognized as sustainable future protein mainly because of the non-use of the already exploited arable lands. Furthermore, the nutritional and functional benefits resulted from their incorporation in staple foods make algae an important tool for improving human health thanks to their rich composition in macro- and micronutrients, and also bioactive compounds. However, the potential sustainability of algae as a source of alternative vegetable proteins is debatable considering the impact of the used production pathways, the industrial needs for a scale up, and the challenges related to the safety of the final products. Accordingly, the industrial utilization of algae biomass is still under investigation by the scientific community which needs to provide further studies regarding the feasibility of this alternative protein source.

4.3 – Fungi-based alternatives

Likewise for algae, the current knowledge gap regarding the sustainability of fungi-based meat alternatives is still limited since Quorn represents the only fungi-based product able to resemble meat quality traits. To date, among the filamentous fungal species available in the global market, only *Fusarium venenatum* has been widely exploited and used to successfully create the most notable fungi-based meat alternative so far (Xiong, 2023). However, also the aforementioned five fungal species (*N. intermedia*, *P. eryngii*, *L. edodes*, *A. bisporus*, and *P. sajor-caju*) are increasingly utilized by food industries due to the similar properties of *F. venenatum* (Kee et al., 2022). Since mycoproteins are considered the building block in the formulation of fungi-based meat alternatives, the available studies regarding nutritional compositions and effects on human health are focused on their properties.

As mentioned in the previous chapter, mycoproteins derive from the production high-protein biomass starting from the vegetative mycelia of filamentous fungi. Their great potential as an ingredient for meat alternatives is related to their nutritional composition which is also suitable in a healthy diet. Indeed, mycoprotein is a whole food characterized by high protein and fibre content as well as low levels of fats and energy (Hashempour-Baltork et al., 2020). Considering the protein content, mycoprotein is more protein-rich than other fungal or plant protein source, although conventional meat remains higher (Derbyshire & Delange, 2021). When dried, the total mass of mycoprotein is composed of 45% of protein on average, of which 46% are EAAs (Van der Heijden et al., 2023). The highest protein content among the exploited six fungal species is found in *F. venenatum*, whereas the lowest in *L. edodes* (Kee et al., 2022). Although mycoprotein EAA contents are lower than traditional meat (Hashempour-Baltork et al., 2020), their profile is generally considered “balanced” compared to the WHO/FAO recommendations (27,7% of total proteins). Digestibility studies are limited since DIASS scores are still unknown; however, PDCAAS scores are 0,99 on average therefore very close to animal proteins (Van der Heijden et al., 2023). The average fat content among the six species is relatively low (ranging from 0,15% to 4,4%) when compared to meat, albeit this comparison is not entirely correct since meat fat content varies greatly based on the type and parts of the animal considered (Kee et al., 2022). In addition, mycoprotein presents an interesting fatty acids profile primarily composed of PUFAs, including both ω-3 and ω-6 fatty acids (Van der Heijden et al., 2023). Another distinctive feature of mycoprotein relies on its high fibre content, enough to fall into the food category of “high in fibre food” established by the EC. Mycoprotein fibre consists primarily of β-glucans whereas the rest of chitin (Kumar et al., 2022a). Regarding micronutrients, compared to meat, mycoprotein generally presents higher contents of zinc, selenium, and in almost all the B-complex vitamins, whereas presents lower contents of iron, sodium, and vitamin B_{12} (Hashempour-Baltork et al., 2020). Similar to algae, mycoprotein contains several pigments including flavins, quinones and melanins with attractive properties. The mycelium of certain fungi can also contain thiols: sulphur-containing alcohol analogues imparting useful antioxidant effects. Ergothioneine and glutathione are two thiols that can be found in high concentrations in some filamentous fungi, both important to face certain oxidative stress-related diseases (Derbyshire & Delange, 2021).

The available literature assessing the effects of mycoprotein on human health strongly establishes the occurrence of several health benefits related to their consumption. The first occurring benefit during mycoprotein consumption is the markable decrease of energy, resulting from the low energy density of mycoprotein coupled with its effects on satiety (Derbyshire & Delange, 2021). Indeed, acute mycoprotein intake is strongly associated with a significant decrease in appetite, a feeling that is commonly regulated by many Short Chain Fatty Acids (SCFAs) produced through the caecal fermentation of fibres (Hashempour-Baltork et al., 2020). Once digested and absorbed, mycoprotein affects other metabolic pathways, particularly those of cholesterol and insulin. Regarding cholesterol, clinical trials revealed that mycoprotein consumption is associated with a decrease in both total and LDL cholesterol, as well as an increase in HDL cholesterol (Hashempour-Baltork et al., 2020). These benefits on cholesterol can be also attributed to the SCFAs produced starting from mycoprotein fibres (Derbyshire & Delange, 2021). Mycoprotein presents beneficials effects also on glycaemic response thanks to its high fibre content. During digestion, soluble fibres slow down the bolus in the gut thus resulting in a decreased absorption rate of glucose by the intestinal cell walls. The delayed breakdown and absorption of carbohydrates operated by the mycoprotein fibres are linked to an improved glycaemic index (Hashempour-Baltork et al., 2020). Other clinical studies, carried out on the muscle anabolic response after the consumption of mycoprotein-based meals, found that the bioavailable EAAs provided by mycoprotein, coupled with the metabolic effects of the fibre-derived SCFAs, led to an overall improvement in the muscle synthesis rates (Van der Heijden et al., 2023).

Despite its varied beneficial effects, mycoprotein is also associated with several hazards that mostly interest allergenic effects and mycotoxin occurrence. Some safety studies revealed that mycoproteins may elicit allergic reactions among consumers, mainly resulting in adverse gastrointestinal reactions ranging from moderate itching and nausea to severe anaphylaxis and vomiting. Although the reported intolerance cases, allergic reactions towards mycoprotein showed lower occurrences than for soy and egg, thus remaining low and uncommon (Hashempour-Baltork et al., 2020). Among the six promising species, *N. intermedia*, *P. eryngii* and *P. sajor-caju* were not associated with any type of allergenic reactions. Conversely, *F. venenatum*, *L. edodes* and *A. bisporus* reported effects on human health despite the classification as GRAS by the FDA. *F. venenatum*, and thus Quorn, has been associated with classic allergic reactions including itching, nausea, and diarrhoea. Despite being one of the most consumed mushrooms in the globe, *L. edodes* (also known as shiitake) has been responsible for causing many allergenic reactions as well as the so-called shiitake dermatitis when non adequately cooked. Finally, *A. bisporus* is another well-established edible mushroom (also called champignon) correlated to some cases of anaphylaxis and stomach bloating. Furthermore, *A. bisporus* is the only one containing a mycotoxin (agaritine) that was classified by the IARC as potential carcinogen (class 2B). Although the presence of mycotoxin, champignon is highly consumed in the world as agaritine concentration can be strongly reduced through correct boiling and frying (Kee et al., 2022). The mycotoxin content and the reported effect on human health are summarized in the Table 4.3.1. To conclude, the presence of allergens in mycoprotein should not generate alarm since, as mentioned before, the occurrence of allergic responses after fungi consumption is quite uncommon. However, adequate knowledge about preparation and cooking methods can help consumers to minimize fungi-related hazards, especially when handling mycotoxin-containing species.

Table 4.3.1 – Mycotoxin content and potential health responses with the consumption of the assessed fungal species (Kee et al., 2022).

Fungal species	Mycotoxin	Effect/Response
Fusarium venenatum	Absent	Allergic reactions
Lentinula edodes	Absent	Allergic reactions, shiitake dermatitis
Agaricus bisporus	Present (agaritine)	Stomach bloating, anaphylaxis
Neurospora intermedia	Absent	-
Pleurotus eryngii	Absent	-
Pleurotus sajor-caju	Absent	-

Despite its unique and beneficial structure, mycoprotein does not receive much attention compared to other alternative protein sources. An example can be found in the low occurrence of updated literature and in the scarcity of funding from the food industries to promote further research and the development of mycoprotein-based food products. The results obtained from several randomized controlled trials showed that mycoprotein introduction in conventional diets is beneficial for the consumer's health, especially in the presence of important metabolic diseases such as obesity. Mycoprotein incorporation is, in fact, associated with a decrease in the ad libitum energy intake and an improvement of the lipoprotein profile, particularly in overweight and obese patients presenting altered total cholesterol levels. Finally, mycoprotein consumption results in a slower and more sustained insulin increase, proving its importance in the regulation of insulin levels. Moreover, this regulating effect may be more marked in conditions of hyperinsulinemia, therefore in overweight and obese consumers. These promising health effects however need broader knowledge since some findings, in particular about glucose and insulin levels, resulted to be not statistically significant. Overall, long-term randomized trials would be helpful to further investigate the potential role of mycoprotein in the human diet and its related sustainability (Derbyshire & Delange, 2021).

4.4 – Insect-based alternatives

The edible species assessed so far have been considered sources of highly valuable nutrients, which make them promising ingredients to use in the reformulation or fortification of existing food products, as well as in the production of insect-based meat alternatives. However, these processed foods represent a novelty for the food industries therefore the technological and sensorial effects derived from insect incorporation in staple foods, as well as their effects on human health, are yet poorly documented. The available literature on insect-related properties is, indeed, limited to easily retrievable data such as nutritional composition and cultivation footprint. Therefore, similarly to algae and fungi, sustainability assessments are limited to the raw materials. At the nutritional level, insects are characterized by an extremely heterogeneous nutritional composition, varying due to both internal factors, such as species and the metamorphosis stages (i.e., larvae, pupae, and adult), and external factors including origin, feed, and cultivation method (Borges et al., 2022). Protein represents the main macronutrient whose contents range from 40% (in Isoptera) to 64% (in Blattodea) of DW, with also great variability within orders. In general, insect protein contents are higher than conventional meat since insects are commonly consumed as dried food. As indicated in the Table 4.4.1, most of insect proteins are considered balanced when compared to animal proteins, indeed, all the orders (except for Hemiptera) have an EAA composition that meet the WHO/FAO recommendations. However, recurrent deficiencies can be found in the Blattodea, Hemiptera, and Isoptera orders, where one or more EAAs are lacking.

Table 2.4.1 – Essential Amino Acid composition (expressed as % of total proteins) of various insect orders compared to two animal protein sources and the WHO/FAO recommendations (Van der Heijden et al., 2023).

Protein source	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl alanine	Threonine	Trypto phan	Valine	EAAs
Beef	3.5	4.6	8.3	9.0	2.7	4.0	4.4	1.0	5.0	42.5
Chicken	3.2	5.0	8.0	8.9	2.7	4.0	4.4	1.2	5.1	42.6
Blattodea	1.8	2.6	5.0	4.4	2.3	2.7	3.0	0.6	4.7	27.2
Coleoptera	2.9	4.9	7.7	5.4	1.7	4.3	3.8	1.0	5.6	37.2
Diptera	3.4	3.7	6.0	6.0	2.3	4.5	3.8	2.8	5.0	37.5
Hemiptera	1.6	3.1	5.0	2.8	2.2	3.4	3.0	1.0	4.4	26.6
Hymenoptera	2.7	4.8	7.9	5.3	2.4	4.6	4.2	1.0	6.0	39.1
Isoptera	4.4	4.8	8.1	6.0	1.2	4.5	4.3	1.0	6.1	40.4
Lepidoptera	2.7	4.3	6.4	6.2	2.2	4.5	4.2	1.5	5.5	37.5
Orthoptera	2.5	4.1	7.6	5.6	1.6	4.2	3.9	0.7	5.3	35.5
WHO/FAO essential amino acids recommendations										**27.7**

Regarding digestibility, human data are still limited: DIAAS scores have not yet been documented, whereas PDCAAS ratings have been established only for some beetles, silkworms, and mealworms. However, from the limited available data, insect proteins have shown a lower digestibility than animal-derived proteins due to the presence of chitin in the exoskeleton, though the PDCAAS scores remain higher than vegetal proteins (Van der Heijden et al., 2023). After proteins, lipids are the second main macronutrient with contents varying from 10% to 70% of DW, with the highest levels during the nymphal phase. Lipids present an attractive fatty acid profile that is mostly composed of PUFAs and varies based on both internal and external factors. In general, naturally harvested insects present lower lipid contents and a better profile than commercially raised insects, which contain higher amount of linoleic acid mainly due to diets usually rich in cereal-based products. Besides fatty acids, insect lipid fraction also includes phospholipids, waxes, sterols, glycolipids, and triacylglycerols. Insects are also a source of fibres, mainly composed of chitin which is an essential component of insect cuticles. Likewise meat, insects provide poor quantities of carbohydrates, generally found as leftover food in their gastrointestinal tract (Oonincx & Finke, 2021). The micronutrient profile varies greatly among orders, especially due to external factors such as the diet. Considering minerals, insects are considered good sources of magnesium, potassium, and sodium, since most species meet the dietary requirements of these three minerals. Conversely, calcium contents are generally low given the absence of a mineralized exoskeleton. Furthermore, trace minerals like iron, zinc, manganese, and selenium can be found in adequate levels in most insect species. However, overall mineral bioavailability can be impeded by the presence of phytates, tannins and oxalates. These classes of antinutritional factors tend to concentrate in the insect gastrointestinal tract, since derive from diets predominantly composed of plants. Besides minerals, insects are also an important source of vitamins including vitamin A (in the form of retinoids), D (*de novo* synthesized by some species), E, and all the B-complex vitamins, except for thiamine (vitamin B_1). Like minerals, wild insects present higher concentrations of all the vitamins, though mainly A and E, than most cultivated insects since the presence of relevant dietary differences. Furthermore, the content of B-complex vitamins heavily relies on the degree of processing, since they can end up being destroyed by heat, light or oxygen exposure along the production chain (Oonincx & Finke, 2021). In addition to their nutritional value, insects also provide several bioactive compounds with activities that include antioxidant, anti-inflammatory, antimicrobial, antifungal, antitumor, and cardioprotective. In particular, the typical high protein contents of insects made them an important source of BAPs, especially those presenting antiradical and anti-inflammatory activity. Other bioactive compounds can be found in the lipid fraction, many of which are EFAs. Among them, the linolenic fatty acids are compounds with well-established beneficial effects towards cardiovascular health. The content of the various EFAs represents a critical point during insect cultivation, since the diet can profoundly change the entire lipid profile, thus affecting the lipid nutritional value. In addition to the EFAs, also some phospholipids, sterols and waxes provide important effects on human health. Lastly, chitin is another bioactive molecule that acts as a dietary fibre, thus providing its related beneficial effects when digested. Moreover, when partially digested by the microbiota, chitin and the derived chitosan express important biological activities including immune-boosting properties and antioxidant, antifungal and antitumor effects. The bioactive compounds provided by insects make them promising ingredients that could be used as nutraceuticals or food additives in the reformulation of staple foods (Acosta-Estrada et al., 2021). Besides beneficial compounds, insects can also be sources of hazardous substances, mostly represented by allergens and environmental residues such as pesticides, mycotoxins, and heavy metals. The occurrence of allergen compounds is a common concern in the safety of insect-based products since they have already been reported in many edible species. The documented allergenic reactions have been predominantly elicited by reactive proteins such as tropomyosin and arginine kinase: two proteins that commonly acts as allergens after arthropod consumption (including also crustaceans and dust mites). A short-term measure to reduce the occurrence of allergic reactions could be providing proper food labelling through appropriate legislation (Van Huis, 2020). The most common reported adverse reaction towards these allergens is anaphylactic shock. Purines represent another concern, especially for people suffering from gout and kidney stones. Indeed, a recurrent consumption of insects could be detrimental since high levels of purine can be found in some common species such as mealworms and house crickets (Acosta-Estrada et al., 2021). Regarding contaminants, some heavy metals has been reported to bioaccumulate, impairing both insect growth and mineral profile. The studies conducted on different species revealed that heavy metals represent a food safety risk for insects, especially for those grown up in contaminated areas (Oonincx & Finke, 2021). On the other hand, the bioaccumulation of pesticides has been poorly documented. However, a study conducted on black soldier fly larvae revealed that both insecticides and fungicides did not accumulate nor altered larvae growth when added to the diet. Likewise, mycotoxins appeared to not accumulate when added to the insect diet, however the effects of the metabolites derived from their digestion have not been assessed yet (Van Huis, 2020). Nevertheless, the occurrence of contaminants in insect-based products need further investigation, with a proper focus on the possible effects on human health. Aside from allergens and contaminants, the main safety issue related to insect is represented by the microbial hazard. Several studies established that insects, regardless of the species, generally present elevated microbial contamination (about 7 – 8 $\log_{10}$ CFU/g), higher than any animal-derived products. The main reasons behind this high level of contamination are the following: firstly, insects are commonly not eviscerated for sale, thus most of the microbial can derive from their bowels. Secondly, the maintenance of adequate environmental conditions along all the production chain, from rearing to shipping, can have a profound impact on the microbial proliferation. According to the EU Regulation of hygiene criteria for minced meat, which also includes insects, fresh insects commonly present levels of microbial contamination that are above the established thresholds. The most recurrent microbes found in insect-based products are food spoilages, among which the genus Enterobacter sp., Enterococcus sp. and Lactococcus sp. are dominant. Besides spoilages, a wide range of pathogenic microorganisms can also be found in fresh insects (including also Salmonella sp. and Escherichia sp. genus), making insects potential vectors of foodborne diseases. Consequentially, all the production chains that handle insects must include additional processing steps aimed to reduce the microbial hazard. The most common antimicrobial procedures include drying, acidification or thermal treatments (like blanching or sterilization), all cost-effective practices that guarantee the safety of the final product (Acosta-Estrada et al., 2021).

Despite the related hazards, the attractive nutritional composition, together with the technological properties and the rearing advantages, represent valuable reasons to further investigate on this alternative protein source. Moreover, the possibility of insect incorporation in staple foods is beneficial for food industries since insects can convey their benefits in a format able to mislead consumer’s perception. Insects are still in a developmental phase in many Western countries, whereas challenges at both nutritional and technological level need to be faced. However, the potential offered by insects is fuelling the research to cover the scientific and sustainability gaps that currently impede (together with the negative consumer perception) the scale up of this emerging alternative protein source.

4.5 – Cell cultured-based alternatives

To date, despite several decades of research and innovations, comprehensive food safety trials regarding cell cultured-derived products have not been published yet. The reason is that most of these products are unsuitable for the market since they fail in the recreation of traditional meat quality traits. Furthermore, all the countries (except for Singapore and the US) still lack a defined legislation regarding both production and sale of cultivated foods (Chodkowska et al., 2022). The inability to run randomized controlled trials based on the consumption of these lab-derived foods generates a big conundrum on their long-term effects on human health. To date, the scientific communities have assessed cell-based food sustainability in a limited way, namely considering only the production stages of cell cultured-derived foods (FAO & WHO, 2023). The data retrieved from the production chain represent the currently available knowledge of these products, which includes nutritional composition, production footprint, and potential benefits and hazards. Therefore, the overall product sustainability remains unknown or based on assumptions.

From the nutritional standpoint, cell cultured-derived foods present a similar, but not identical, composition compared to meat. The composition can vary to some extent based on the various processing methods used and the types of isolated cells. The latter have recently been confirmed by the scientific community which shown that satellite cells taken from bovines and chickens present a different amino acid profile and taste in respect to their conventional counterparts. It remains quite unclear whether cultured cells will be able to cover these nutritional gaps or fortification will be the only way to reach the desired composition (Thorrez & Olenic, 2023). A particular feature of these cultivated products is that, during food processing, their nutritional composition can be adjusted through different engineering technologies or by simply adding the missing nutrients. However, the effect of these adjustments on the overall product sustainability is still unknown (Wood et al., 2023). The composition of cell-grown foods can be further affected by the scaffold, depending on the materials and technologies used for its realization. Indeed, during the growth, the cellular biomass may interact with the scaffold thus resulting in exchanged compounds that alter the product composition. As a solution, animal proteins such as collagen or fibrin could be useful as scaffold material since these proteins are naturally occurring in meat; however, this solution is not suitable in the production of animal-slaughter-free products. An interesting alternative to animal-derived materials could be vegetable polysaccharides, which can supplement the product with dietary fibre. However, despite its nutritional benefits, the provided fibre would change the composition of cell-grown foods therefore resulting in products not resembling conventional meat (Seah et al., 2022). When compared to meat, the protein content of cultured products is usually the factor that resembles meat the most since both products have the animal cell as a building block. However, considering the applied processing methods, even protein content can vary to some extent from meat. Regarding fats, they are obtained through the cultivation of adipose stem cells that synthesize both saturated and unsaturated fatty acids. The complex fatty acid profile of meat, however, results are impossible to replicate using only this process. To solve these fat deficiencies, the addition of animal- or plant-derived fats to the cell biomass could be a valuable solution. However, the end-stage manipulation of the fatty acid profile is still under investigation and requires further research since the simple addition of fats to the media could have adverse effects on cellular growth as well as raise the environmental and economic costs of the food production. Conversely, due to the current knowledge gap, fat addition to the media can perhaps be more convenient than cultivating adipose stem cells (Fraeye et al., 2020). Another important of conventional meat is the content of important high-quality minerals, which, in cell cultured-derived products, are provided by the culture media. However, media not always present a complete micronutrient profile, with some minerals completely absent or present in lower concentrations when compared to meat (the lacking minerals are generally iron, zinc, and selenium). Therefore, the deficiencies are supplemented through direct addition in order to maximize cell proliferation. Although the ease of this method, is still unclear whether the mineral uptake and accumulation in the final product would reach comparable levels to meat. Besides minerals, another limiting factor of culture media is represented by vitamins, particularly by the vitamin B_{12}. Indeed, this vitamin can be found exclusively in animal products since it is produced by some microorganisms and then used by the animal. In a laboratory this important step is missing, thus vitamin B_{12} need to be supplemented to the media as essential for cell growth. However, like minerals, the uptake of vitamin B_{12} remain an important challenge for food processing since this vitamin requires a specific binding protein for its uptake. Therefore, further investigation is needed to understand whether the end-stage addition of nutrients to the media is a cost-effective strategy to resemble meat at nutritional level (Fraeye et al., 2020). Aside from macro- and micronutrients, cell cultured-derived foods also lack several bioactive compounds of which meat is an essential source. The two most important compounds are taurine and creatine. The first molecule is a free amino acid produced in the human body by the liver and brain, which intake has been associated with beneficial effects on the human health, including cardiovascular protection. Furthermore, adding taurine to the “differentiating media” could improve cellular growth since this molecule has an important role in skeletal muscle cell differentiation. The second bioactive compound is also produced in the human body to be accumulated in the skeletal muscles which provides the first energy substrate for muscle contraction. Like taurine, creatin supplementation in the “differentiating media” enhances the differentiation of skeletal muscle cells into myotubes, in addition to provide well-known nutritional benefits after its intake. Despite the nutritional and differentiation advantages provided by the supplementation of these bioactive compounds to the culture media, numerous uncertainties remain regarding the economic feasibility of this strategy (Fraeye et al., 2020).

Cell culture-derived foods are produced in strictly controlled environments to improve cell growth as well as guaranteeing food safety. However, despite these strict measures, cell-based foods are not free from hazards. In the recent paper published by the FAO and the WHO (2023), up to 53 potential health hazards have been identified along the four production stages of cell-grown foods, namely cell selection, production, harvesting, and food processing. As depicted in the Figure 4.5.1, hazard identification is only the first of four steps in order to make a proper risk assessment, which consists of a scientific evaluation of the potential adverse health responses after the exposure to food-derived hazards. Together with risk communication and management, risk assessment is essential to carry out a food safety risk analysis (FAO & WHO, 2023).

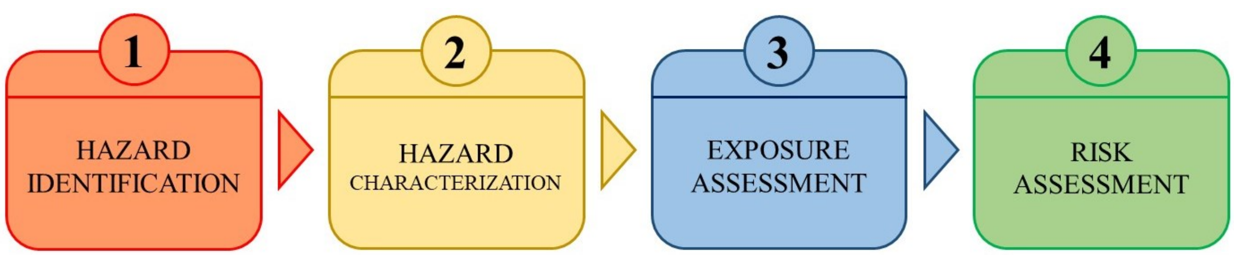

Figure 4.5.1 – Schematic diagram depicting the principal steps needed for a risk assessment (FAO & WHO, 2023).

The expert panels established that many hazards such as microbial contamination might occur during the production of conventional food, whereas some other hazards are uniquely related to cell-based foods. The first production phase is cell selection which is composed of several steps such as animal biopsy, cell isolation, preparation, and storage. During this phase, the main hazards encountered include microbial (both spoilage and pathogen microorganisms) and chemical contamination, which might be present in the biopsied tissue. Both hazards can be introduced in the production chain at any of its steps since cell cultivation is highly susceptible to any kind of contamination. Common prophylaxis measures towards these hazards include follow Good Hygiene Practices (GHPs, including sterilization of environment and work tools), a regular monitoring, and the usage of antibiotics to prevent microbial growth. However, antibiotic drugs represent a growing hazard for consumers since they are associated with the generation of drug-resistant microbial strains, which are increasingly considered a life-threatening issue towards humans. Furthermore, antimicrobial drugs represent also potential allergens towards some consumers. However, when the culture conditions are safe and controlled, antibiotics can be eliminated or substituted by other chemical preservatives to reduce human exposure to them. After animal biopsy, the obtained tissue undergoes further processing steps to isolate determined cells and then stored in a controlled environment. During these steps the used ingredients (like culture media) can introduce other health hazards including microplastics, heavy metals, chemical contaminants, and food allergens. The second phase of the production process is cell growth and production in proper bioreactors. Besides microbial and chemical contamination, another important hazard that could be encountered during cell growth are genetic drifts caused by an accumulation of adverse mutations over time in cell lines. After cell growth, the obtained biomass must be harvested therefore separated from the culture media and its ingredients. However, after separation, components and residues of the media could remain in the biomass. In particular, hormones and growth factors are biological components of the media that might reach the consumer whether harvesting is inadequately performed. These active molecules represent a health hazard since they have been associated with metabolic alterations and cancer development. Furthermore, to facilitate the separation of the biomass from the media, several chemical compounds or enzymes (such as dissociation agents and proteases) are utilized. In case of inadequate harvesting, some of them could remain in the biomass and elicit allergic reactions once consumed. The harvested biomass is then processed into cell-based foods through the addition of several ingredients and food additives such as binders, flavour enhancers, preservatives, and emulsifiers. Among these ingredients, some of them can act as allergens when consumed as well as the residues that might be derived from the scaffold removal. Before packaging, cell-based foods undergo further mechanical and thermal processing and perhaps resulting in the formation of adverse by-products derived from undesired oxidation and degradation reactions. These by-products can result in a worsening of the product quality traits as well as representing a hazard to human health. Furthermore, as for conventionally produced foods, GHPs must be followed to minimize any kind of contamination. Finally, during packaging, each product must be clearly labelled to properly warn consumers and thus minimize allergies occurrence. Accordingly, all the ingredients, nutrients, substances, and food additives utilized along the whole production chain must be properly labelled (FAO & WHO, 2023).

Despite the accuracy of this work, the identification of hazards related to cell-grown foods is still incomplete since the post-consumption effects have not been assessed yet. Moreover, since hazard identification represents only the first of four steps, further data must be collected in order to carry out a proper risk assessment of cell cultured-derived foods (FAO & WHO, 2023). Considering the global population trends, in-depth investigations must be conducted soon to assess whether lab-derived foods represent a valid and healthy source of nutrients and therefore a sustainable solution towards conventional meat.

Chap. 5 – SWOT analysis and discussion

After the assessment of the benefits and hazards conveyed by meat alternatives, much remain to be inferred to understand whether these novel food products really represent a real replacer for their animal counterparts and whether they represent the ideal solution to face the ongoing challenges of the growing population and the climate changes. Currently, there are many barriers that interpose between meat alternatives and their integration into the diet of most consumers. Optimistic predictions made by some meat alternative supporters show that these products will reduce the meat's market shares rather than completely replace it, with 60% of the global meat market covered by meat alternatives in 2040. However, contrasting predictions were made considering some crucial issues that still restrain the diffusion of these products, in particular the capacity of providing the equivalent sensory properties of meat and their selling price (Siegrist & Hartmann, 2023). The following SWOT analysis (Figure 5.1) summarizes everything said so far on meat alternatives and dividing it into four categories, namely Strengths, Weaknesses, Opportunities and Threats.

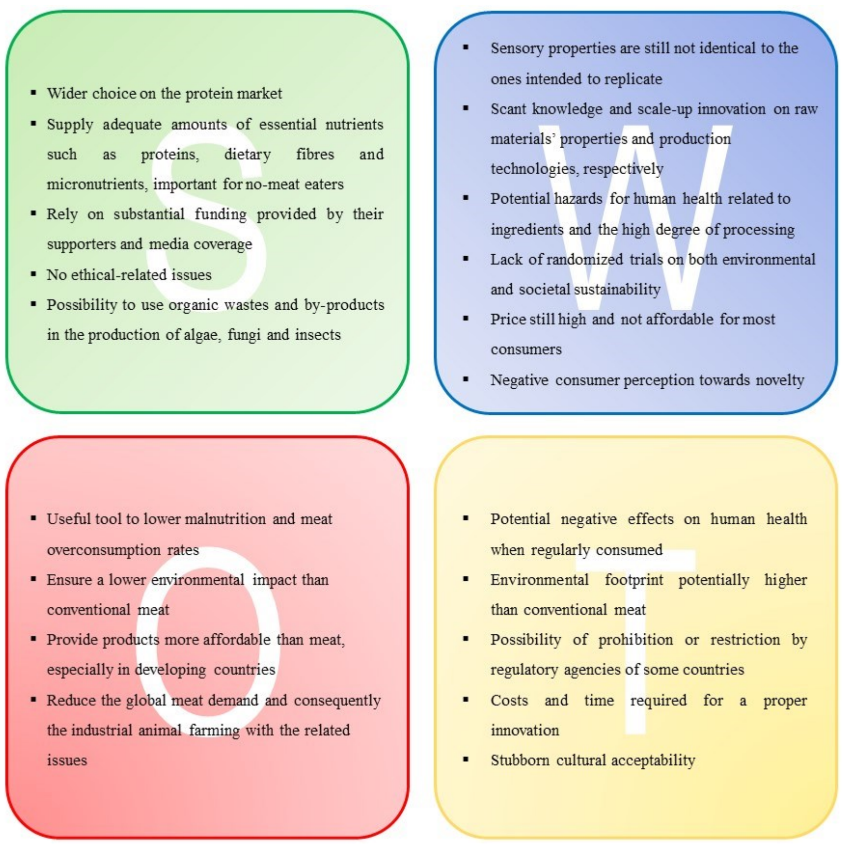

Figure 5.1 – SWOT analysis related to the future of the assessed meat alternatives.

Meat alternatives represent novel products able to enlarge the protein market towards all consumers while providing essential nutrients, especially to no-meat-eater who tend to be deficient in different animal-derived nutrients. Furthermore, the market development of meat alternatives can rely on significant funding since their supporters (including many billionaires and stakeholders) quicken the academic research by investing significant amounts of money in it, in exchange of future profits (Leroy et al., 2023). Besides the economic return, the research is also aimed to exploit the several opportunities offered by the meat alternatives, namely the production of foods providing essential nutrients to treat malnutrition, in addition to environmental, societal, and economic advantages. These advantages could be a lower environmental footprint and selling price than meat, as well as replace the market shares of industrially produced animal-derived foods and lower the growing meat overconsumption rates. However, the several weaknesses and threats depicted in the Figure 5.1 severely slow down the development and diffusion of meat alternatives. While the knowledge and technological gaps prevent the food industries to recreate the meat sensory properties, drawbacks like the high prices, the negative consumer perceptions, the different hazards found in the products and the lack of long-term randomized trails, represent prominent barriers for the global diffusion of meat alternatives. Furthermore, there are several factors threatening both the development and diffusion of meat alternatives. Future studies assessing the overall sustainability could claim meat alternatives as foods potentially hazardous when regularly consumed or having a higher environmental impact than meat. Finally, the diffusion of meat alternatives could be blocked or limited in countries characterized by a strict food legislation or a common negative behaviour towards novelties deeply rooted in consumers.

Based on this analysis, the fate of the meat alternative market seems more doubtful than the one claimed by their industrial producers. Despite the many barriers hampering their market development, several supporters have established optimistic expectations showing the end of the meat market already in the near future. However, these claims have been made despite the current incomplete literature as well as ignoring several opposing factors. Besides the trials on consumer acceptance (Siegrist & Hartmann, 2023), meat alternative supporters tend to ignore also that conventional farming is deeply involved in a variety of societal roles beyond the production of animal-derived foods that include the conversion of non-edible food into high quality food products; the production of useful by-products (e.g., manure, wool, horns, etc.); the animal contribution in many ecosystem services; the provision of labour and workforce; and, lastly, the cultural meaning resulted after centuries of domestication (Wood et al., 2023). Another factor persistently ignored by supporters is the fact that most of the growing population, and the consequent protein demand, will be in developing countries, where very few studies on consumer acceptance have been conducted so far. Finally, the food industries should also consider that important shifts in consumer dietary habits usually take a long time, just like shifting towards diets consisting of less animal-based products (Siegrist & Hartmann, 2023).

To conclude, considering the weaknesses and threats depicted in the Figure 5.1 and the abovementioned claims commonly ignored by the food industries, it is understandable that predicting any future trend of meat alternatives is quite complex, if not risky. To make truthful predictions, much research should be carried out in the future and focused on covering the significant gaps found in the current literature. The numerous strengths and opportunities that currently fuel the innovation of meat alternatives could not be enough to guarantee a future market development. Indeed, the food industries should address their fundings to strategies that go beyond advertising and focus primarily on technological solutions to overcome much of the barriers encountered so far.

Conclusions

The food system faces two significant challenges: the increasing global demand for proteins and the impacts of climate change. These challenges underscore the overall lack of sustainability in current food systems, encompassing economic, social, and environmental aspects. The overconsumption of meat, a dietary pattern increasingly prevalent in developed countries, is strongly associated with various side effects, including the rise of industrial animal farming and its related environmental impact, as well as negative health effects. Consequently, meat alternatives have been introduced to consumers as a promising approach to enhance food system sustainability, offering a healthier and more ethically conscious option compared to conventional meat. Supporters of the food industry claim that meat alternatives will serve as the sustainable future of protein, providing slaughter-free products with the same nutritional profile as meat, albeit with a lower environmental impact.

However, considering the barriers outlined in the SWOT analysis, the possibility of meat alternatives completely dominating the meat market remains highly unlikely, at least in the foreseeable future. Among the protein-rich materials currently utilised in the production of meat alternatives, including plants, algae, fungi, insects, and cell cultures, none have thus far been able to replicate the sensory properties of conventional meat. Quality traits such as texture, taste, and flavour continue to be challenging to recreate using current industrial processing methods, primarily due to limited knowledge and technological innovation. Each raw material presents several drawbacks that significantly hinder their innovation. Nonetheless, these alternative proteins also offer several advantages that warrant further investigation. Each protein source possesses unique nutritional and functional properties that should be valued rather than altered and processed solely to replicate the sensory properties of an existing product, especially when excessive processing tends to degrade many of these distinctive features. Indeed, these raw materials should be appreciated and marketed as alternative proteins, rather than being exploited solely to resemble other products.

Still, the need to replicate the familiar and beloved qualities of meat is crucial for the success of these alternatives, particularly to capture market shares among non-meat-eater consumers. It is important to note that the ideal of successfully replicating meat-like quality traits represents a double-edged sword, as the high degree of processing required for this purpose also transforms these raw materials into ultra-processed foods. Therefore, food industries should reconsider prioritising profits and instead evaluate whether recreating the sensory properties of meat is a cost-effective strategy worthy of further research and funding. In food production, healthiness should never be sacrificed for taste, as this does not provide a long-term sustainable solution.

Currently, it is challenging to determine whether meat alternatives represent the most sustainable solution for our future food system, as existing knowledge and technological gaps hinder accurate predictions by the scientific community. Overall sustainability presents a significant conundrum for these meat alternatives, and its resolution requires time, which is a resource that is increasingly scarce. In conclusion, considering these prominent barriers, it seems more reasonable to focus on improving the current food system rather than adopting a novel and experimental system that perceives animal farming as a problem to be minimised. Enhancing the livestock system while mitigating the overproduction and overconsumption of meat, characteristic of Western countries, could be the strategy that currently presents the highest chance of success. Meat is considered a nourishing and culturally significant unprocessed food by the majority of populations worldwide. For these reasons, it cannot be simply eliminated from everyone's diet, and more importantly, meat should not be permanently replaced by ultra-processed foods, including meat alternatives, as the extensive list of adverse health effects is closely associated with this category of food products.

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