

Review

Advancements in texturization processes for the development of plant-based meat analogs: a review

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Meat analogs are developed in response to environmental concerns, health conscious, and ethical consumer choices. Advancements in texturization processes with respect to the optimization of extrusion cooking, shear cell, and 3D printing are thought to be essential to maintain future consumer interest. Here, we describe both thermal and nonthermomechanical treatments based on peculiar gelation mechanisms of ingredients. The combination of two or more different technologies is a promising strategy to enhance textural, nutritional, and sensory properties. Examples include integrating extrusion with shear cell, using new rotating die geometries, or combining fermentations with structuring processes. Other innovations explore sustainable protein sources and additives and nonprotein ingredients to improve textural properties, although the latter may raise concerns about acceptance and sustainability.

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Current Opinion in Food Science 2024, 58:101192

This review comes from a themed issue on **Innovations in Food Science**

Edited by **Davide Giacalone**

Available online 26 June 2024

<https://doi.org/10.1016/j.cofs.2024.101192>

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The motivations and the drivers behind the shift toward plant-based food are given in excellent recent studies [1,2,3,4••,5], and they are summarized in Figure 1.

Compared with traditional meat substitutes, such as seitan, tempeh or tofu [5], meat analogs are specifically designed and developed to resemble meat product characteristics, such as texture and taste. This imitation also considers the visual appearance and the cooking experience of the products [1], aiming to mimic all the phenomena occurring during preparation, such as color changes, fat melting, and aroma development. This choice is driven by the need of capturing meat consumers that seek for meat analogs [5], or flexitarians consumers who selectively and limitedly consume meat products. A high similarity with meat products helps consumers to become familiar with these new products, considering that sensory barriers are one of the major limitations for the acceptance of meat analogs [4••,5], in addition to person-related factors [4••]. Consequently, the technological advancements in this sector are mainly aimed at enhancing the sensory quality of these products, with a particular emphasis on protein texturization.

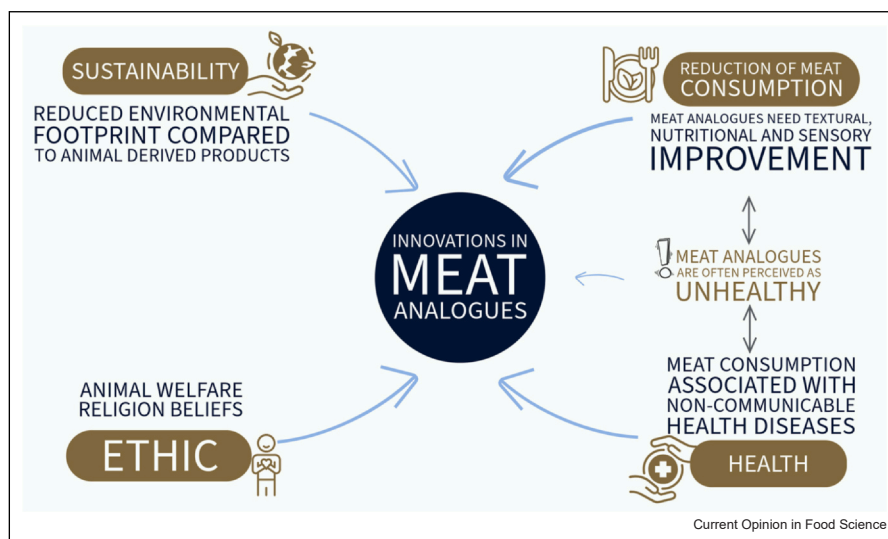
Most meat analogs use texturized proteins to obtain the fibrous texture resembling the meat muscle [1,5–8]. To tune the texture and juiciness, additional ingredients, such as fats, polysaccharides, hydrocolloids, colorant and flavoring agents, are used [1,7,9••]. It may be surprising to read that protein texturization has been used for decades. The first texturized proteins were developed in 1960s using low-moisture extrusion (LME) with single-screw extruders [5,8]. Then, during 1990s, high-moisture extrusion (HME) technologies emerged, with twin-screw extruder equipped with a long cooling die [8,10], aimed at producing texturized protein with well-defined anisotropic structure. Nowadays, HME is one of the most studied technologies to produce meat analogs [3]. More recently, texturization processes including shear cell, 3D printing, and controlled gelation have been described (Figure 2), which all will be discussed in the next sections.

Introduction

The global demand for plant-based protein sources and food has demonstrated a remarkable increase in recent years, driven by growing environmental concerns, health-conscious consumer choices, and ethical considerations.

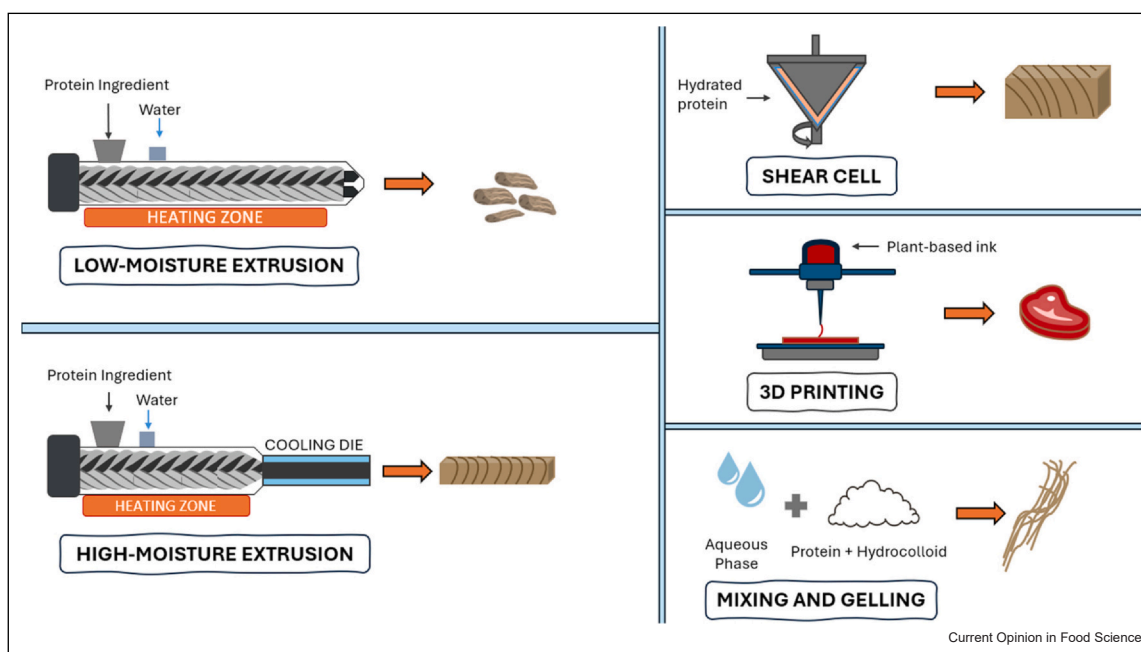
In this review, the recent technological advancements for the development of meat analogs are discussed, highlighting the challenges and the opportunities that need to be addressed to support the transition toward diets lower in meat. In the dynamic scenario of

Figure 1



Main drivers and factors that stimulate the innovations in meat analogs.

Figure 2



Texturization technologies for the development of meat analogs and materials with fibrous texture.

innovations, we give our viewpoint and perspectives concerning the most promising strategies and the challenges for the development of this sector.

Advancements in protein texturization

It is possible to distinguish two categories of meat analogs [1,11••]. The first including burgers, sausages, or

other reconstructed products obtained with texturized proteins and other ingredients. The second one is aimed at mimicking whole-muscle meat products, specifically fillet of meat. Figure 2 depicts the technological processes used to produce texturized protein and processes used to prepare meat analogs. Both LME and HME can be used to obtain texturized proteins, whereas whole-cut

meat analogs can be only obtained with HME cooking operating with a cooling die with suitable dimensions. The demand for whole-cut meat analogs stimulated the development of alternative technologies in addition to extrusion cooking, such as shear cell and 3D printing, together with pioneering approaches exploiting the gelation properties of ingredients and that will be discussed next in the manuscript. The development of new meat analog product is probably key to maintain future consumer interest.

Innovations in thermomechanical treatments

High-moisture extrusion cooking

Table 1 summarizes the processing conditions and ingredients used in the extrusion experiments. The thermomechanical treatment occurring in the extrusion barrel is responsible for protein unfolding, cross-linking and aggregations [9••,10]. The screw configuration determines not only mixing intensity but also the product temperature and residence time. Then, the long cooling die plays a crucial role in the texture formation through the deformation and elongation of the protein phase [9••,10,12•]. Recently, the overall thermal process intensity was quantified by Near Infrared spectroscopy [13], providing an online process monitoring that can guarantee the constant and the correct conditions during texturization, improving process standardization. Given its conformation and set-up, the innovations in HME can regard both the barrel section and the cooling die.

Zink et al. [14•] proposed the nitrogen injection as a technology to tailor a microfoaming mechanism during the extrusion. The idea behind nitrogen injection is that the texture formation could be modulated by creating microbubbles in the melt [14•,15]. By adjusting the gas pressure and the die temperature, it was possible to obtain meat analogs with different textures. An increase in nitrogen pressure led to a decrease in meat analog density while simultaneously resulting in a decrease in hardness due to the aerated structure [15]. The different densities of meat analogs may also influence their physicochemical properties. Specifically, the water absorption of meat analogs is influenced by their porosity and density [6], offering interesting possibilities to tune the final product properties. Zheng et al. [16] recently investigated the possibility of modulating the flow in the cooling die using a multihole nozzle at the junction between the extrusion end and the cooling die. The multiholenozzle allows for the creation of long and straight fibers that are solidified at a temperature of about 40°C [16]. Contrary to the anisotropic structure commonly found in high-moisture meat analogs, which have a V-shaped or parabolic pattern, this peculiar fiber conformation was found to enhance freeze–thaw stability and increase the rehydration capacity, which was explained by improved water distribution in the samples

[16]. Snel et al. [12•] designed an in-house die that consists of a cylinder with two-rotating inner parts, combining the well-defined shear typical of the shear cell with the advantages of the extrusion technologies, such as continuous processing. This design efficiently cools the melt at the barrel exit while maintaining well-defined shear and allows adjustment of the shear rate during cooling. Consequently, the modulation of fiber formation can be achieved either independently of, or in addition to, traditional extrusion parameters such as screw speed and barrel temperature. The rotating die also facilitates the production of extrudates of considerable dimensions, potentially suitable for whole-cut meat analogs. From an industrial implementation standpoint, integrating both gas injection and the rotating cooling die is relatively straightforward. Gas injection requires only an injection port and a pressure control valve, while the rotating die can be attached to existing equipment. Finally, the introduction of these new process variables calls for further optimization studies involving processing conditions and the behavior of protein ingredients. Moreover, it would be interesting to investigate the economical and the environmental implications of these innovations at both pilot-scale and industrial-scale levels.

The exploration of oil addition during extrusion has emerged as a promising strategy to enhance the textural properties of meat analogs, particularly improving tenderness and juiciness. However, challenges arise regarding effectively incorporating oil during processing, while the occurrence of lipid oxidation due to the thermomechanical treatment is not perfectly clear. However, adding oil at the end of the extruder barrel, in correspondence with the mixing zone, has been shown to enhance the fibrous structure formation, generating small-sized oil droplets [17], which are well-included in the protein matrix. Interestingly, it is reported that preparing an oil-in-water emulsion allows to double the maximum quantity of oil that can be added during extrusion without compromising the textural properties because the oil was distributed in small droplets within the protein matrix [18].

Finally, the modulation of structure formation and textural properties can also be achieved by adjusting environmental conditions during extrusion, varying the pH [19•,20]. It affects some physicochemical properties of proteins, including (i) the higher reactivity of disulfide bonds at higher pH conditions and (ii) the higher protein solubility and lower water holding capacity at higher pH [19•]. In fact, disulfide bonds have a recognized role in the fibrous structure formation [9••]. Nisov et al. [19•] highlighted the necessity of increasing the extrusion temperature under acidic conditions (pH 5), finding an overall enhanced fibrous structure formation at higher pH levels (pH 7). These results are particularly relevant from the perspective of producing meat analogs using

Table 1

A summary of the raw materials and processing conditions used for HME and LME of meat analogs.

Protein ingredient	Moisture content (%)	Additives/nonprotein ingredient	Innovation	T last heating zone (°C)	Water feed (kg/h)	Protein feed (kg/h)	Screw speed (rpm)	Cooling die (°C)	Reference
HME									
SPC	57.7	Canola oil	N ₂ gas injection	140	Total = 35		275	n.a.	[14•]
SPI-SPC	70	/	N ₂ gas injection	125	n.a.	0.5	200	35, 50, 65	[15]
SPI	65	/	Multihole nozzle in the cooling die	120	n.a.	n.a.	175	20–60	[16]
PPI, SPC, WG, and their blends	≈55–65	0.7% NaCl	Rotating cooling die	125	9	6	240	85–10°C and 45, 75, 105 rpm in the cooling die	[12•]
Rice protein, WG, PPC, PPI	n.a.	1 M HCl, 1 M NaOH	pH shifting from 5 to 7	4	0.19–0.31	0.18–0.28	200–350	30–45	[19•]
Rapeseed	n.a.	HCl, NaOH	Lactic acid fermentation of protein and pH adjustment	145–165	0.18–0.30	0.25–0.30	200–300	30	[20]
Sunflower protein:PPI 1:1	n.a.	HCl, NaOH	Lactic acid fermentation of protein and pH adjustment. By product addition	125	0.24–0.33	0.25	250	n.a.	[40•]
PPI with 5, 10, 15% <i>Penicillium limosum</i>	60	/	Mycoprotein Addition	140	0.72	0.40	100	n.a.	[43]
Peanut protein	55	0–0.3% Transglutaminase	Enzymatic treatment	110	6.5	6	210	70	[32]
WG	51–53	2, 4, 6% MCT oil	Oil addition point	120–140	Total = 10		200, 400, 800	40	[17]
SPI:WG 70:30	60	O/W emulsions with maize oil	Oil added as emulsions	140	n.a.	0.3	400	40	[18]
PPI	54	0–0.15% L-cysteine	Additive for texturization	140	n.a.	10	162	50	[29]
Mung bean	50–60	/	Dry-fractionated protein	115–145	Total = 0.7		800	10	[38]
Dry- and wet-fractionated hemp protein	52–60	20% Maize starch	Dry-fractionated protein	150	Total = 10.8		550	24	[37]
PPI, PPC, Wheat and oat brans	46–56	/	By-product addition	120–145	0.21–0.37	0.25–0.30	250–300	30	[41]
SPI	60	5–20% Tomato peel powder	By-product addition	90	n.a.	3	300	n.a.	[42]
LME									
Faba bean:oat 80:20	28.5–38.4	/	Dry-fractionated protein	133–167	0.26–0.46	3.32–6.68	900	/	36
Dry-fractionated pea:oat protein 70:30	25	/	Dry-fractionated protein	140	Total ≈ 5.6		800	/	[6]
PPI:oat protein 70:30	25–30	/	Lactic acid fermentation and phytase treatment	140	Total = 5		600–1200	/	[39]

Abbreviations: PPI: pea protein isolate; PPC: pea protein concentrate; SPI: soy protein isolate; SPC: soy protein concentrate; WG: wheat gluten; n.a.: not available.

fermented raw materials, as will be discussed in a next section of the manuscript. Moreover, both the oil addition and pH modulation can justify further investigation to assess the stability and shelf-life of the final products. Indeed, oxidative degradation of lipids during the structuring process, as well as the shelf-life of meat analogs affected by processing conditions and pH, remain largely unexplored areas in current literature.

Low-moisture extrusion cooking

Although HME is attracting most of the interest in scientific literature, texturized proteins produced through LME are extensively utilized in the food industry for burgers, patties, and other food resembling preparations based on minced meat. However, as reported in Table 1, recent studies investigating protein texturization via LME are limited. It might be hypothesized that being a well-known process, as described in the introduction, academic interest has now shifted toward HME and alternative structuring technologies. LME refers to extrusion processes in which materials with a moisture content below 40% are processed. The exact moisture content depends on the water absorption capacity of the protein ingredients [6], while the die design is less relevant compared with high-moisture processes. It is interesting to note that the environmental impact of a certain plant-based product depends on the type of structuring process, with HME leading to a lower impact compared with low-moisture one [21••], which can be explained by the fact that LME extrudates are dried, while HME products are not. Consequently, these findings suggest that further research efforts should focus on optimizing processing conditions to minimize process-related impacts. This underscores the necessity of adopting a multidisciplinary approach to comprehensively understand the structuring processes while also considering the environmental and economic impacts.

Beyond extrusion: shear cells and 3D printing

A recent review on the shear cell devices and on their unit operations for protein structuring is given by Cornet et al. [10]. Like extrusion, shear cell technology exploits a thermomechanical process to create a fibrous texture from plant proteins [10,22,23]. However, contrary to extrusion, shear cell allows to apply a well-defined shear during processing. It is worth noting that shear cell technology is relatively recent compared with extrusion, which explains the lower number of articles. However, research activities cover several aspects, as reported in Table 2, which summarizes the recent developments involving shear cell technology. For example, the influence of processing conditions on the structuring mechanisms and product properties has been investigated. It was reported that the effect of mixing time on product structure can vary depending on the protein type [23]. While hydration time has a limited influence on product quality, it remains important to ensure complete

Table 2

Summary of the raw materials and processing conditions for shear cell structuring process.

Protein ingredient	Moisture content	Additives/Nonprotein ingredient	Processing conditions	Reference
SPC, SPI, PPI, WG PPI:WG 1:1	60% 60%	1% NaCl 1% NaCl	120–140°C for 15 min at 30 rpm 120°C for 15 min at 5–100 rpm 120°C for 15 min with 8 min shearing at 5–100 rpm 120°C for 15 min with 5–100 rpm during cooling	[23] [22]
PPI:WG 1:1	60%	0.2% L-cysteine	100–150°C for 15 min at 30 rpm, cooling for 10 min	[30]
SPI	60%	2% high acyl gellan gum, low acyl gellan gum, high methoxyl pectin, low methoxyl pectin, and xanthan +0–1% CaCl ₂ and NaCl	120°C for 15 min at 30 rpm, cooling for 10 min	[31•]
PPI; MBPI; PPI:WG 1:1; MBPI:WG 1:1	60%	Transglutaminase 0–0.7%	120°C for 15 min at 0–30 rpm, cooling for 5 min	[33]
Biomass from <i>Methylobacillus flagellatus</i> OCB6	60%	≈1% NaCl	140°C for 15 min at 30 rpm	[45]

Abbreviations: PPI: pea protein isolate; SPI: soy protein isolate; MBPI: mung bean protein isolate; WG: wheat gluten.

hydration of the protein mix [23]. Then, cooling of the protein melt is also a critical aspect to consider during shear cell processing because the degree and strength of the fibrous structure can be tailored by applying a certain shears during cooling [22]. These findings also contribute to a deeper understanding of the phenomena occurring in the cooling die during HME. Since the experiments were conducted at well-defined shear rates and temperatures in a shear cell, these results can be transposed to HME due to the similarities in the structuring mechanism [10]. Specifically, it is suggested that fibrous structures can form in the cooling die at low shear rates and that increasing the temperature could lead to stronger extrudate [22]. Other studies investigating additives to aid protein texturization in shear cell will be discussed in an upcoming section.

3D printing is particularly interesting for its versatility in creating desired shapes, textures, and sensory properties. While innovations in 3D printing technologies have been comprehensively and recently discussed elsewhere [24,25], this article aims to highlight the challenges of this technology not only from an engineering perspective but also from an economic viewpoint. One distinguishing aspect of 3D printing is that protein ingredients may not be the primary focus as in the other discussed technologies. Instead, gelatinized starch, hydrocolloids, additives, and enzymes play a critical role in ensuring the printability of the ink and guaranteeing the optimal flowability [24–26]. In fact, given the low shear stresses and temperatures, protein are not subjected to denaturation, unfolding and aggregation reactions [25] contrary to extrusion and shear cell. Regarding the temperature, it is interesting to report that most of the studies on 3D printing are often performed at room temperature, after which the printed product is cooked [24–26]. Further research should focus on the optimization of meat analog inks and the development of 3D printers that can enhance the versatility of this technology regarding the types of ingredients that can be utilized [25].

From a critical perspective, it is significant to note that, unlike extrusion processes, shear cells and 3D printing operate in batches, potentially resulting in longer production times and thus higher associated costs for meat analogs. For instance, shearing time in a shear cell can extend up to 15 min, with additional time needed for material cooling and equipment discharge (Table 2). However, the solution to mitigate the limitations of these single technologies involves combining the working principles of two or more technologies. As discussed above, an example is already available for shear cell and extrusion cooking. Conversely, 3D printing requires further optimization of the equipment and ingredients of the ink. In fact, the printing speed can be $10\text{--}40\text{ mm s}^{-1}$, with an extrusion rate variable between 0.3 and 6 ml min^{-1} [25]. Therefore, assuming that 1 ml of

ink weighs 1 g, producing 125 g steak would require from 20 to >400 min. Despite this, 3D printing has the unique ability to personalize shapes, thicknesses, and surface characteristics of products. Furthermore, this technology is particularly promising to address specific needs of consumer groups such as dysphagic patients or elderly people [25].

Nonthermomechanical processes: mixing and gelation

Cutting-edge studies involve the maximal exploitation of the functionality of the ingredients. In a patent from 2011 [27], it was described that mixing milk protein, a hydrocolloid that precipitates with metal cations and water, with a solution containing a metal cation can lead to the formation of a fibrous product. It can be expected that the technology can also be used for plant-based protein because the structuring relies mainly on the hydrocolloid properties. Recently, Dobson et al. [11••] studied an innovative structuring process that does not rely on extrusion or shear processing. Instead, they leveraged specific physicochemical properties of zein, which can form fibers using a supporting matrix when plasticized. Their procedure is that the dry ingredients (proteins and starches) are mixed with an aqueous solution in a ratio of 1:1.125 (w:w), stirred and manually extended to develop a fibrous network. The experimental methods described here align with recent theories involving the exploitation of the gelation properties of the ingredients, suggesting the possibility of creating meat analogs through simple mixing and mild thermal treatments [2,28]. In a pioneering perspective, this opens to the development of ingredient mixes that can be marketed as powdered materials and that consumers can directly use in their domestic preparations to create their desired products. Hypothetically, this shift would focus the attention on the functionality of the ingredients, rather than on the structuring processes.

Future perspectives for meat analogs: additives, new protein sources, biotechnological processes, and combined technologies

In addition to optimization of the technologies described above, there are new developments that can lead to new or better meat analog products. In this section, we first discuss the developments with respect to new additives and enzymes, then we focus on development in proteinaceous ingredients and their production including fermentation. Finally, we describe the potential of using combinations of processes.

Additives and enzymes [9••] can enhance the product properties because they can alter the properties of the protein ingredients through, for example, cross-linking and disulfide bonds formation. L-cysteine has been

studied during HME [29], shear cell processing [30,31•], and 3D printing [26]. An important reason to apply L-cysteine is that it can enhance the aroma of the products, with a consequent improvement of the sensory perception [26]. However, the presence of L-cysteine was effective in a specific and narrow range of temperatures, comprised between 120 and 130°C [30], and concentrations, typically 0.2% [31•] to 0.4% [26]. Transglutaminase can also have beneficial effects on the texture formation in meat analogs, as reported in previous studies [32,33]. Specifically, Zhang et al. [32] proposed that transglutaminase promotes conformational changes in the secondary protein structure, favoring the formation of β -sheet structures in the extrudate. This is suggested to stabilize the protein conformation and thus product structure in addition to the formed hydrogen and disulfide bonds. Interestingly, Schlangen et al. [33] stated that the use of a cross-linking enzyme should be tailored on certain protein types and the state of the proteins based on their findings that transglutaminase acts differently on the same proteins that only differ in their state, especially degree of denaturation. Hydrocolloids, for example, gums, pectin and other polysaccharides can improve textural properties of meat analogs, and their effect can be also modulated by the salt addition [31•].

Next to additives, many new proteinaceous ingredients are emerging currently. One route to make new ingredients is through the use of new sustainable protein extraction technologies. For instance, dry fractionation is a sustainable protein extraction method [34], and it is highly versatile and adaptable to diverse protein sources [35]. Dry-fractionated proteins have been recently used to texturize proteins using both LME [6,36] and HME [37,38] (Table 1), overall suggesting the feasibility of these proteins for texture development, together with the necessity of considerably improving the sensory profile. Dry-fractionated protein has a characteristic beany and green flavor, which is challenging for the consumer acceptability due to lack of intensive washing processes in case of dry fractionation. Besides dry fractionation, also wet fractionation and washing processes are studied as a route to make new ingredients. Studies describe how better ingredients can be produced in a more sustainable manner. The advantage of wet fractionation is that the purity can be controlled, and resulting ingredients are mostly more neutral in taste and color. Fermentation protocols can be also applied to protein ingredients to enhance both nutritional properties by reducing the antinutritional factors, [39,40•] and sensory characteristics through mitigating the off-notes characteristics of the raw materials [20]. This is especially important when considering proteinaceous ingredients from by-products derived from the food industry. Often, lowering the presence of unwanted compounds that compromise nutritional value and sensory properties is required to make the ingredients

suitable for use in food products [40•]. After fermentation, often with lactic acid bacteria, a neutralization to pH 7 is applied to reduce the sour taste of the products, making it similar to the nonfermented ones [40•]. Then, the ingredients can be further processed using for example HME. This concept was successfully applied to valorize sunflower press cake [40•].

Other authors suggested that wheat and oat brans, derived from milling operations, could be directly incorporated with pea proteins, preferably not exceeding 15% in HME [41]. This also increases the fiber content, with consequent nutritional benefits. Lyu et al. [42] explored the utilization of tomato peel powder, from the tomato industry, to attain the desired color in meat analogs, thereby enhancing the nutritional profile. However, it is essential to acknowledge that protein hydrolysis [20] and the macromolecules degradation [39] during fermentation may impede fibrous structure formation and weaken the product, suggesting the importance of further investigation and optimization of fermentation protocols tailoring them for texturization.

Emerging frontiers involve the utilization of cellular proteins derived from mycoproteins as an ingredient for extrusion [43] or as a whole biomass [44]. Microbial biomass cultivated on independent-crop substrates such as renewable methanol made from agricultural by-products could be structured using, for example, shear cell [45]. The limited adoption of these innovative protein sources is primarily due to limited availability of the products in addition to nutritional concerns [43,45], which call for further research in this field.

When overviewing all information above, we conclude that there are many possibilities for innovation in meat analogs and clear directions for future research. Especially, we think that the combination of technologies could be the most promising strategy for the development of meat analogs with improved texture, sensory and nutritional properties. Examples include integrating extrusion with shear cell, using the rotating die, or the fermentations with the structuring processes.

Additives enhance the structural properties of meat analogs in terms of visual fibrousness, tensile strength, and springiness. However, an increase in the number of ingredients could affect consumer acceptability [4••] and the overall environmental footprint. Oil addition during extrusion requires investigations on the oxidative degradation of lipids during the process and the oxidative status of oil-added extrudates. Finally, chemical modification of pH may impact the product stability, with optimal structure typically achieved at higher pH values. Conversely, fermented raw materials with low pH may positively affect meat analogs shelf-life, which is an area still unexplored in current literature.

Conclusion

In this review, we discussed the advancements in the plant-based meat analogs sector and examined the thermo-mechanical treatments such as extrusion cooking and shear cell and innovative nonthermomechanical processes based on the molecular properties of the ingredients. Within this landscape of innovations, we conclude that there is not a single most promising direction for the sector to effectively reach a sustainable alternative product for meat. In contrast, combining two or more different technologies probably offers great opportunities in meat analog development. In this way, the limitations of individual technologies, such as the textural properties of extruded proteins, the batchwise conditions of the shear cell, and the need to improve the nutritional and sensory properties of plant-based proteins, could be mitigated by integrating multiple processes. Possible combinations include extrusion with shear cell and the application of biotechnological protocols before the structuring processes. Moreover, the importance of optimizing the structuring process has emerged. The low specific mechanical energy of shear cell processing suggests that structuring processes can be optimized to lower the mechanical stresses given to the ingredients, which can lead to less damage to their functional properties. As a perspective, this optimization could also reduce the energy consumption and operational costs. This aspect could be further investigated, even in comparison to 3D printing.

The importance of additives and nonprotein ingredients to enhance the structural properties of meat analogs was discussed. However, this is in contrast with the purpose of reducing the ingredients contained in plant-based meat to enhance the acceptance and sustainability of these products. This consumer wish is particularly challenging for reconstructed products such as burger and sausages, whereas it might be simpler for whole-cut products that do not need binders to resemble the minced meat behavior. Fermentation to make the proteinaceous ingredients or to improve the nutritional value can be a clean label option to stimulate new developments in the area of meat analogs.

Overall, the efforts in the development of processes for meat analogs and the continuous research for the improvement of the sensory and nutritional qualities could encourage the shifting toward a diet less rich in meat products. This development should be supported by more comprehensive and multidisciplinary approach to better understand the nature of the structuring process as well as the impacts in terms of environmental and economical sustainability.

CRedit authorship contribution statement

Davide De Angelis: Conceptualization, Investigation, Writing – original draft preparation, Writing – review & editing, Project administration; **Atze Jan van der Goot:**

Conceptualization, Investigation, Writing – review & editing; **Antonella Pasqualone:** Writing – review & editing; **Carmin Summo:** Writing – review & editing.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

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

Acknowledgements

This research was carried out within the ERC Seeds project funded by the University of Bari, grant number 2023-UNBACLE-0243473.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. McClements DJ, Grossmann L: **A brief review of the science behind the design of healthy and sustainable plant-based foods.** *NPJ Sci Food* 2021, **5**:17.
2. McClements DJ: **Soft matter physics approaches for creating plant-based meat analogs.** *Cur Opin Food Sci* 2024, **55**:101120.
3. Miller O, Scarlett CJ, Adhikari B, Akanbi TO: **Are plant-based meat analogues fulfilling their potentials? An Australian perspective.** *Future Foods* 2024, **9**:100305.
4. Giacalone D, Clausen MP, Jaeger SR: **Understanding barriers to consumption of plant-based foods and beverages: Insights from sensory and consumer science.** *Cur Opin Food Sci* 2022, **48**:100919.
- This paper describes the challenges to potentially improve the sensory quality of plant-based food, discussing the drivers and the barriers related to the consumer acceptance of these products. The discussion about the person- and nonperson-related factors is of relevant interest for readers.
5. He J, Evans NM, Liu H, Shao S: **A review of research on plant-based meat alternatives: driving forces, history, manufacturing, and consumer attitudes.** *Compr Rev Food Sci Food Saf* 2020, **19**:2639-2656.
6. De Angelis D, Kaleda A, Pasqualone A, Vaikma H, Tamm M, Tammik ML, Squeo G, Summo C: **Physicochemical and sensorial evaluation of meat analogues produced from dry-fractionated pea and oat proteins.** *Foods* 2020, **9**:1754.
7. Kyriakopoulou K, Keppler JK, van der Goot AJ: **Functionality of ingredients and additives in plant-based meat analogues.** *Foods* 2021, **10**:600.
8. Ubbink, Muihalidin BJ: **Protein physical state in meat analogue processing.** *Cur Opin Food Sci* 2022, **45**:100822.
9. Van der Sman RGM, van der Goot AJ: **Hypotheses concerning structuring of extruded meat analogs.** *Curr Res Food Sci* 2023, **6**:100510.
- The paper comprehensively investigates the phenomena that lead to protein texturization during HME, giving six hypothesis that can explain the structuring process. The discussion about the gaps that need to be filled by researcher can motivate future investigations in this field.
10. Cornet SH, Snel SJ, Schreuders FK, van der Sman RG, Beyrer M, van der Goot AJ: **Thermo-mechanical processing of plant**

- proteins using shear cell and high-moisture extrusion cooking. *Crit Rev Food Sci Nutr* 2022, **62**:3264–3280.
11. Dobson S, Stobbs J, Laredo T, Marangoni AG: **A facile strategy for plant protein fiber formation without extrusion or shear processing.** *Innov Food Sci Emerg Technol* 2023, **86**:103385.
 - The authors describe a cutting-edge technology that does not rely on extrusion or similar shear processing for the development of a fibrous structure. This opens to interesting perspectives for creating complex food structures by exploiting the molecular properties of proteins, hydrocolloids, and starch.
 12. Snel SJ, Bellwald Y, van der Goot AJ, Beyrer M: **Novel rotating die coupled to a twin-screw extruder as a new route to produce meat analogues with soy, pea and gluten.** *Innov Food Sci Emerg Technol* 2022, **81**:103152.
 - In this research, a rotating cooling die was studied to improve the textural properties of meat analogs. The study is particularly interesting because it points out the advantages of combining different technologies to improve the texturization process. Moreover, studying different rotational speed in the cooling die, it was possible to tune the micro- and macro-texture and anisotropy.
 13. Köllmann N, Vringer R, Mishra P, Zhang L, van der Goot AJ: **Near-infrared spectroscopy to quantify overall thermal process intensity during high-moisture extrusion of soy protein concentrate.** *Food Res Int* 2024, **186**:114320.
 14. Zink JI, Zeneli L, Windhab EJ: **Micro-foaming of plant protein based meat analogues for tailored textural properties.** *Curr Res Food Sci* 2023, **7**:100580.
 - The HME was implemented with a nitrogen injection system able to introduce a microfoaming process during soy protein texturization. The lightness and the textural properties can be tailored by adapting the concentrations of injected nitrogen, pointing out interesting insights for the product development.
 15. Ghanghas N, Nadimi M, Paliwal J, Koksel F: **Gas-assisted high-moisture extrusion of soy-based meat analogues: impacts of nitrogen pressure and cooling die temperature on density, texture and microstructure.** *Innov Food Sci Emerg Technol* 2024, **92**:103557.
 16. Zheng Y, Xu J, Sun C, Zhao Y, Cao Y, Lu W, Zhang Y, Fang Y: **Multihole nozzle-mediated high-moisture extrusion of soy proteins into fiber-rich structures.** *Food Hydrocoll* 2024, **151**:109819.
 17. Kendler C, Duchardt A, Karbstein HP, Emin MA: **Effect of oil content and oil addition point on the extrusion processing of wheat gluten-based meat analogues.** *Foods* 2021, **10**:697.
 18. Wang H, Zhang L, Czaja TP, Bakalis S, Zhang W, Lametsch R: **Structural characteristics of high-moisture extrudates with oil-in-water emulsions.** *Food Res Int* 2022, **158**:111554.
 19. Nisov A, Nikinmaa M, Nordlund E, Sozer N: **Effect of pH and temperature on fibrous structure formation of plant proteins during high-moisture extrusion processing.** *Food Res Int* 2022, **156**:111089.
 - The effect of pH modulation on the fibrous structure was investigated during HME. The authors observed an enhanced texture formation when the pH was shifted from 5 to 7, regardless of the isoelectric point of the proteins. This finding was explained by the authors, who correlated it with the higher reactivity of disulfide bonds at pH 7, resulting in improved texture.
 20. Nisov A, Valtonen A, Aisala H, Spaccasassi A, Walser C, Dawid C, Sozer N: **Effect of peptide formation during rapeseed fermentation on meat analogue structure and sensory properties at different pH conditions.** *Food Res Int* 2024, **180**:114070.
 21. Saerens W, Smetana S, Van Campenhout L, Lammers V, Heinz V: **Life cycle assessment of burger patties produced with extruded meat substitutes.** *J Clean Prod* 2021, **306**:127177.
 - This research offers interesting insights regarding the environmental impact of plant-based patties produced with texturized proteins derived from different sources and texturization technologies. Compared with a meat-based product, the environmental impact of plant-based patties is at least 10-fold lower. Nevertheless, it was reported that LME had a higher environmental impact compared with high-moisture processes, highlighting the need of optimizing the texturization processes.
 22. Köllmann N, Schreuders FK, Zhang L, van der Goot AJ: **On the importance of cooling in structuring processes for meat analogues.** *J Food Eng* 2023, **350**:111490.
 23. Köllmann N, Sivakul K, Zhang L, van der Goot AJ: **Effect of mixing and hydrating time on the structural properties of high-temperature shear cell products from multiple plant-based ingredients.** *J Food Eng* 2024, **369**:111911.
 24. Ramachandraiah K: **Potential development of sustainable 3D-printed meat analogues: a review.** *Sustainability* 2021, **13**:938.
 25. Wen Y, Chao C, Che QT, Kim HW, Park HJ: **Development of plant-based meat analogs using 3D printing: status and opportunities.** *Trends Food Sci Technol* 2023, **132**:76–92.
 26. Chao C, Park HJ, Kim HW: **Effect of L-cysteine on functional properties and fibrous structure formation of 3D-printed meat analogs from plant-based proteins.** *Food Chem* 2024, **439**:137972.
 27. Kweldam AC: **Method for the Preparation of a Meat Substitute Product, Meat Substitute Product Obtained With the Method and Ready to Consume Meat Substitute Product;** U.S. Patent No US20110244090A1, 2011.
 28. Ryu J, McClements DJ: **Control of plant-based biopolymer composite gel texture: combining potato proteins with different high acyl-low acyl gellan gum ratios.** *Food Hydrocoll* 2024, **149**:109636.
 29. Peng H, Zhang J, Wang S, Qi M, Yue M, Zhang S, Song J, Wang C, Zhang D, Wang X, Ma C: **High moisture extrusion of pea protein: effect of L-cysteine on product properties and the process forming a fibrous structure.** *Food Hydrocoll* 2022, **129**:107633.
 30. Dinani ST, Allaire N, Boom R, van der Goot AJ: **Influence of processing temperature on quality attributes of meat analogues fortified with L-cysteine.** *Food Hydrocoll* 2023, **137**:108422.
 31. Dinani ST, Zhang Y, Vardhanabhuti B, van der Goot AJ: **Enhancing textural properties in plant-based meat alternatives: the impact of hydrocolloids and salts on soy protein-based products.** *Curr Res Food Sci* 2023, **7**:100571.
 - This research investigates the effects of high acyl gellan gum, low acyl gellan gum, high methoxyl pectin, low methoxyl pectin, and xanthan gum and two types of salts (CaCl₂ and NaCl) at three concentrations on the textural properties of meat analogs produced with shear cell. The results suggested that texture formation can be modulated by salt concentration and by the type of hydrocolloids, offering important insights for the development of products with improved textural properties.
 32. Zhang J, Chen Q, Liu L, Zhang Y, He N, Wang Q: **High-moisture extrusion process of transglutaminase-modified peanut protein: effect of transglutaminase on the mechanics of the process forming a fibrous structure.** *Food Hydrocoll* 2021, **112**:106346.
 33. Schlangen M, Ribberink MA, Dinani ST, Sagis LM, van der Goot AJ: **Mechanical and rheological effects of transglutaminase treatment on dense plant protein blends.** *Food Hydrocoll* 2023, **136**:108261.
 34. Lie-Piang A, Yang J, Schutyser MAI, Nikiforidis CV, Boom RM: **Mild fractionation for more sustainable food ingredients.** *Annu Rev Food Sci Technol* 2023, **14**:473–493.
 35. De Angelis D, Latrofa V, Caponio F, Pasqualone A, Summo C: **Techno-functional properties of dry-fractionated plant-based proteins and application in food product development: a review.** *J Sci Food Agric* 2024, **104**:1884–1896.
 36. do Carmo CS, Rieder A, Varela P, Zobel H, Dessev T, Nersten S, Gaber SM, Sahlström S, Knutsen SH: **Texturized vegetable protein from a faba bean protein concentrate and an oat fraction: impact on physicochemical, nutritional, textural and sensory properties.** *Future Foods* 2023, **7**:100228.
 37. Nasrollahzadeh F, Roman L, Swaraj VS, Ragavan KV, Vidal NP, Dutcher JR, Martinez MM: **Hemp (Cannabis sativa L.) protein concentrates from wet and dry industrial fractionation: molecular properties, nutritional composition, and anisotropic structuring.** *Food Hydrocoll* 2022, **131**:107755.

38. De Angelis D, Opaluwa C, Pasqualone A, Karbstein HP, Summo C: **Rheological properties of dry-fractionated mung bean protein and structural, textural, and rheological evaluation of meat analogues produced by high-moisture extrusion cooking.** *Cur Res Food Sci* 2023, **7**:100552.
 39. Kaleda A, Talvistu K, Tamm M, Viirma M, Rosend J, Tanilas K, Kriisa M, Part N, Tammik ML: **Impact of fermentation and phytase treatment of pea-oat protein blend on physicochemical, sensory, and nutritional properties of extruded meat analogs.** *Foods* 2020, **9**:1059.
 40. Pöri P, Lille M, Edelmann M, Aisala H, Santangelo D, Coda R, Sozer N: **Technological and sensory properties of plant-based meat analogues containing fermented sunflower protein concentrate.** *Future Foods* 2023, **8**:100244.
- This research was aimed at the valorization of a sunflower press cake derived from the oil extraction. Despite being rich in protein, this by-product is characterized by the presence of a high content of anti-nutritional factors. Consequently, the authors studied the lactic acid fermentation to reduce the content and utilized the fermented raw material in combination with pea protein to produce meat analogs through HME. The authors emphasized the importance of neutralizing the raw material after fermentation to improve sensory properties and suggested the need for optimizing the fermentation protocol to further enhance the product's flavor.
41. Rekola SM, Kårlund A, Mikkonen S, Kolehmainen M, Pomponio L, Sozer N: **Structure, texture and protein digestibility of high moisture extruded meat alternatives enriched with cereal brans.** *Appl Food Res* 2023, **3**:100262.
 42. Lyu X, Ying D, Zhang P, Fang Z: **Effect of whole tomato powder or tomato peel powder incorporation on the color, nutritional, and textural properties of extruded high moisture meat analogues.** *Food Bioproc Technol* 2024, **17**:231-244.
 43. Zhang C, Wu X, Chen J, Zhou J: **Novel fungal alternative proteins from *Penicillium limosum* for enhancing structural and functional properties of plant-based meat analogues.** *Food Chem* 2024, **444**:138627.
 44. Okeudo-Cogan MC, Yang S, Murray BS, Ettelaie R, Connell SD, Radford S, Micklethwaite S, Benitez-Alfonso Y, Yeshvekar R, Sarkar A: **Multivalent cations modulating microstructure and interactions of potato protein and fungal hyphae in a functional meat analogue.** *Food Hydrocoll* 2024, **149**:109569.
 45. Jia W, Pouvreau L, van der Goot AJ, Althuis TY, Virant D, Kruis AJ, Kosec G, Claassens NJ, Keppler JK: **Renewable methanol utilizing bacteria as future meat analogue: an explorative study on the physicochemical and texturing properties of *Methylobacillus flagellatus* biomass and fractions.** *Food Hydrocoll* 2024, **151**:109832.