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Fermented meat sausages and the challenge of their plant-based alternatives: A comparative review on aroma-related aspects

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ABSTRACT

Traditional fermented meat sausages are produced around the world due to their convenience and sensory characteristics which are responsible for their high acceptability. They constitute a cultural heritage as shown by the high diversity of products around the world. Recent trends are addressing issues regarding innovation in their formulation by reduction of salt, fat and additives (curing salts). However, the current trend towards a reduction in the consumption of meat has produced an increase in the formulation of meat product analogues. This trend is the main focus of producers to offer new attractive products to consumers even though the aroma profile of traditional fermented meat sausages is not reached. In this manuscript, we review and discuss the chemistry of aroma formation in traditional fermented meat sausages in contrast to the potential of plant-based ingredients used in meat analogues.

1. Introduction

Fermented sausages are produced around the world due to their convenience and sensory characteristics (Leroy et al., 2018). They constitute a cultural heritage as seen by the high diversity of products in Asia, Europe and the Americas. Essentially, they are composed by a meat batter of minced lean meat and fat mixed with other ingredients (sugar, herbs and spices, starter cultures, etc.) and additives (curing salt, antioxidants, etc.), that are stuffed into casings, fermented and then, submitted to drying and maturation process. Multiple variations in terms of ingredients and processing are found around the world, thus generating a large variety of fermented meat sausages like the well-known salami, salchichón, saussiçon *sec*, and chorizo in Europe, and summer sausage and pepperoni in the USA (Ockerman & Basu, 2014). In the last years, the innovation on fermented sausages is aimed to improve their healthy profile while maintaining their sensory characteristics (Vitale et al., 2020). In this sense, many efforts have been focused on reducing the sodium and fat contents, as well as the presence of additives, such as curing agents (nitrite) (Perea-Sanz, López-Díez, Belloch, & Flores, 2020). However, changes in ingredients or processing affect greatly the flavour of these products, and consequently, consumer preference, which is highly influenced by cultural habits and experience (Iaccarino, Di Monaco, Mincione, Cavella, & Masi, 2006).

In the last years, the interest of the consumers towards meat-free foods has increased considerably. A consumer study carried out in the UK about consumers' interest in meat free foods showed an increase of 40% value and 26% volume growth of the meat-free product market from 2014 to 2019 (Mintel, 2019). This reflected the change in consumer behaviour regarding meat consumption, with an increase from 28 to 39% in the percentage of meat eaters who have reduced their meat consumption in the same period. Regarding the percentage of people following a flexitarian, vegan, vegetarian or pescatarian diet in Europe, this varies depending on the country: 29% in Germany, 25% in the UK, 20% in Spain and 18% in Italy (IPSOS, 2018).

The main concerns about the consumption of animal meat products are related to the effect on the environment, animal welfare and the consumption of healthier food products. These trends have been reflected into the dietary policies that advice to reduce the consumption of meat and its products. This fact has created a great controversy because of the potential loss of traditional meat products in favour of new plant-based alternatives, and the subsequent loss of local traditions and habits (Leroy & Hite, 2020). Nevertheless, the attention on an adequate nutrition through the use of proteins from animal or vegetal origins is the main target. Some consumer studies regarding preferences and demand for plant-based meat analogues over meat products have been done taking into account information regarding environmental impact

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or the processing technology used to produce the meat analogues (Van Loo, Caputo, & Lusk, 2020). These authors showed that, although most of the consumers from the USA preferred the meat products (78%), those choosing their meat analogues were driven by their awareness about animal welfare and environmental issues, rather than protein origin. Therefore, innovations are focused on the manufacture of meat analogues even though its sensory acceptability is a difficult task to achieve.

As shown above, several studies have been directed to determine the consumer's opinion and preferences of meat and meat alternatives (Van Loo et al., 2020), as well as the quality attributes with physicochemical methods (McClements, Weiss, Kinchla, Nolden, & Grossmann, 2021). However, small attention has been paid to the flavour of fermented meat alternatives, except for the removal of off-flavours in the plant material used during processing (Tangyu, Muller, Bolten, & Wittmann, 2019) and those related to flavour interactions with the plant proteins (Guo, He, Wu, Zeng, & Chen, 2020). Indeed, aroma has been a big challenge for the formulation of fermented meat analogues. Current “practices” are focused on the direct addition of flavourings or flavour precursors into the meat analogue, although natural alternatives to produce meaty flavours are searched. This is the case of the use of soy leghemoglobin isolated from genetically modified yeast to emulate not only the colour of meat, but also meat flavour, as claimed by the authors (Fraser, O'Reilly-Brown, Karr, Holz-Schietinger, & Cohn, 2017a). Albeit, the contribution of this hemoprotein to flavour is not clear because its use is combined with the use of other flavour precursors (like sugars and amino acids, among other) added into the beef-like product as indicated in the US Patent No. 9700067 B2 (Fraser, O'Reilly-Brown, Karr, Holz-Schietinger, & Cohn, 2017b). Therefore, until now there is not any plant-based products that have been directly related to the production of meaty flavours in meat products alternatives. Thus, the aim of this review was to provide an overview of the formation of aroma compounds in fermented meat sausages and to study the potential of plant-based ingredients in the formulation of analogues to these meat products.

2. Flavour precursors in fermented meat sausages

The presence of flavour precursors is essential for the formation of aroma in meat, and hence this must be taken into account when formulating meat analogues. The main ingredients used in the preparation of fermented dry and semidry sausages, i.e., meat and fat, have a great effect on their aroma, as well as their preservation (Leroy, Geyzen, Janssens, De Vuyst, & Scholliers, 2013; Toldrá & Flores, 2014). Main components of meat are protein and fat, apart from water. During meat product processing, fat can be added up to as much as 50% of the meat batter, whereas protein can represent more than 20% of lean meat. Carbohydrates are present in very low amounts in meat (approximately 0.5 g per 100 g in meat). Nevertheless, variations in meat composition are highly affected by animal species, breed, feeding and genetic factors as well as location in carcass (Toldrá & Flores, 2014). These variations in the ingredients can lead to differences in the aroma of fermented products, as for example in fermented sausages from pigs raised following different husbandry systems (Škrlep, Čandek-Potokar, Atorek-Lukač, Tomažin, & Flores, 2019). Other factors, like the presence of carbohydrates and starter cultures, are essential for flavour formation in fermented sausages. Meat proteins and lipids are hydrolysed during fermentation and drying and generate aroma precursors such as free fatty acids, and free amino acids. All of them are substrates of chemical and microbial reactions producing volatile aroma compounds (Flores, 2010).

Free fatty acids (FFAs) and amino acids are derived from the degradation of fat and protein, the main ingredients present in the meat product (Flores & Olivares, 2014). Therefore, its composition is essential for the flavour characteristics of the meat product. The effect of meat lipid composition on meat flavour is well known in cooked meat products where lipid degradation and oxidation together with the Maillard reaction act as the main sources of meaty aroma compounds (Elmore & Mottram, 2006). In fermented meat sausages, muscle and subcutaneous fat tissues are the source of free fatty acids by the lipolysis of the triglycerides and phospholipids. These fatty acids will participate in further oxidation reactions producing aroma compounds. During the manufacture of fermented sausages, triglycerides comprise the most abundant fat fraction, and they also release the biggest proportion of fatty acids during the fermentation and ripening processes (Marco, Navarro, & Flores, 2006; Molly, Demeyer, Civera, & Verplaetse, 1996). On the other hand, lipolysis is affected by the processing applied not only due to the lipolytic enzymes present in the microorganisms used, but also for the physicochemical conditions and ingredients used in formulation (Marco et al., 2006). Nevertheless, the potential of the phospholipid fraction to produce aroma compounds in fermented products should not be disregarded, even if present in a lower proportion, because of the presence of polyunsaturated fatty acids with high impact to produce meaty aromas (Wood et al., 2008).

The degradation of proteins by proteolysis produces a source of amino acids as well as small peptides that participate in the generation of aroma compounds (Corral, Leitner, Siegmund, & Flores, 2016; Flores & Olivares, 2014). Myofibrillar and sarcoplasmic proteins are hydrolysed by endogenous and microbial proteases (*endo*- and exoproteases) (Toldrá & Flores, 1998). The endogenous proteolytic activity is the main contributors to the free amino acid production as microbial enzymes are inhibited due the acid pH produced during fermentation (Montel, Masson, & Talon, 1998). Nevertheless, the presence of high content of branched amino acids as well as the sulphur amino acids methionine and cysteine are essential for the generation of meaty aroma compounds in fermented meat sausages (Flores, 2018). The conversion of these precursors into volatile compounds is carried out by the microorganisms added as bacterial starter cultures, which include enzymes capable of their degradation (Flores, 2018; Sunesen & Stahnke, 2003).

Other precursors of meaty aroma compounds are those derived from thiamine degradation. The contribution of thiamine to flavour formation in cooked meat products is well known and several intense meat aroma compounds were detected: 2-furylmethanethiol, 2-methyl-3-furanthiol, 2-methyl-3-(methyldithio) furan, bis(2-methyl-3-furyl) disulfide and 2-acetyl-2-thiazoline (Thomas, Mercier, Tournayre, Martin, & Berdagué, 2015). The concentration of thiamine in meat products depends highly on product composition, although fresh pork meat is characterised by a high thiamine content (Flores, 2018). Unfortunately, little is known about the effect of the fermentation process on its degradation.

3. Microbiota participation in flavour formation in fermented dry sausages

There is a wide variety of fermented sausages in the market with very different appearance, texture, colour, and flavour, derived from the different processing technologies applied (use of starters, temperature applied, surface moulds, smoking, etc.) and ingredients used. The differences between traditional and industrial sausages are based on the manufacture parameters. For instance, traditional sausages are manufactured at low temperatures, without the addition of starter cultures, and using natural casings. However, most of the industrial sausages produced worldwide involve the use of starter cultures (Toldrá & Flores, 2014). Nevertheless, the different processing parameters applied among European fermented sausages (e.g., use of salt, temperatures applied) have an effect on the fermentative microbiota (prevalence of lactic acid bacteria and coagulase negative staphylococci), even when bacterial starter cultures are used revealing an effect of the product geographical origin (Van Reckem et al., 2019).

Microbiota in fermented dry sausages participates in the fermentation of carbohydrates and in the proteolysis and lipolysis reactions providing aroma precursors (Janssens, Myter, De Vuyst, & Leroy, 2012; Ravyts, De Vuyst, & Leroy, 2012). The development of aroma occurs mainly during the ripening process of sausages. Different volatiles can contribute to a variety of aroma notes in fermented products, such as fruity, sweet, toasted, green, acid, savoury, spicy, sulphur, etc. The microbial metabolism reactions that participate in fermented sausage flavour are carbohydrate fermentation, amino acid degradation, lipid β-oxidation, and esterase activity (Flores & Olivares, 2014). The contribution of each reaction to the aroma depends on the bacterial diversity of the sausage that is highly affected by the processing conditions used during fermented sausage manufacture. In the last years, many attempts have been done to correlate bacterial diversity with the formation of volatile aroma compounds in fermented dry sausages in European traditional fermented sausages (Belleggia et al., 2020; Ferrocino et al., 2018; Iacumin et al., 2020) and Asian sausages (Hu et al., 2020; Yu et al., 2021). However, the complex volatile profile together with the high diversity of bacteria present in the sausages has produced different assumptions. In spontaneously fermented Italian sausages, the carbohydrate and amino acid metabolism and formation of volatile compounds from these reactions was related to the presence of *Lactobacillus sakei* (Ferrocino et al., 2018). Also, the most significant effect that allowed the distinction between spontaneously fermented versus starter inoculated sausages was the presence of lactic acid bacteria (LAB) and in particular L. *sakei*. Besides, the presence of esters produced from lactic acid bacteria (LAB) esterase activity was reported. *L. sakei* is characterised by its fast growth and a subsequent steep decrease in pH which has been related to a high concentration of volatile metabolites, especially organic acids that affected consumer preference negatively (Ferrocino et al., 2018). However, it was found that in other traditional Italian fermented dry sausages the volatilome depended on microbial metabolism, or in the case of Ciauscolo salami, the aroma was mostly driven by the spices used in the formulation (Belleggia et al., 2020). Nonetheless, the reason behind these results in Ciauscolo salami might be partly due to the volatile extraction technique used (i.e., headspace-SPME-GC–MS) that did not allow to isolate key aroma compounds. The impact of spices in the aroma of fermented sausages is enormous as demonstrated by the identification of many different terpene compounds derived from a variety of spices and other flavourings, such as black pepper and garlic used in Italian sausages (Belleggia et al., 2020), or aniseed, fennel, cardamom, ginger, chili and black pepper in Chinese fermented sausages (Hu et al., 2020; Yu et al., 2021).

Concerning Asian fermented dry sausages, there is a high production and variability in China. Recent studies have been done on fermented dry sausages from Northeast China produced by a 10–15 day air-drying process and submitted to spontaneous fermentation (Hu et al., 2020). The study reported the relationship between microbiota and volatile compounds in samples of sausages collected from different areas of Northern China with a similar process and formulation. The small calibre sausages were produced including wine, monosodium glutamate and a mix of spices as ingredients at different proportions. They included some of the following spices: aniseed, fennel, pepper, cardamom, angelica, *Amomum villosum*, ginger, *Pericarpium zanthoxyli*, and *Ilicium verum*. The volatile profile was analysed by solid-phase microextraction (SPME) and GC–MS and in order to correlate the microbiota to the volatile compounds, compounds derived from spices were not included. In any case, the authors found a relationship between *Lactobacillus* sp. and acid and alcohols compounds, while the contribution of *Staphylococcus xylosus* to the formation of esters was not confirmed due to its absence in some of the samples analysed. Nevertheless, the authors showed the complexity of the spontaneous fermentation on the formation of flavour in sausages.

Another recent study from China showed the correlation between microbiota and volatile compounds measured using gas chromatography and ion mobility spectrometry (GC-IMS) in Xiangxi sausages from different areas in the western Hunan province in China (Yu et al., 2021). Spices like chili and black pepper were used in their formulation and then, sausages were fermented, smoked and dried for 50 days. However, the interpretation of the reported results is difficult due to the absence of description about manufacture differences between sausages, as well as the few indications about physicochemical characteristics (pH, moisture, etc.). Nevertheless, the volatile profile of the sausages was mostly characterised by the presence of esters and other volatile compounds derived from spices, with clear differences between samples (Yu et al., 2021). In addition, the correlation between microbiota and volatile compounds indicated the positive contribution of several groups like *Debaryomyces* yeasts to the development of major volatile compounds. However, it is necessary to elucidate the key aroma compounds characteristic to this type of fermented dry sausages.

4. Aroma compounds in fermented dry meat sausages

Among the hundreds of volatile compounds identified in foods (Dunkel et al., 2014) and specifically in fermented sausages, only those with a concentration over its odour threshold may produce an impact in the fermented dry sausage aroma (Flores, 2018). The use of olfactometry techniques revealed the aroma compounds with an impact in dry fermented sausage and demonstrated the effect of different formulations and ripening processes on aroma formation (Flores & Corral, 2017).

The extraction of the volatile compounds from Spanish (Perea-Sanz et al., 2020) and Italian (Aquilani et al., 2018) fermented dry sausages using the same SPME extraction conditions revealed differences in the aroma profile. The Spanish fermented sausage was a starter-inoculated fermented sausage stuffed into a large diameter casing and dried for 2 months, whereas the Italian sausage was a small calibre spontaneously fermented sausage dried for 1 month. The main difference in aroma compounds between both sausages was the presence of compounds derived from the spices that produce characteristic aroma notes into the Italian sausages, not so intense in the Spanish ones (Table 1). Furthermore, the compounds derived from amino acid degradation, such as Strecker aldehydes, alcohols and acids, were abundant in the aroma profile of Italian sausages, while the only compounds from this group producing an impact in the Spanish sausage was 3-methylbutanal. Nevertheless, the contribution of ester compounds (fruity compounds) and those compounds derived from lipid oxidation (grassy, “green” compounds) to the aroma profile was more important in the Spanish than the Italian sausage. Finally, several key aroma compounds produced an impact on both sausages such as 2-acetyl-1-pyrroline and methional (Table 1).

5. Composition of meat analogues, plant isolates and flavouring ingredients used in meat analogues

As already discussed, the composition of the ingredients used in the preparation of fermented dry sausages affects greatly the final composition in terms of volatile aroma-active compounds. For this reason, the concentration of certain aroma precursors (mainly amino acids, fatty acids, sugars) in plant-based ingredients must be considered when formulating a meat-free analogue aiming to resemble the aroma of its meat counterpart. The main ingredients of meat analogues are plant protein-rich ingredients, such as protein isolates and concentrates from soy and wheat, but also legumes like pea and lupin, or rice and potato. In meat analogues, the protein ingredients used are the most important component for differentiation of the product because of the relationship with the meat-like structure aimed and their nutritional intake (Bohrer, 2019). Other ingredients are lipids and polysaccharides which produce the consistency of the meat analogue and help mimic the texture of the meat product. Taste, aroma, and colour resembling those of meat are usually simulated by the addition of substances like flavourings, spices, and herbs, and colouring agents into the matrix (Boukid, 2021).

In contrast with proteins from animal origin, which had a more complete amino acid profile, plant proteins show some limitations in terms of nutritional requirements for humans (Donadelli, Aldrich, Jones, & Beyer, 2019). Several plant protein sources contain a limited amount of certain amino acids, for example, the low proportion of lysine and methionine in soy. The amino acid composition of some legumes was

Table 1
Odour-active compounds identified by GC-Olfactometry in fermented dry sausages.

Volatile compound	Spanish fermented dry sausage[1]			Italian dry fermented sausage[2]		
	Odour description	LRI	DF	Odour Description	LRI	DF
Amino acid degradation						
Methanethiol	rotten, unpleasant	474	9	–		
2-Methylpropanal	–			acid, floral, green	590	4
3-Methylbutanal	sweet, green, spicy	690	5	caramel, chocolate, grass	691	6
2-Methylbutanal	–			sweet, floral, fruity	699	6
3-Methylbutanol	–			sweet, spicy, floral	793	4
2-Methylpropanoic acid	–			cheese, green, slightly sweet	864	6
Ethylbenzene	–			earthy, fresh, green	881	4
3-Methylbutanoic acid	–			cheese, rancid	941	8
2,5-Dimethylpyrazine	–			meaty, cooked potatoes	943	7
2-Acetyl-1-pyrroline	roasted, fried corn, roasted nuts	962	11	roasted nuts, popcorn	961	12
Methional	cooked potatoes, vegetable, meaty	969	11	mashed potato, cooked onion, roasted meat	964	9
Dimethyl trisulfide	pungent, rotten, vegetable	1011	11	–		
Tetramethylpyrazine	–			earthy, green, wetness	1118	7
2-Acetyl-2-thiazoline	toasted, fried corn, caramel, bread	1180	8	–		
Carbohydrate fermentation						
2-Butanone	wet, fresh, sweet	628	3	sweet, green, grass	629	5
2,3-Butanedione	cheese, butter, dairy	633	11	–		
2-Butanol	–			sweet, caramel, malt	643	5
Acetic acid	acid, fermented, vinegar	701	11	grass, vegetable, fresh	718	5
3-Hydroxy-2-butanone	cardboard, green	779	3	–		
Lipid β-oxidation						
2-Pentanone				floral, green, fresh	731	8
1-Octen-3-ol	mushrooms, fresh	1032	5			
Lipid oxidation						
1-Propanol	acid, fermented	611	4	vegetal, green, pungent	611	5
Propanoic acid	–			green, cheese, pungent	806	4
1-Pentanol	–			vegetable, pungent, cabbage	823	6
Hexanal	green, fresh cut grass, fatty	835	12	green, grass, vegetable	839	8
1-Hexanol	fatty, rancid, rotten fruit	925	12	–		
Heptanal	cured, rancid	942	6	–		
Octanal	sweet, citrus, floral	1046	7	–		
Hexanoic acid	cheese, floral fresh	1068	3	–		
Nonanal	fresh, herbaceous, green	1150	7	–		
Ester activity						
Ethyl 2-Methylpropanoate	sweet, fruity	783	4	–		
Ethyl butanoate	sweet, fruity, fresh	824	5	–		
Ethyl 2-hydroxypropanoate	fresh, floral, acid	861	3	–		
Ethyl 2-methylbutanoate	pineapple, sweet, acid	872	10	–		
Ethyl 3-methylbutanoate	sweet, fruity, acid	875	7	–		
3-Methylbutyl acetate	–			sweet, fresh, floral	905	7
Thiamine degradation						
2-Methyl-3-furanthiol	fatty, medicinal, sulphur	899	3	–		
Methyl-2-methyl-3-furyl disulfide	meaty, wet wood, fermented, rotten	1225	9	–		
Spices						
α -Thujene	–			sour, unpleasant, fruity,	934	5
β-Myrcene	green	1002	3	irritating, spicy, pepper	1003	10
3-Carene	earthy, green, fresh	1021	12	–		
α-Terpinene	–			pungent, pine, woody, earthy	1035	12
Terpene	–			earthy, green, vegetable, fresh, fruity	1075	5
Unidentified terpene	spicy, fresh, floral	1092	3	–		
Terpinolene	–			floral, rose, grass, green	1106	11
Linalool	–			fresh, floral, soap	1145	7
β -Terpinene/γ-terpinene	–			cooked, cooked vegetable, pungent, resin	1158	8
Unknown						
Carbon disulfide	–			burnt, malt	537	4
Unknown	–			cured, meaty, fresh	762	6
Unknown	–			roasted, fried nuts, biscuits	1190	11
4-Methylphenol	unpleasant, rotten, sulphur	1198	6	–		

Compounds not detected in one of the samples are represented by a dash (–). LRI: linear retention index in a DB-624 capillary column; DF: detection frequency by GC-O. References: [1] Perea-Sanz et al. (2020); [2] Aquilani et al. (2018).

recently indicated by Guyomarc'h et al. (2021), remarking the composition in essential amino acids of these sources.

The lack of specific essential amino acids, but also their lower digestibility (protein digestion and absorption kinetics), has increased the interest in enhancing the composition of plant proteins. These facts have been shown to have a direct effect in the use of plant proteins in food supplements like those indicated for muscle mass maintenance and growth (Van Vliet, Burd, & van Loon, 2015). Therefore, different strategies have been proposed to enhance the anabolic properties of plant proteins, like fortification with the amino acids methionine, lysine, and/or leucine; selective breeding to improve amino acid profiles; increase in plant consumption and ingesting multiple protein sources to provide a more balanced amino acid profile. These strategies, of course, can be applied for the preparation plant-based protein products with specific amino acid composition for the development of aroma. In this sense, especial interest is directed to the soluble amino acids present in meat and meat products as a result of proteolysis as it happens in fermented meat products as indicated above. Nevertheless, in comparison to the flavour precursors present in meat and fat used in fermented dry sausages (Section 2), plant protein isolates and concentrates show an amino acid and lipid profiles different to those of meats and a lack of thiamine, what directly affects the formation of meaty aromas. For this reason, the improvements achieved in the amino acid profile of plant proteins for other purposes can be used for the preparation of meat-free products with precursors of meaty aromas naturally present.

6. Flavour of plant protein isolates

Plant proteins are usually characterised by the presence of off-flavours, like the typical bean aroma of soy or pea-based products. These off-flavours can be translated to the final product, which in certain cases can produce rejection by some consumers. The presence of green and beany off-flavours in legume proteins is considered an issue for food applications. In soy protein isolates, off flavours are mainly derived from lipid oxidation but also from the presence of phytochemicals. The residual amount of phospholipids that contain polyunsaturated fatty acids (PUFAs) are oxidised during storage producing off-flavours (Damodaran & Arora, 2013). Bitter and astringent taste compounds have also been detected due to the presence of residual polyphenolic compounds (phenolic acids) that are not completely eliminated during protein extraction. Different methodologies are proposed to remove off-flavours or their precursors such as residual phospholipids by using a combination of technologies based on ultrasound, enzyme treatment, and molecular inclusion technologies (Damodaran & Arora, 2013).

Odour-active off-flavours in plant-based foods made from pea and soy have been identified by olfactometry studies (Zhang, Hua, Li, Kong, & Chen, 2020). In general, the beany, grassy and earthy odours are produced by the presence of hexanal, (*E*,*E*)-2,4-nonadienal, and (*E*,*E*)-2,4-decadienal. However, several differences have been observed depending on the plant-based raw material. For example, in pea milk other compounds like 2-methoxy-3-isopropyl-(5 or 6)-methylpyrazine produces off-flavours while in soy milk a characteristic earthy off-flavour is produced by 1-octen-3-one (Zhang et al., 2020). However, the presence of off-flavours in pea and soy proteins depends mainly on the lipoxygenase activity present in the plant material while the lipid content produces a minor effect on their formation (Zhang et al., 2020).

Current studies are focused on the functionality of plant protein ingredients for use in non-milk beverages or meat analogues (Akharume, Aluko, & Adedeji, 2021). Functionality is directed to the improvement of organoleptic properties but essentially in hydration and structural capacities of plant proteins. In addition to the application of physical techniques for modification of plant-based proteins many advancement has been done regarding fermentation and development of fermented milk-like beverages (Tangyu et al., 2019). In this fermentation reactions, the microorganisms used are strains of LAB, yeast and fungi where the main purpose is the reduction of protein isolates off-flavours, increase texture properties and nutrient profile (Akharume et al., 2021). Regarding flavour formation, the fermentation process improves the plant protein solubility and amino acid composition, and availability for flavour reactions. However, in the field of meat analogues few studies are focused on fermentation and flavour improvement of meat analogues.

7. Flavour improvement of plant protein isolates by fermentation

Apart from reducing or eliminating off-flavours in plant proteins, fermentation has been widely used for providing other aromas and tastes very appreciated by the consumers. Indeed, this can be seized for the design of fermented dry sausage analogues prepared entirely from plant-based ingredients. The use of fermentation of plant-based foods to generate different flavour profiles is widely known in Asia since ancient times. Several of these fermented foods have been described as having a taste profile with umami characteristics as that expected in an animal-derived food product and have been characterised in terms of their aroma profile and taste. That is the case of Guilin Huaqiao white sufu, a traditional fermented soybean curd from China, also known as "Chinese cheese" (He, Wan, Liu, & Chen, 2020). It is a soybean curd, fermented by mould, salted and ripened for several months. In its manufacture other ingredients are added, including Chinese liquor, salt, sugar, flour paste, and spices. The manufacture process produced a fermented plant-based product with an appreciated fermented, alcoholic, fatty and sweet aroma notes, and umami properties (He et al., 2020). Another well-known soy fermented product is miso, the Japanese fermented soybean paste that imparts an umami after taste. It is produced from soybean and salt and fermented with *Aspergillus oryzae* ("koji"), which is then ripened for months (Inoue et al., 2016). The flavour of soy miso is not only attributed to the fermentation but also to the ripening process. A large variety of odour-active volatile compounds found in miso, mainly organic acids, higher alcohols and aldehydes, but also some sulphur compounds like methional, and dimethyl di- and trisulfides. In addition, many different types of miso are produced with different flavour profiles depending on ingredients and process and the further cooking of the product.

In order to obtain more digestible soy protein isolates to be used in vegetarian foods, some researchers have prepared and characterised protein isolates from fermented soy products. That is the case of a study where a protein isolate was prepared from tempeh, a soybean product fermented in solid state with the fungus *Rhizopus* strains (Wan Saidatul Syida, Noriham, Normah, & Mohd Yusuf, 2018). In the fermented tempeh the dominant aroma compounds were identified as 2-methylpropanal, 1-octene-3-one and methional. Moreover, the frying process applied to tempeh for consumption produced the change of impact aroma compounds, being the dominant compounds 2-acetyl-1-pyrroline, 2-ethyl-3,5-dimethylpyrazine, dimethyl trisulfide, methional, 2-methylpropanal and (*E*,*E*)-2,4-decadienal (Jeleń, Majcher, Ginja, & Kuligowski, 2013). This demonstrated the contribution of pyrazines to the roasted aroma of fried tempeh.

The fermentation of other plant products, like cereal, has the capacity to produce pleasant aroma compounds of potential interest in the design of fermented dry sausage analogues. In cereal-based liquid foods, the fermentation process produces some volatile compounds similar to those reported in fermented meat products. The presence of compounds derived from carbohydrate fermentation and amino acid metabolism in fermented beverages is common and depends on the microorganism used and the physicochemical conditions and nutrients available for fermentation (Peyer, Zannini, & Arendt, 2016). One of the advantages of cereal fermentation and microbial acidification is the activation of the endogenous acid proteases and peptidases present in cereals. At the same time, LAB produce exogenous proteases which participate in the fermentation process and the production of flavour compounds derived from amino acid metabolism (Peyer et al., 2016). However, beverage fermentation has several aroma problems due to aging and formation of stale off-notes. Several compounds have been related to the staling process, such as the presence of 3-methylbutanal, 2-furfural, benzaldehyde, phenylacetaldehyde, hexadienal, heptanal, methional, and (*E*)-β-damascenone (Peyer et al., 2016). Several studies have shown the ability of the fermentation process to reduce off-flavour in plant protein isolates and its ability to be used in "plant milks" (El Youssef et al., 2020; Schindler et al., 2012) (Table 2).

Soy and pea protein isolates are widely used in meat analogues (Boukid, 2021), although the application of a fermentation process for meat analogues production is scarce. Regarding the elimination of off-flavours, since 1979 the enzymatic treatment of soy with aldehyde

Table 2
Flavour changes in fermented plant protein isolates used in meat analogues processing.

Protein isolate	Microorganism	Volatile compounds	Aroma descriptors	Reference
Pea (*Pisum sativum*)	LAB (*Lactobacillus plantarum*)	Hexanal 1-pyrroline dimethyl trisulfide 1-octen-3-one 2,5-dimethyl pyrazine 3-octen-2-one (*E*)-β-damascenone guaiacol	green sperm sulphur, faecal mushroom nutty mushroom floral smokey	Schindler et al., 2012
Soy protein isolates (SPI)	- Enzymatic treatment: Alcalase, Papain, Flavourzyme + Papain - Fermentation with *Lactobacillus perolens*, *Actinomucor elegans*, and *Rhizopus oryzae*	Not determined	Reduction of beany flavour	Meinlschmidt et al., 2016
Pea protein isolate	Commercial starter (DuPont Danisco): VEGE 047 LYO Yeast: *Kluyveromyces lactis* Clib *Kluyveromyces marxianus* *Torulaspora delbrueckii*	Off flavours: 2-methylpropanal, *trans*-2-methyl-2-butenal, hexanal, (*E*)-2-hexenal, heptanal, (*E*)-2-octenal, nonanal, butanal, (*E*)-2-heptenal, decanal, 1-penten-3-ol, 1-octen-3-ol, 1-hexanol, 1-octanol, 6-methyl-5-hepten-2-one, 2-octanone, 2-nonanone, 2-n-heptylfuran, 2-ethyl-furan, and 2-pentylfuran Esters: isoamyl acetate, 2-methylbutyl acetate, 2-phenylethyl acetate, isobutyl acetate, ethyl octanoate, ethyl hexanoate, hexyl acetate, ethyl isobutyrate, ethyl propionate, propyl acetate, and ethyl acetate	Off-flavours reduction in inoculated isolates. Ester compounds present in samples yeast inoculated	El Youssef et al., 2020
Pea-oat protein blend	Commercial starter from DuPont Danisco® VEGE 022 VEGE 053 (lactic acid bacteria: Streptococcus, Lactobacillus, Lactococcus and bifidobacteria)	Remove of aldehydes from raw material Increase in Maillard compounds (pyrazines) Increase in: carboxylic acids (butanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, nonanoic acid), alcohols (1-hexanol, benzyl alcohol), acetoin, and 2,4-decadienal.	Increased flavour. Undesirable odours: medicinal, soapy, citrus-like.	Kaleda et al., 2020

dehydrogenase was proposed as a strategy to reduce the hexanal content and therefore, the beany flavour (Chiba, Takahashi, & Sasaki, 1979). In addition, the application of fermentation using *Lactobacillus* or *Streptococci* strains or other microorganism without lipoxygenase activities was indicated to reduce the presence of these off-flavours in pea isolates (Schindler et al., 2012). The fermentation of pea proteins by LAB produced a protein extract with a more pleasant aroma and light milky attribute than the unfermented product, although both of them were characterised by a nutty, green, and cereal-like odour (Schindler et al., 2012). The improvement in odour of the fermented pea isolate was attributed to a reduction in the concentration of hexanal and the ability of several compounds to mask the green note produced by it.

Recent studies have proposed the fermentation of pea proteins with a combination of LAB (commercial starter culture VEGE 047 LYO) and yeast to reduce the off-flavours produced by the presence of hexanal and other oxidation products (El Youssef et al., 2020) (Table 2). The fermented product tried to emulate the sensory perception of a "yogurt-like" product by the acidification produced by LAB. The off-flavour was reduced by fermentation but not completely eliminated and suggested the presence of other potent aroma compounds producing the beany off-note. The presence of yeast strains in the fermentation of pea proteins introduced a different aroma profile characterised by the presence of esters, essentially acetate and ethyl esters (El Youssef et al., 2020). However, not all the yeast strains produced the same odour effect as it was shown by the highest ability to produce ester compounds by *Kluyveromyces* sp. compared to *Torulaspora* yeast strain. Moreover, the combination of different microorganisms for fermentation (LAB and moulds) was proposed as a way to improve the taste of soy protein isolates when it was used in combination with enzymatic hydrolysis (Meinlschmidt, Schweiggert-Weisz, & Eisner, 2016). The purpose of this study was to reduce the allergenicity of the soy isolates but, at the same time, the process improved the taste and functionality (emulsifying and foaming properties) of the isolate. In addition, the fermented isolates showed a reduced beany flavour.

In 2020, Kaleda et al. reported the use of a fermentation process of pea-oat protein blend as a treatment before texturisation using extrusion cooking (Table 2). They reported that the fermentation process affected the extrusion process because a more intense treatment was required to form the fibrous structure. The high temperature applied during the extrusion process eliminated many of the volatile compounds present in the raw materials (aldehydes), whilst it simultaneously increased the content of pyrazine and thiophene compounds due to Maillard reaction. This effect was correlated to the increase in free amino acids produced by the fermentation process. However, the fermented extrudate contained several compounds that negatively affected the flavour producing medicinal, soapy, and citrus-like odours generated from the presence of carboxylic acids (butanoic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, nonanoic acid), alcohols (1-hexanol, benzyl alcohol), acetoin, and (*E,E*)-2,4-decadienal. These volatile compounds were the result of the activity of the commercial fermented starter (VEGE 053) used that contained LAB (*Streptococcus*, *Lactobacillus*, and *Lactococcus*, among others) and bifidobacteria. As shown in the literature, the fermentation of vegetable proteins has beneficial effects, such as production of pleasant aromas, and improvement of functionality and nutritional values. This proves the great potential of these ingredients in the preparation of plant-based fermented dry sausage analogues with a formulation designed to provide a natural pleasant flavour produced by the fermentation of the precursors already present in the product.

8. Conclusions

The formation of aroma in traditional fermented meat sausages is a complex process affected by composition, processing and conditions. The intrinsic characteristics of plant isolates cannot reproduce the aroma of fermented meat sausages, mostly due to their composition in terms of aroma precursors (amino acids, carbohydrates, and fatty acids). The offer of new attractive meat analogues to consumers must be based on the use of plant protein isolates by using other processing techniques. Fermentation process can be a strategy to reproduce the fermented meaty aroma. However, there is a need to control the formation of aroma compounds as well as the potential formation of stale odours that would be responsible for off-flavours after processing, ripening or storage conditions. Therefore, studies should be directed to the use of different microorganisms to produce a high diversity of aromas to obtain fermented plant proteins with potential use in meat analogues. Besides, the used of fermented protein isolates as ingredient or the fermentation of the final product should also be considered when planning the production strategy. Nevertheless, until now the fermented plant protein isolates studied has been focused on off flavour removal but not on the production and generation of meaty aroma compounds. The potential of plant ingredients in the preparation of fermented meat analogues is great, but ingredients, microbial cultures and processes need to be carefully designed in order to obtain a product with organoleptic characteristics that consumer will appreciate. Thus, further research is required to fill the gap of knowledge in the area, mostly identified by the lack of studies focused on aroma and sensory properties.

Declaration of Competing Interest

The authors declare no conflict of interest.

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