

Fermentation : To enhance organoleptic property of plant-based meat analogues

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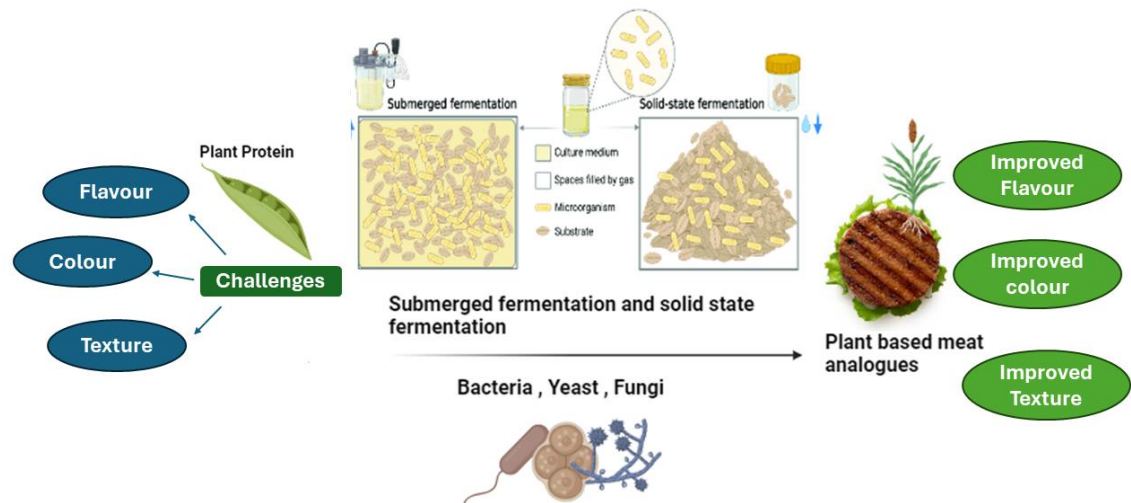
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Abstract

Plant-based meat analogues (PBMA) are attracting scientific and commercial attention because of rising consumer demand for ethical, eco-friendly, and healthy meat alternatives. Nevertheless, their consumption remains constrained due to undesirable flavours, primarily associated with plant proteins, attributed to aldehydes, ketones, furans, and alcohols. Despite substantial research advancements in the understanding of the formation, and possible modification, of flavour, texture, and colour of plant proteins, the PBMA food sector still struggles with delivering the desired organoleptic properties. Subsequently, PBMA companies are pushing boundaries to replicate the meat-eating experience, by enhancing flavour, texture, colour, and nutritional value, while minimising the usage of chemicals. To overcome , these challenges, fermentation has recently emerged as a chemical-free sustainable method. Thus, the main objective of this review is to discuss the challenges and potential applications of fermentation in enhancing the flavour, texture, and colour of plant proteins for the development of PBMA.

Key Words: Plant-based meat analogues; Flavour; Colour; Texture; Fermentation

1. Introduction

By definition Plant based meat analogues (PBMA) (also called meat substitutes, mock meat, or faux meat) that mimic the appearance, flavour and the fibrous texture of animal meat (Boukid, 2020). In recent years, there has been a significant surge in the market for PBMA products, driven by a notable increase in consumer demand (Andreani et al., 2023). For example, the global PBMA market is expected to generate around USD 30.9 billion by 2026, with a compound annual growth rate of around 8.6% from 2019 to 2025 (Andreani et al., 2023). With this rapid expansion, it is anticipated that there will be heightened competition in the market in terms of the food quality as well as nutritional attributes of PBMA.

The major nutritional components of PBMA are plant proteins (20–50%), vegetal lipids (0–5%), polysaccharides (2–30%) and other ingredients such as flavour, colour, and binding agents (Kyriakopoulou et al., 2021; Yang et al., 2023). The major constraint of using plant proteins in developing PBMA is the off-flavour associated (Pérez et al., 2023; Rajpurohit & Li, 2023). In

1979, (J.J. RACKIS, 1979) has published a review addressing flavour-related challenges inherent in vegetable protein substrates, which shows the longstanding issue within the domain. Currently, the addition of artificial flavours has been identified as the commonly used method to overcome the flavour issue in plant protein in developing PBMA. However this solution has been reported to associate with chronic health problems (Kale et al., 2022) (Boukid et al., 2023). These factors are greatly impacting on consumers' concerns and potential demand on PBMA purchasing. Hence there is always an increase interest in sustainable methods to enhance the organoleptic properties of plant proteins.

Modern microbiologists have shown that fermentation can effectively eliminate or mask the off-flavours present in plant proteins which eventually improves the quality of PBMA (El Youssef et al., 2020) (Emkani et al., 2022; Leonard et al., 2022). Currently, the most used microorganisms in flavour enhancement of plant proteins are bacteria which are mostly represented by lactic acid and fungi in the form of yeast and mold such as *Mucor*, *Aspergillus* species (Elhalis, 2023) . Besides ongoing technical development of the flavour and taste of PBMA, it is equally important to consider other organoleptic attributes, which are also very important for consumer choice, such as colour and texture. Nevertheless, currently there is limited information on the utilisation of fermentation for the improvement of texture and colour in PBMA. This review aims to fill the knowledge gap by gathering and summarizing available scientific information on the challenges associated with the main sensory attributes flavour, texture, and colour of plant-based meat analogues (PBMA). It also focuses on the potential of fermentation techniques to enhance these sensory attributes in plant proteins, contributing to the development of organoleptically improved PBMA.

1. Importance of organoleptic Properties in PBMA

The organoleptic properties of food are a complex series of sensory characteristics that can be perceived through the five senses. These characteristics include the colour, smell, taste, and texture. Sensorial property of a food plays an important role in consumer acceptance. Unpleasant or unexpected taste in food can be a barrier to acceptance. A survey conducted by the International Food Information Council (IFIC), USA in 2019 of US adults showed that 86% of consumers considered taste to be the important factor of purchase intention of a food item. Data collected from a consumer survey in the U.K. and The Netherlands

showed that purchase intention of meat substitute ultimately depended on the product's sensory attributes more than ethical aspects (Hoek et al., 2011). Thus, these surveys reinforce the importance of understanding and managing the organoleptic properties of food is essential for food producers and manufacturers to ensure consumer satisfaction and market success.

Meat and meat products are highly appreciated by consumers due to their sensory properties and nutritional composition, which include high-quality proteins, minerals (e.g., iron, zinc, and selenium), and vitamins (e.g., A, B6, and B12) (Y. Wang et al., 2022). When PBMA are considered, the past studies have shown that sensory attributes and consumer acceptance is strongly associated with the choice of plant protein source as it was targeted to vegetarians and vegans (Fiorentini et al., 2020). Upon broadening the PBMA market by a wider audience of meat eaters, there was an extra pressure on manufacturers to match texture, appearance, aroma, and taste to resemble to those of equivalent authentic meat products, before, during, and after cooking (Andreani et al., 2023). Recently, few sensory related studies were designed using PBMA burgers to compare with real meat burgers on consumer demand. For example, (Grasso et al., 2022) showed that consumers had higher sensory expectations for a beef burger than for a plant-based or hybrid patty; however, in terms of acceptability and purchase intentions, the hybrid one (60% beef and 40% vegetables) was the most preferred after the tasting. Similarly, a study by (Caputo et al., 2022) focused on US consumers preference on 100% beef burger, a plant-based burger using pea protein, a plant-based burger using animal-like protein, and a blended burger with 70% beef and 30% mushroom in terms of sensory. The results showed that the consumers liked the beef burgers upon all the alternatives including the plant-based burger made with pea protein in terms of sensory.

Further, a survey on Australian consumers have shown that still consumers prefer meat over meat alternatives for several reasons, with fewer vegetarian products to choose from, not enough nutrients, such as iron and amino acids, and most importantly, PBMA that do not mimic the flavour of real meat (Miller et al., 2024). Overall, the studies consistently indicate that consumers generally prefer traditional meat products or hybrid meat over their plant-based alternatives emphasizing the importance of organoleptic properties in customer acceptance. Therefore, it is crucial to understand the underlying challenges associated with developing the main sensory aspects to develop new sustainable technologies.

2.1 Flavour

Flavour is one of the most relevant and critical attributes determining the consumer's food choice (Pérez et al., 2023). Flavour is a combination of aroma, taste, and mouthfeel, and plays a vital role in the customer acceptance of any food item, including PBMA. Plant proteins are the main source of raw material for PBMA including soy protein, wheat protein, pea protein as well as peanut protein (Bakhsh, Lee, et al., 2021b; Wang et al., 2023; Yu et al., 2023). Consequently, flavour of PBMA depends on the flavour of the plant protein used. Plant proteins do not inherently possess flavour. However, they can function as a flavour carrier by combining or absorbing flavour compounds, thereby altering flavour perception during consumption. Retaining desirable flavours can enhance the overall flavour of the food produced. Conversely, protein molecules can also bind to off-flavour molecules, which can adversely impact the sensory experience of the developed food products (Yang et al., 2023). The commonly identified off-flavour compounds in plant proteins are summarized in (Table 1). Protein-flavour interactions are mainly caused by reversible hydrophobic interactions, hydrophilic interactions, Van der Waal's forces, and ionic bonds, but can also be caused by irreversible covalent binding (Pérez et al., 2023) (Saffarionpour, 2023)(Longbei Xiang et al., 2023).

2.1.1 Challenge in Flavour development in PBMA.

When meat is considered, there are about 1000 flavour components which contribute to its unique taste and aroma. Even though raw meat does not possess any inherent flavour, the heating process triggers a series of complex decomposition, oxidation, reduction, and chemical reactions, such as the Maillard reaction, resulting in the formation of various volatile compounds (alcohol, alkenes, aldehydes, ketones, ester, acids, and ether)(Van et al., 2012) . The biggest challenge identified in developing PBMA using plant protein is the off flavour associated with plant proteins. The term “off-flavour”, refers to an unpleasant flavour, which includes unpleasant aroma, taste, and other undesirable perceptions (Mittermeier-Klessinger et al., 2021) (Longbei Xiang et al., 2023) (Leonard et al., 2022). Off- flavours can be categorized to volatile and non-volatile compounds. Volatile Aldehydes, alcohols, ketones, acids,

pyrazines, furans, or sulphide compounds are attributed to the green grassy and beany off-odour and non-volatile compounds such as saponins, phenolic compounds, peptides/amino acids, or alkaloids are attributed to the long-lasting bitter and/or astringent off-taste (Mittermeier-Klessinger et al., 2021; Saffarionpour, 2023). Recent reviews by (Y. Wang et al., 2022; Yang et al., 2023) have broadly discussed the origin of different off-flavours in plant materials from various mechanisms. In summary, Lipids are the main cause of off-flavour formation in plant protein ingredients. The oxidation of unsaturated fatty acids, together with the hydrolysis of lipids, is an important reaction in the development of volatile and non-volatile off-flavour compounds, which may or may not require enzymes (Y. Wang et al., 2022). In addition, pyrolysis of amino acids and peptides also form off- flavour compounds in plant proteins (Rajpurohit & Li, 2023) (Saffarionpour, 2023) . To overcome the above off-flavour- protein challenge, there are few technologies been employed.

The technologies on eliminating and masking off-flavour present in plant protein can be categorized into three main categories: physical, chemical, and biological methods . However, the methods have its own advantages and disadvantage arising a constant need for a sustainable method. Mostly used physical methods include heat treatment, vacuum treatment, and adsorption, etc., which essentially blunt the lipoxygenase and reduce the enzymatic reaction (Nedele et al., 2022). The advantages of physical methods include simple operation and low cost, however as discussed in some cases they are declared likely to denature the protein and negatively affect the nutrition and flavour. Chemical methods usually include organic solvent extraction, acid, and alkaline treatment, antioxidant and reducing agent treatment, etc., but potential issues such as chemical residues are parallel in line. After using these methods to remove beany flavour, food flavour will become lighter, and additional flavouring agents might be required to re-enhance the flavour of the processed food (Yu et al., 2023) (Saffarionpour, 2023). Given the advantages and disadvantages associated with physical and chemical methods, there is an increased need for more sustainable approaches to achieve the desired flavour profile in PBMA. One promising biological method that has emerged is microbial fermentation, which will be discussed in detail in the subsequent chapter. This method leverages the natural metabolic processes of microorganisms to improve the organoleptic properties of plant proteins.

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Table 1: Main off-flavour odorants in plant-based proteins and their odour thresholds (OTs) in water (adapted and modified from (Fischer et al., 2022; Wang et al., 2021) (Y. Wang et al., 2022)

Odorants	OTs/ppb	Aroma attributes	Molecule of origin
Aldehyde			
(E)-2-hexenal	0.082	leafy	Linolenic acid
(E)-2-heptenal	0.013	soap, fat	Linolenic acid
(E)-2-octenal	0.003	fatty, green, cucumber	Linoleic acid
(Z)-3-hexenal	0.00012	green	Linolenic acid
pentanal	0.008	irritant	Linoleic acid
hexanal	0.0045	cut-grass, green	Linolenic acid, Linoleic acid
heptanal	0.003	dry fish	
nonanal	0.001	green, fatty	Oleic acid
octanal	0.0007	fatty, pungent	Linoleic acid ,Oleic acid
(E)-2-nonenal	0.0004	beany, green	

benzaldehyde	0.003	almond	Phenylalanine ,
(E,E)-2,4-nonadienal	0.0001	fatty	Amino acid
(E,E)-2,4-heptadienal	0.00256	fatty, fishy	
(E,E)-2,4-decadienal	0.000027	spices	
pyrazine			
2-isopropyl-3-methoxypyrazine	0.0008	pea-like, earthy	Serine , Amino Acid
2-butyl-3-methoxypyrazine	0.001	musty pea like, green vegetable	
Acid			
Acid acetic	180	sour	
Ketone			
2-heptanone	3	fragrance	
1 -penten-3-one	0.0009	spicy, onion	
1-octen-3-one	0.000003	green, beany	
2-octanone	0.05	soapy, floral	

Furan			
2-penthyfuran	0.0058	beany off-flavours	
2-ethylfuran	0.0023	earthy, malty, sweet	
Alcohol			
1-octen-3-ol	0.007	mushroom	Linoleic acid
1-pentanol	0.1502	green, wax	Linoleic acid
1-penten-3-one	0.3581	beany, green	
hexanol	0.5	green	Linolenic acid
3-methyl-1 -butanol	0.004	balsamic	Leucine , Amino Acid
Sulfide			Methionine
Dimethyl sulfide	0.3	Sulfury onion, cabbage, radish, tomato green	,Amino Acids

Dimethyl disulfide	7	Sulfurous Vegetable cabbage, onion
Dimethyl trisulfide	0.01	Sulfurous cooked onion, savory, meaty

2.2 Texture

The textural profile (firmness, juiciness, springiness, and cohesiveness) is the main parameter for the quality and acceptability of a product and consumers are even willing to pay more to receive a product with better texture (Kumari et al., 2023). The concept of texturization was first developed in the 1970s to develop a texturizing technique by using plant proteins, mainly soy (Bakhsh, Lee, Lee, Sabikun, et al., 2021). Since then, a range of technologies are used to mimic a fibrous meat texture in meat analogues. Generally, mixing, heating, and low and high moisture extrusion is known as the most established texturization methods to create meat like structures of plant protein meat analogues. In addition, there are novel structuring technologies such as shear cell, spinning and 3D printing (Schmid et al., 2022) (Zahari et al., 2022). Among all the methods high and low moisture extrusion is the most popular method of texturization (Liu et al., 2023). In the extrusion process initially plant protein is mixed with constructional ingredients (binders, flavouring and binding agents) and water at low temperature. That step confirms the hydration of the protein. In the latter stages of the extrusion due to continuous heating and shear force, proteins get denatured and forms a fiber like structure (Baune et al., 2022). Shear structuring (shear-cell) is a new technique compared to excursion. The design of the instrument is like rheometer where it is either cone to cone or cylinder to cylinder. The bottom cone of the instrument rotates at a defined rotating speed while the top cone remains stationary. The plant protein is exposed to shear force and high temperature for 15-20 min followed by an hour resting period to achieve the fibril texture. 3D printing a less complex method compared to other texturizing methods. A paste of plant protein with additional ingredient is used to form the fibre like structure through a layer-by-layer deposition process (Ozturk & Hamaker, 2023). However, all these texturization technologies has its own pros and cons which is necessary to be considered before choosing a method (Table 3). Despite of many technological developments texture improvement remains a challenge to date.

39 Table 3: Advantages and disadvantages of different texturizing technologies in the production of plant- based meat analogues (References
 40 (Boukid, 2020) and (Yu et al., 2023)) .

Technology	Advantage	Disadvantage
Extrusion	1. High productivity 2. Low cost 3. Versatility 4. Energy efficiency 5. Anti-nutritional factors denaturation 6. Increase protein digestibility	1. Changes in colour due to Maillard reaction, caramelization, hydrolysis, and degradation of pigments 2. Requires high temperature and pressure 3. Needs the highest mechanical energy 4. Difficult to modify the formulations and texture of products.
High-temperature induced shearing	1. Cost-effective 2. Produce defined fibrous structure	1. Difficult for scaling
Wet spinning	1. Produce defined fibrous protein products	1. Requires pure proteins 2. Low pH 3. High salt concentrations and chemical additives 4. Large amounts of wastes
Electrospinning	1. Cost-effective	1. Difficult to control

	<ol style="list-style-type: none"> 2. Scalable 3. Production of very thin fibrils 	<ol style="list-style-type: none"> 2. Difficulty to electro spin plant proteins
Freeze structuring	<ol style="list-style-type: none"> 1. Modulation of textural properties of plant proteins 	<ol style="list-style-type: none"> 1. Need further freezing conditions to optimize and monitor
Mixing plant proteins and hydrocolloids	<ol style="list-style-type: none"> 1. Formation of fibrous structure that can be modulated. 	<ol style="list-style-type: none"> 1. Require hydrocolloids and divalent metal cations for the precipitation.
Bioprinting (3D printing technology)	<ol style="list-style-type: none"> 1. Enable the design of products with texture similar to muscle fibers 2. Tailor the nutritional content of the product 3. Small production space and ease of operation 	<ol style="list-style-type: none"> 1. Require maturation under specific conditions. 2. High production cost 3. Low extrusion force of 3D printer. 4. Limitations in printable plant materials 5. High requirements on the flow characteristics of inks.

2.2.1 Challenge in texture development in PBMA.

In red meat, textural and taste parameters are important to the consumers and represent high economic value as some cuts bring exorbitant prices (Bakhsh, Lee, et al., 2021a). In contrast, meat analogues lack these features and generally regarded as substandard or cheaper meats. In terms of mimicking animal meat, some of the reconstituted ground meat products have been commercially produced, but the whole meat (e.g., steak) has a complex hierarchy of muscle tissue, adipose tissue, and connective tissue surrounding muscle fibers. The complexity of muscle fibers makes it extremely difficult to understand their physical, chemical, and functional properties (Kumari et al., 2023). Transforming plant globular proteins into meat-like fibers that meet textural requirements is one of the biggest challenges in developing meat analogues (Kumari et al., 2023). Further, It has been observed that texturized proteins produced using current texturizing technologies tend to have a spongier structure rather than a fibrous, meat-like texture (Elhalis et al., 2023). Achieving a good mouthfeel of the product after cooking is important. Supporting the fact, a recent study by (Zhou et al., 2022) compared the cooking behaviours of meat and PBMA in terms of appearance, texture, and fluid holding properties. The study revealed that plant-based burgers to be softer than meat burgers after cooking in addition to showing differences in colour and other physiochemical properties concluding the need for more technological enhancement in PBMA in mimicking real meat.

One of the other biggest challenges in plant-based protein formulations is the crumbling problem of products after cooking, which is directly related to poor textural attributes. Without the addition of binders, plant-based meat analogue (PBMA) products, such as burger patties formed with texturized particles, tend to fall apart during cooking. To address this issue, ingredients such as methylcellulose with its derivatives (e.g., hydroxypropyl methylcellulose, carboxymethylcellulose), laccase, k-carrageenan, and konjac glucomannan are added to provide viscoelasticity, cohesiveness, and binding ability (Yu et al., 2023). However, methylcellulose is criticized for not being “clean label” due to its synthesis chemically from cellulose in the presence of concentrated sodium hydroxide solution (Ozturk & Hamaker, 2023). Hence currently there is a search for natural and chemical free method to address the above challenges in the plant-based meat analogues in terms of texture.

2.3. Colour

Besides flavour and texture, the colour of PBMA s also plays a pivotal role in influencing consumer's purchasing decisions, directly impacting their overall acceptance (Ryu et al., 2023). PBMA s are coloured either by applying colouring solutions to the extruded meat analogues or mixing colouring ingredients with plant protein material before extrusion process or injecting into the barrel of extruder (Ishaq, 2022). Most of the colorants used in PBMA s formation are chemically synthesized or naturally derived components. Heat-stable colouring ingredients like caramel colours, malt, annatto, turmeric, cumin, erythrosine, and carotenoids (such as carotene, canthaxanthin, and lycopene) have been observed to offer the desired hues like in real meat products (Kyriakopoulou et al., 2021). In addition, carotenoid-rich extracts like paprika oleoresins and annatto seed extracts as well as lycopene-rich extracts from tomatoes have been used in PBMA s such as fermented sausages (Kyriakopoulou et al., 2021).

2.3.1. Challenges in colour development in PBMA s

Unlike animal meat, the colour of PBMA s is yellow due to the absence of myoglobin. Myoglobin, a globin protein consisting of a heme group, is the main colour pigment of animal meat, resulting in fresh meat's light, bright-red colour (Suman & Joseph, 2013). During cooking, myoglobin is denatured by heat, resulting in a dull brown (Suman & Joseph, 2013). In fact, the yellow colour of PBMA s makes it difficult to be perceived as real meat and provide a meat-like sensory experience to consumers (He, Evans, Liu, & Shao, 2020). Therefore, colour is an important hurdle to overcome in PBMA s development. Despite the availability of different colouring methodologies and the variety of colourants, the final PBMA s products do not always meet the expected quality. In numerous applications, mismatches between pH range of the colourant and the pH of the meat analogue are identified as the main cause of colour problem (Kyriakopoulou et al., 2021). Hence, acidulants such as citric acid, acetic acid, lactic acid, and their combination are added to the process of manufacturing PBMA s (Elhalis et al., 2023) (Kyriakopoulou et al., 2021). However, the pH change also interferes with the proteins and affect the protein structure and the taste in the PBMA s. Due to proteins buffering capacity, large quantities of additives are required to induce pH changes. Further, to retain the colour of

the colouring agent, colour retention aids such as maltodextrin and hydrate alginate are used. Therefore, there is a need for further studies to develop new techniques for enhancing PBMA colour properties with fewer chemical additives (Kyriakopoulou et al., 2021).

Thus far, this review has examined the primary sensory attributes and the challenges associated with developing these attributes in plant-based meat analogues (PBMA) derived from plant proteins. A comparative analysis of the existing novel techniques for enhancing sensory qualities in plant proteins underscores the persistent need for a novel, sustainable method. Consequently, the remainder of this review will focus on the potential application of fermentation as an innovative, green, and sustainable approach to improve the sensory properties of plant proteins in the development of PBMA.

2. Overview of fermentation

Since the Neolithic era, fermentation has been a natural process passed down through generations to produce a diverse array of foods and beverages. This ancient technique has been employed primarily to extend shelf life and preserve food. Fermentation has been used in the production of numerous staple food products, including cheese, yogurt, kimchi, and tempeh. Additionally, fermented meats such as salami have gained worldwide popularity. Beyond its role in animal-based products, fermentation is also extensively applied to plant-based sources. This process has been used to enhance the nutritional value, aroma, taste, texture, and stability of foods such as coffee, bread, chocolate, wine, and olives. Overall, fermented foods play a crucial role in the human diet, contributing significantly to nutritional diversity and sensory satisfaction (Boukid et al., 2023).

Recently fermentation has been employed to plant based alternatives to improve the organoleptic property by reducing unpleasant off-flavour and creating a distinctive fermented flavour through microbial metabolism along with nutritional value (Saffarionpour, 2023). Mainly, the starter culture fermentation technique has been employed to achieve the desired flavour in plant-based meat substitute. A starter culture contains a wide range of active microorganisms, including bacteria and fungi, that are added to initiate desirable changes during the product manufacturing process. However, the use of fermented plant materials and starter culture technology in the production of PBMA has received little attention. Therefore,

subsequent chapters will focus on showing the potential of using fermentation in the improvement of flavour , texture, and colour in plant proteins to develop PBMA s.

3.1 Fermentation to enhance flavour in plant protein to develop PBMA s

The fermentation process can produce new aromatic compounds, eliminated, reduced, or mask undesirable flavours due to the metabolic effect of microorganisms (Emkani et al., 2022; Kale et al., 2022). There has been a volume of studies reporting the use of microorganisms to improve the flavour of pulse protein. However, research couple with mechanism are rare to be found. This review is also not scoped to discuss the mechanism in depth, however a recent review by (Fischer et al., 2022) focused on underlying mechanism behind the fermentation for mitigating twenty-six common off-flavour compounds found in plant proteins. The review summarizes that strains possessing active forms of Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes to have a measurable impact on volatile compounds, thereby enhancing the overall aromatic profile of the products.

Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) enzymes abundantly available in yeast and lactic acid bacteria (*Lactobacillus acidophilus* , *Limosilactobacillus fermentum*, *Lactiplantatibacillus plantarum*, *Streptococcus thermophilus*, *Sacharomyces cerevisiae* and *Gluconobacter suboxydans*). ADH possesses the ability to convert aldehydes to primary alcohols and ketones to secondary alcohols. On the other hand, ALDH can convert aldehydes to carboxylic acids. Hexanal (odor threshold: 4.1 ppb) being one of the key beany flavour compounds in pea protein can be catalyzed by ADH to produce hexanol with a relatively high odour threshold (odor threshold: 200 ppb) or by ALDH to produce hexanoic acid (odor threshold: 93 ppb), thereby improving the inclusive flavour profile.(Longbei Xiang et al., 2023)

The mitigation of off-flavour in plant protein using microorganisms can also be further elaborated in different strategies such as biotransformation , decomposition, and masking. Biotransformation as described by (Vong & Liu, 2017) involves the interaction between microbial enzymes with the aroma precursors in the plant protein to produce variety of desirable flavour compounds. In a specific study by (Nedele et al., 2022), the focus was on addressing the presence of aldehydes with a green note in soy by fermenting it with *Lycoperdon pyriforme*. Over a fermentation period of 28 hours, a noticeable reduction in off-aroma intensity was observed. The study suggested that the conversion of saturated aldehydes into corresponding alcohols, which are perceived to have less off flavour, contributed to the

enhancement of flavour. This instance exemplifies the concept of biotransformation in action, where microbial activity alters the flavour profile of the plant protein, making it more appealing to consumers. Following biotransformation, another effective strategy for mitigating off-flavours in plant protein involves decomposition of off-flavour precursors, such as phospholipids (PLs) and free fatty acids (Tao et al., 2022). (Zhu & Damodaran, 2018) reported phospholipase A2 (PLA2) and cyclodextrin (CD) mixed with soybean meal in a water bath had a promising removal rate of off flavour. This method has involved two principles such as hydrolysis of PLs by PLA2, followed by removal of the hydrolysed products by cyclodextrin (β CD) via forming an inclusion complex. This approach highlights the significance of decomposing off-flavour precursors as an effective means of improving the overall flavour profile of plant proteins. Some microbial strains can produce new compounds with aroma after fermentation while reducing the content of compounds with beany smell which is known as masking. (Pei et al., 2022) conducted a study where they fermented pea protein with *Lactobacillus rhamnosus* L08, resulting in the production of new compounds with desirable aroma characteristics while simultaneously reducing compounds associated with unpleasant beany smells. The fermentation process led to an increase in acids and esters, contributing to flavour enhancement, while simultaneously reducing the levels of undesirable compounds such as nonanal, decanal, octanal, 1-hexanol, and 2-ethyl-1-hexanol. This study stands an example for the masking strategy on flavour enhancement. Further (Table 2) summarizes the use of different type of microorganisms in different plant protein sources in reducing unendurable flavours.

While fermentation is recognized for its ability to enhance the flavour of plant proteins, it also comes with limitations. One of the challenges associated with fermentation is the potential for excessive accumulation of aroma compounds, particularly esters, which can result in an overly fruity aroma in plant-based meat analogues (PBMA). This mainly occurs due to the excessive enzymatic activities which break down peptide, amino acids, and fatty acids and convert the nitrogen compounds into ammonia that is recognized as an undesirable pungent and fishy odour (Elhalis et al., 2023). As an example, *Aspergillus* species, such as *A. sydowii*, was shown to produce excessive amounts of extracellular endo- and exo-peptidase enzymes correlated to an undesirable rancid odour during cheese ripening processes (Takenaka et al., 2021). Similarly, alkaline *Bacillus* fermentation is associated with the creation of excessive amounts of ammonia, and polypeptide of glutamic acid, giving the final product a notably stringy and

208 pungent smell, which may be undesirable in PBMAAs (Elhalis et al., 2023). Hence it is vital to
209 choose the optimal fermentation conditions during the flavour enhancement process.

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212 Table 2: Selective fermentation methods to decrease undesirable flavour in plant-based meat alternatives.

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	Matrices	Strain	Fermentation Condition	Method	Function	Sensory property	Reference
<i>Lactobacillus</i>	Soymilk	<i>Lactobacilli</i> and <i>Streptococci</i>	37°C 12 h	Decomposition	Reduce or even eliminate the concentrations of volatile components that have been associated with the beany flavour of soymilk, such as methanol, acetaldehyde, ethanol, and hexanal.	The heat treatment caused a severe cooked flavour. The resulting fermented product not suitable for sensory analysis.	(Blagden & Gilliland, 2006)
	Lupin protein extracts (LPE)	<i>L. plantarum</i> L1047 and <i>Pediococcus pentosaceus</i> P113	—	Decomposition and masking	Decrease the concentration of n-hexanal and prevent its re-formation;	More pleasant odour of the fermented protein extracts, compared to the	(Schindler et al., 2011; Schindler et al., 2012)

					Change the aroma profile which may mask off Flavors.	unfermented protein extracts is explained by its different aroma profile.	
	Pea (<i>Pisum sativum</i>) protein extract	<i>L. plantarum</i> L1047 or <i>P. pentosaceus</i> P113	37°C 48 h	Decomposition and masking	Decrease the n-hexanal content; Reduce or mask undesirable green notes.	Improve the aroma profile.	(Schindler et al., 2012)
	Pea protein isolates	<i>L. plantarum</i> , <i>L. casei</i> and mixed strains of probiotics	37°C 5, 10, 15, 20, 25, and 30 h Anaerobic conditions	Decomposition	Remove around 42% aldehyde and 64% ketone content; Produce a small amount of alcohol.	Decrease the overall unpleasant aroma and flavour intensity.	(Shi et al., 2021)
	Mung bean	LAB (<i>L. plantarum</i>)	37 ± 1°C 48 h	Biotransformation	Transform aldehydes into esters.	The esters give the fermented products a pleasant fruity odour's.	(Yi et al., 2021)
	<i>Lupinus angustifolius</i> L.	Five lactic acid bacteria	28°C 48 h	Decomposition and masking	Reduce aldehydes, especially hexanal, which	Increase sourness and “vinegar” odour; Reduce	(Laaksonen et al., 2021)

					possesses “green” odour; Create new pleasant aromatic compounds	the “beany” flavour as well as the unpleasant off flavour	
Pea flour (<i>Pisum sativum</i> L.)	<i>L. rhamnosus</i> L08	37°C 2 days	Decomposition and masking	Increase the variety of acids and esters. Reduce the unpleasant flavor compounds such as nonanal, decanal, octanal, 1-hexanol and 2-ethyl-1-hexanol; Produce phenylethyl aldehyde that could bring pleasant aromas	Reduce the unpleasant beany flavor; Produce floral and honey-like aromas.	(Pei et al., 2022)	
Pea protein isolate	<i>Lactobacillus plantarum</i> DSM-20174 <i>Lactobacillus</i>	30°C and 37 °C aerobe and	Decomposition and masking	Not analysed	After 24 h of fermentation, all the characteristic	(Garcia Arteaga et al., 2021)	

<i>perolens</i> DSM-12744	anaerobe	aroma attributes of pea protein isolate decreased; after 48 h of fermentation, the cheese aroma increased
<i>Lactobacillus fermentum</i> DSM-20391	conditions for 48h	
<i>Lactobacillus casei</i> DSM-20011		
<i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i> DSM-20200		
<i>Pediococcus pentosaceus</i> DSM-20336		

Yeast and Bacteria	Pea protein	<i>S. cerevisiae</i> <i>Lactobacillus plantarum</i>	<i>S. cerevisiae</i> : 35 °C, 150 rpm for 6 h <i>Lactobacillus plantarum</i> : 37 °C for 8 h under static conditions	Decomposition	<i>S. cerevisiae</i> and <i>L. plantarum</i> fermentation removed 79.65% and 78.94% of the major beany flavour respectively.	Reduce beany flavour	(L. Xiang et al., 2023)
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	Pea protein isolate	<i>VEGE 047 LYO</i> <i>Kluyveromyces lactis</i> Clib 196 <i>Kluyveromyces marxianus</i> 3810 <i>Torulaspora delbrueckii</i> TD 291	30°C Fermentations were stopped at pH 4.55	Decomposition	Aldehydes, ketones, furan off-flavour compounds were significantly removed	The green and Leguminous properties were significantly reduced after fermentation	(El Youssef et al., 2020)
Yeast	Okara Soybean residue (okara)	Yeast (<i>Lindnera Saturnus</i>) Four “dairy yeasts” (<i>Geotrichum candidum</i> , <i>Yarrowia lipolytica</i> , <i>Debaryomyces hansenii</i> and <i>Kluyveromyces lactis</i>) and six “wine yeasts” (<i>Saccharomyces cerevisiae</i> ,	36–48 h 30°C 48 h Solid-state fermentation	Biotransformation	Main off-flavour hexanal and 2-hexanal has significantly reduced. There was a notable increase in ethyl and acetyl esters along with 13 more ester compounds.		(Vong & Liu, 2017)

		<i>Lachancea thermotolerans</i> , <i>Metschnikowia pulcherrima</i> , <i>Pichia kluyveri</i> , <i>Torulaspora delbrueckii</i> , and <i>Williopsis saturnus</i>)				
Fungi	Okara	<i>Wolfiporia cocos</i> CGMCC 5.55, <i>Wolfiporia cocos</i> CGMCC 5.528, <i>Wolfiporia Cocos</i> CGMCC 5.78 and <i>Tremella fuciformis</i> CGMCC 5.466	25 ± 1 °C, 120 rpm for 7 days	Biotransformation	Off-flavour compounds decreased, and new aromatic compounds were generated. The content of hexanal decreased to 0.37%, and cis-6-nonenal was not detected.	(Z. Wang et al., 2022)

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3.2 Fermentation to improve texture to develop PBMA

Research investigating the use of fermenting plant protein or textured plant protein to improve the textural properties of plant-based meat analogues (PBMA) is currently very limited. The reason could be that most of the fermentation would result in a soft texture due to the microorganism's decomposition function on the proteins and other food components, which might not be desirable to form the meat fibrous texture from a plant protein source. We only noted one research on this aspect. (Maung et al., 2020) used soy protein extruded at 40% and 50% moisture content and fermented by *Bacillus subtilis*. Results showed that after fermentation, proteins extruded at 50% moisture content maintained higher chewiness, hardness, integrity index, and layered structure than that extruded at 40% moisture content. The study concluded that fermenting the TVP extruded at 50% moisture content has the potential to produce a better texture property TVP. The study suggested that it is possible to optimize the fermentation conditions to enhance the texture properties of plant proteins in making PBMA, although more research is needed.

3.3 Fermentation in enhancing the colour properties in plant proteins to develop PBMA.

The demand for pigments from natural sources to substitute synthetic ones for application in the food industry has been continuously increasing due to synthetic colours being associated with several health problems including hypersensitivity, carcinogenesis, and negative environmental impacts. Nature is rich in pigment-producing microorganisms. Several fungi, including *Y. lipolytica*, *Aspergillus*, *Monascus*, *Fusarium*, *Penicillium*, *Neospora*, and *Trichoderma* produce various pigments as secondary metabolites during their growth. Especially, *Monascus purpureus* and *Aspergillus nidulans* were reported to produce red pigmentation in several studies and might be suitable to be applied to plant-based fresh meat alternatives (Elhalis et al., 2023). In addition to benefiting the colouring of products, pigments of microorganisms could provide health benefits. (Berger & Krings, 2014). These benefits are attributed to the presence of bioactive compounds produced during the secondary metabolism of microorganisms (Sen, 2019). The pigments produced by microorganisms have also shown low production costs compared with plant pigments

(Egea, 2023). On the other hand, microbial pigments present higher yields, as well as simpler and cleaner extraction and purification processes compared with plant pigments. Pigments from microorganisms show no seasonal variation compared with pigments extracted from plant sources, and demonstrate the possibility of improvements in the process to increase production yield (Egea, 2023). Hence natural pigments derived from microbes can be highly regarded as a better option for synthetic pigments.

Despite the benefits associated with natural pigments derived from microorganisms, studies focused on producing colour in plant materials or plant proteins using fermentation is limited. A study by (Hadiseh Keivani, 2020), pigment production of *Monascus* was investigated under submerged fermentation using soybean meal as a nitrogen source to replace yeast extracts. The research revealed that the maximum production of *Monascus* pigment occurred when soybean meal replaced 79.72% of the nitrogen source. This observation led to the conclusion that a combination of soybean meal and yeast extracts as nitrogen sources is advantageous for pigment growth. Followed by that (Keivani & Jahadi, 2022) did the same study model using solid state fermentation. The study revealed that the maximum colour was produced when soybean was replaced 98.3%. Hence these studies above confirms that soybean meals can be an alternative substrate to grow *Monascus purpureus*. Similarly, (Ou et al., 2023) studied the colour change of soy protein using solid state fermentation with *Monascus purpureus* and *Rhodotorula mucilaginosa*. The results indicated that fermentation technique can produce a colour mimicking the colour and lustre of real meat when () used as the conditions. This study has introduced the potential of fermentation as a promising technique to improve the colour similar to meat in plant protein. However, more studies are needed for a better conclusion of suitability of microbial fermentation in PBMA's colour production.

6. Conclusions and future directions

It has always been a challenge to match the quality of plant-based food to that of animal food. The main challenges of PBMA's to mimic the exact colour, flavour and texture to meat has led to the use of excessive additives and chemicals. As a result, PBMA's packages include a long list of chemicals which could give a sense of processed and unhealthy among consumers. However, the recent demand for PBMA's have led to invention of new

techniques to improve the product quality with less chemical involvement. Among those techniques, fermentation is gaining attention as a sustainable solution to design plant-based alternatives with similar features to those of traditional foods. The most important advantage of fermentation is the familiarity and versatility of microorganisms from natural origin. Further, the incorporation of microorganisms in manufacturing plant-based meat alternatives has improved the flavour by mitigating or masking undesirable flavour in plant proteins. However, more research is required to select suitable starter cultures to improve colour and texture of plant proteins in making PBMA. In addition, qualitative and quantitative sensorial studies should be conducted for a better understanding of consumer acceptance/rejection of fermentation on plant proteins.

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