

Minireview

Fermentation of plant-based dairy alternatives by lactic acid bacteria

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Summary

Ethical, environmental and health concerns around dairy products are driving a fast-growing industry for plant-based dairy alternatives, but undesirable flavours and textures in available products are limiting their uptake into the mainstream. The molecular processes initiated during fermentation by lactic acid bacteria in dairy products is well understood, such as proteolysis of caseins into peptides and amino acids, and the utilisation of carbohydrates to form lactic acid and exopolysaccharides. These processes are fundamental to developing the flavour and texture of fermented dairy products like cheese and yoghurt, yet how these processes work in plant-based alternatives is poorly understood. With this knowledge, bespoke fermentative processes could be engineered for

specific food qualities in plant-based foods. This review will provide an overview of recent research that reveals how fermentation occurs in plant-based milk, with a focus on how differences in plant proteins and carbohydrate structure affect how they undergo the fermentation process. The practical aspects of how this knowledge has been used to develop plant-based cheeses and yoghurts is also discussed.

Introduction

Fermentation of dairy milk by lactic acid bacteria is well documented, but how the molecular processes involved in fermentation occur in plant-based milks has received little attention (Hayes *et al.*, 2007; Mende *et al.*, 2016; Ji *et al.*, 2021). The increasing interest in plant-based alternatives to products such as cheeses and yoghurts is hindered by difficulties in creating products with acceptable textures and flavours (Jaeger and Giacalone, 2021). Understanding how fermentation occurs in plant-based dairy substitutes and how fermentation influences flavour and texture is key to developing products that are desirable to consumers wishing to switch to plant-based alternatives.

Consumption of dairy products is declining in many Western countries, while plant-based milk sales are increasing (Islam *et al.*, 2021). Plant-based milk now makes up 15% of the total milk industry, and other plant-based dairy alternatives are also increasing in popularity (Good Food Institute, 2021). There is now a wide range of plant-based dairy alternative products available, with markets for these products being in the millions or billions of dollars (Fig. 1). Increased awareness around the environmental impacts of dairy farming, ethical concerns, medical reasons such as lactose intolerance and cow's milk allergy, and an increased perception of milk as being 'unhealthy' all contribute to consumers choosing plant-based alternatives to milk and dairy products (McCarthy *et al.*, 2017). Plant-based milks use less energy in production and result in emission of less greenhouse gas per gram of protein than animal-based milks (González *et al.*, 2011), making them an attractive option for those wanting to reduce their carbon footprint. They are also a good alternative for those who are

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unable to consume cow's milk for medical reasons as they do not contain lactose or other allergens present in cow's milk, although some plant-based milks such as soy or nut-based milks contain other allergens. They tend to be lower in fat, especially saturated fat, so can be a good option for those looking for lower-fat alternatives. Additionally, plant-based milks can be produced so as to contain dietary fibre, adding nutritional benefits not found in dairy milk. On the other hand, current plant-based milks are generally lower in protein, except soy, which is comparable with dairy milk, and plant-based milks are commonly fortified with nutrients such as calcium and vitamin B12 to improve nutritional quality [for review see Mäkinen *et al.* (2016); Clegg *et al.* (2021)]. Plant-based milks often contain less complete proteins, for example, cereals can be deficient in lysine, while legumes tend to be deficient in methionine and cysteine (Sim *et al.*, 2021). Strategies to combat this, such as combining plant-based milk types in a product to provide an overall complete amino acid profile, can be used. Plant proteins also tend to be more difficult to digest. This can be due to antinutritional factor compounds present such as protease inhibitors and non-starch polysaccharides, as well as properties inherent to the protein structure such as cross-linking, hydrophobicity and secondary structure elements (Sá *et al.*, 2020).

Lactic acid bacteria (LAB) are the group of species responsible for fermentation in dairy-based foods and have been used in food fermentation for centuries

(Bernardeau *et al.*, 2006). In these foods, fermentation can improve the flavour and texture of products. For example, the breakdown of proteins into amino acids and further into volatile organic compounds is one of the main sources of flavour compounds in cheese (Yvon and Rijnen, 2001; Smit *et al.*, 2005), and the production of exopolysaccharides from sugars improves the texture of cheeses and yoghurts (Korczyk and Varga, 2021). Fermentation has well-documented health benefits, such as the introduction of bioactive small molecules including bioactive peptides and, in some cases, can improve the vitamin content (Şanlıer *et al.*, 2019). Fermentation can also extend product shelf-life. LAB acidify the media, making it a less hospitable environment for other microbial organisms, and also produce a range of compounds which inhibit growth of other organisms such as organic acids, hydrogen peroxide and bacteriocins (Saranraj *et al.*, 2013).

One reason for resistance to the adoption of plant-based milks is the presence of undesirable 'beany' flavours and undesirable textures. These undesirable flavours can largely be attributed to aldehydes, primarily hexanal but also 3-Z-hexenal, as well as alcohols such as *n*-hexanol, *n*-pentanol and *n*-heptanol, ketones such as ethyl vinyl ketone and furans such as *n*-pentyl furan and 2-(1-pentenyl) furan (Rackis *et al.*, 1979). Fermentation of plant-based milks by a range of LAB is able to completely remove hexanal and decrease the concentration of other contributing volatile organic compounds in soy (Blagden and Gilliland, 2005). Antinutritional factors

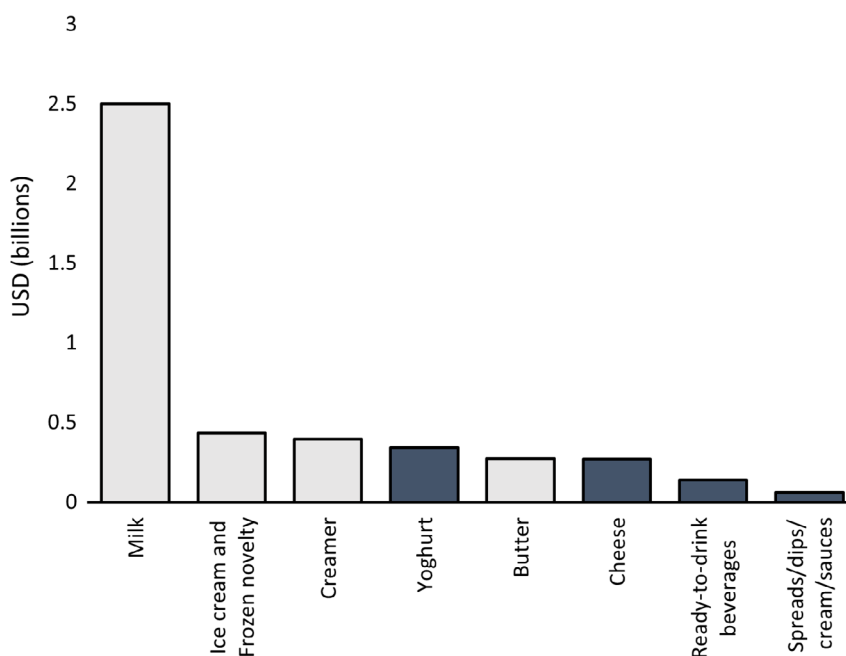


Fig. 1. Sales in USD of different categories of plant-based dairy alternatives in the USA in 2020. Categories in dark grey are those which can utilise fermentation. Adapted from Good Food Institute (2021).

present in plants such as tannins, saponins, phytic acid, α -galactosides and trypsin inhibitors are also broken down during fermentation by LAB, improving the nutritional quality of plant-based products (Lopez *et al.*, 2000; Adeyemo and Onilude, 2013). Therefore, fermentation and the development of fermented products could be an important pathway to improving the nutritional quality and acceptability of plant-based milk products.

This review will discuss how the fermentation of plant proteins has been used to produce plant-based dairy alternative products and highlight the gaps in understanding the molecular-level processes that are occurring during fermentation. The current understanding of how molecular processes such as proteolysis and exopolysaccharide formation occur during the fermentation of dairy milk products will be discussed before relating this knowledge to plant-based systems, in order to compare the differences between the two systems. This will help to demonstrate why the development of plant-based dairy alternatives has faced difficulties in mimicking the flavour and texture profiles of dairy-based products and will highlight potential avenues that could be exploited to improve plant-based dairy products.

Fermentation processes

Proteolysis, lipolysis and carbohydrate metabolism are the major metabolic processes involved in the

fermentation of dairy milk. Proteolysis results in the development of many of the major flavour compounds in hard cheeses (Fox *et al.*, 1996; Smit *et al.*, 2005). Carbohydrate metabolism produces lactic acid, which is the main driver of acidification of the product, diacetyl and acetaldehyde which are key flavour compounds, and exopolysaccharides, which are important for the development of flavour and texture, especially in yoghurts. Lipolysis is critical for flavour development in dairy cheeses, where lipases hydrolyse milk fats to release free fatty acids. These free fatty acids can be flavour compounds themselves and are more importantly precursors to flavour compounds such as methyl ketones, alcohols, lactones and esters [for review, see Jooyandeh *et al.* (2009)]. However, lipases mostly originate from microbial sources other than LAB and will not be discussed further in this review. For a summary of important fermentative characteristics of some commercially relevant LAB species in the fermented dairy industry, refer to Table 1.

Proteolytic system

Due to the limited ability of LAB to synthesise amino acids, and the negligible quantities of free amino acids and available peptides present in dairy milk, LAB from milk require an effective proteolytic system (Fig. 2) in order to grow (Teusink and Molenaar, 2017). Cell envelope proteinases are the large, multidomain serine

Table 1. Commercially relevant lactic acid bacteria species and their fermentation characteristics.

Species name	Bacteria type	Fermentation type	Cell envelope proteinase present	Common commercial usage	Main role in dairy fermentation
<i>Lactobacillus helveticus</i> ^a	Thermophilic	Homofermentative	Prth2 (common to all strains), PrtH, PrtH3, PrtH4	Cheese (esp. mozzarella, Emmental, Grana Padano), fermented milk.	Most proteolytic LAB species commonly used in dairy fermentation
<i>Streptococcus thermophilus</i> ^b	Thermophilic	Homofermentative	PrtS	Yoghurt starter, cheese, fermented milk.	EPS production in yoghurt
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i> ^c	Thermophilic	Homofermentative	PrtB	Yoghurt starter, cheese, fermented milk.	Proteolysis in yoghurt
<i>Lactococcus lactis</i> subsp. <i>cremoris</i> ^d	Mesophilic	Homofermentative	PrtP	Cheese	Development of flavour compounds in cheese
<i>Lactococcus lactis</i> subsp. <i>lactis</i> ^d	Mesophilic	Homofermentative	PrtP	Cheese.	Fast acidification through conversion of lactose to lactic acid
<i>Leuconostoc mesenteroides</i> ^e	Mesophilic	Heterofermentative	n/a	Cheese (esp. Swiss styles, Roquefort)	Produces complex flavours and eye holes in cheese through production of CO ₂ and diacetyl.

a. Sadat-Mekmene *et al.* (2011).

b. Iyer *et al.* (2010).

c. Tian *et al.* (2018).

d. Stanley (2003).

e. Hemme and Foucaud-Scheunemann (2004).

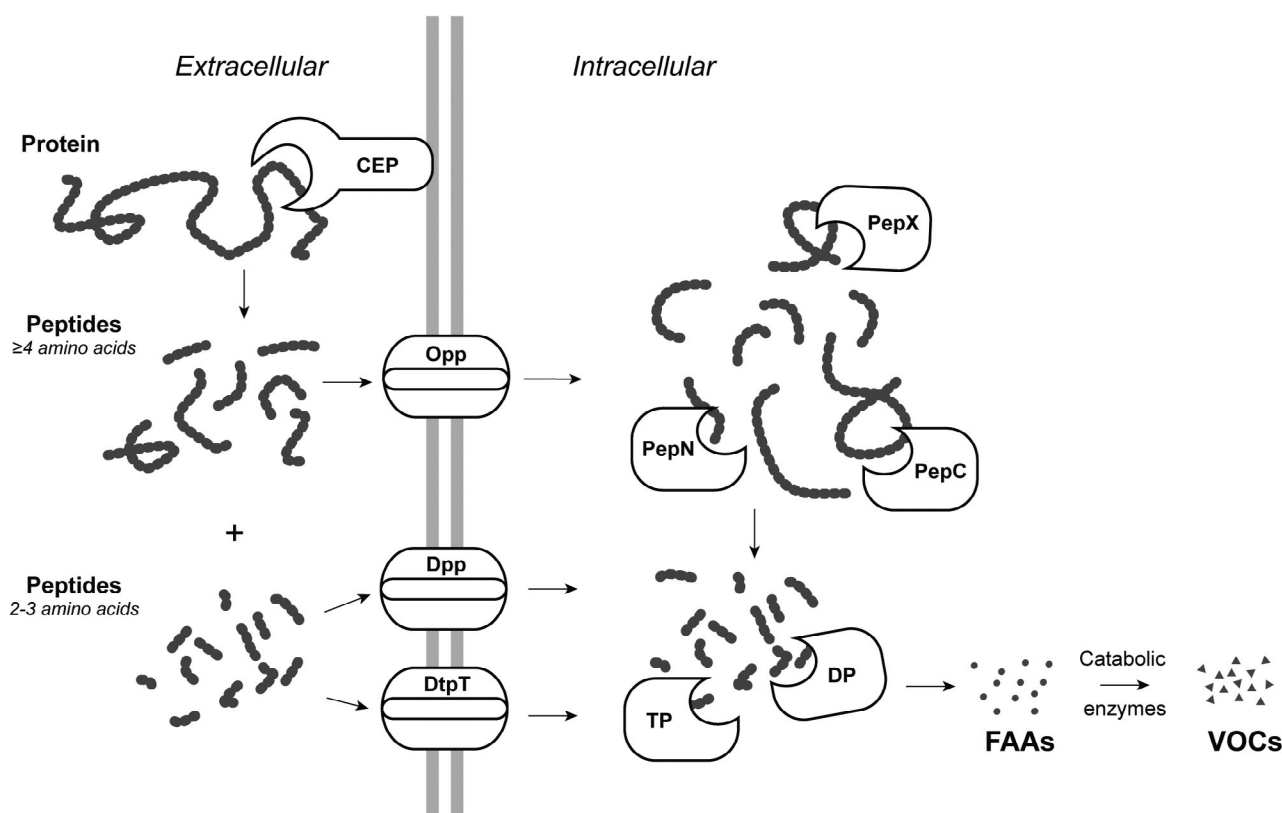


Fig. 2. Generalised depiction of the proteolytic system of lactic acid bacteria. Protein is first broken down by cell envelope proteinase (CEP). Opp, Dpp and DtpT systems transport different sized peptides into the cell. PepX, PepN and PepC intracellular proteases catabolise larger peptides into di- and tripeptides. Dipeptidases (DP) and tripeptidases (TP) break di- and tripeptides down into free amino acids (FAAs). A wide range of catabolic enzymes then break the FAAs down into volatile organic compounds (VOCs).

proteases that catalyse the first step of this process, where large proteins are broken down into peptides. Many different cell envelope proteinases have been described, varying in size, specificity for caseins, and domains present. These are designated as PrtX enzymes, where X corresponds to the specific cell envelope proteinase. Some species express the same cell envelope proteinase, for example, *Lactocaseibacillus paracasei* subsp. *paracasei*, *Lactococcus lactis* subsp. *cremoris* and *Lc. lactis* subsp. *lactis* all express PrtP. *Lactobacillus helveticus* expresses four different PrtH enzymes, and most other LAB express only one unique cell envelope proteinase (Table 1). These are secreted from the cell and anchored to the cell membrane, which ensures that proteolysis occurs close to the cell and allows efficient subsequent transportation. The catalytic domain contains the active site, composed of three amino acid residues (serine, histidine and aspartic acid) that are highly conserved across species (Hansen and Marcatili, 2020). The cell envelope proteinase from *Lc. lactis* subsp. *lactis*, PrtP, degrades caseins into peptides ranging from 4 to 30 amino acids, with no di- or tripeptides being detected, and only trace amounts of

phenylalanine being detected in free amino acid analysis (Juillard *et al.*, 1995). An oligopeptide permease (Opp) transport system then transports oligopeptides across the cell membrane, while an ion-linked transporter (DtpT) and ABC transporter (Dpp) system transport any di- and tri-peptides produced. Once transported into the cell, the oligopeptides are first broken down into smaller peptides by intracellular endopeptidases, aminopeptidases (PepN and PepC) and X-prolyl dipeptidyl aminopeptidase (PepX). The generated smaller peptides are then further broken down by dipeptidases and tripeptidases into individual amino acids [for review of the proteolytic system, see Savijoki *et al.* (2006)]. These amino acids are then further broken down into volatile organic compounds, some of which are responsible for the characteristic flavours of dairy products. The specific compounds responsible for the flavour in several types of cheese are described in Table 2.

Proteolysis in plant-based dairy alternatives

The proteolytic systems of LAB and their function in dairy milk is well known [reviewed in Savijoki *et al.*

Table 2. Volatile organic compounds derived from amino acids which are important to the flavour of cheese. Adapted from Smit *et al.* (2005).

Amino acid	Gouda	Cheddar	Camembert	Swiss
Leucine	3-Methylbutanal 3-Methylbutanol 2-Methylpropanol	3-Methylbutanal	3-Methylbutanal	3-Methylbutanal
Methionine	Methanethiol Dimethylsulphide Dimethyltrisulphide	Methional Methanethiol Dimethyldisulphide Dimethyltrisulphide	Methional Methanethiol Dimethylsulphide	Methional
Valine	-	Isovaleric acid	-	-
Phenylalanine	-	-	Benzaldehyde Phenylacetaldehyde	-

(2006); Ji *et al.* (2021)]. In contrast, very little research has defined how these same systems work on the proteins of plant-based foods. Research so far has focused primarily on soy (Aguirre *et al.*, 2008; Boulay *et al.*, 2020; Shirotani *et al.*, 2021), as one of the most commonly used plant alternatives to dairy products until recently.

Differences in the structures of plant-based and dairy-based proteins mean that the abundance of research into proteolysis in dairy milk is not directly translatable to plant-based milk. The major proteins in dairy milk are caseins, comprising around 80% of the protein in cow's milk. Caseins are a family of flexible phosphoproteins comprising of α_{S1} -, α_{S2} -, β - and κ -caseins. They form large micelles in milk due to the hydrophobic aggregation of the proteins, with negatively charged κ -caseins forming the outside of the micelle (Wei and Yano, 2020). Prt enzymes are classified into two types based on their ability to breakdown caseins – P_I enzymes mainly degrade β -caseins, while P_{III} are able to degrade α_{S1} -, β - and κ -caseins to a similar extent (Visser *et al.*, 1986). P_{III} enzymes also have a broader specificity for β -casein than P_I enzymes (Visser *et al.*, 1991). The high abundance of proline residues in caseins leads to an open and disordered secondary structure, which makes them highly susceptible to hydrolysis by cell envelope proteinases (Savijoki *et al.*, 2006). In contrast, most plant proteins are less accessible to proteases, due to being large, multimeric, globular proteins that fold into ordered, tightly packed structures. This is due to the disulphide bonds, hydrophobic effects, electrostatic forces, hydrogen bonding and van der Waals forces present (Sim *et al.*, 2021). Table 3 illustrates some of the key differences between the casein proteins and some selected plant proteins to highlight how proteolysis is more easily facilitated in milk proteins than plant proteins. Carbonaro *et al.* (2012) investigated the relationship between the digestibility of proteins and their β -sheet content in legume, cereal, meat and milk proteins and found that digestibility had a strong inverse relationship to the β -sheet content. The authors hypothesised that this was due to the hydrophobic characteristics of these

structures, which also make them prone to aggregation upon denaturation. As many plant proteins, especially globular proteins, have high β -sheet content (Table 3), this contributes to the low digestibility of these proteins. There have been no plant proteins described within the literature that possess flexible micellar structures analogous to casein, making it difficult to mimic the characteristics and structure of dairy products with plant-based products (Sim *et al.*, 2021).

Casein bears several key similarities, however, with plant prolamins, the seed storage proteins of cereals, such as zein (found in maize) and gliadins (found in wheat). As demonstrated in Table 3, they are a similar molecular weight, possess high proportions of proline residues, are relatively disordered and spontaneously form large functional structures in their native biological systems. Some research suggests gliadin and gliadin-derived peptides are suitable substrates for cell envelope proteinases. Pescuma *et al.* (2013) found that cell envelope proteinases from eight different species of LAB were able to hydrolyse gliadins from wheat. Gobbetti *et al.* (1996) found that a cell envelope proteinase isolated from *Fructilactobacillus sanfranciscensis*, one of the dominant LAB in sourdough fermentation, had improved proteolysis of gliadins compared with caseins, and that a dipeptidase and aminopeptidase isolated from *F. sanfranciscensis* had strong affinity for the hydrophobic peptides released from gliadins. However, the application of prolamin proteins in plant-based milks may be limited, due to their role in triggering coeliac disease (Shan *et al.*, 2002) and their low solubility in water (Shewry, 2019). Despite this, the similarity in some structural features of prolamins with caseins means these proteins and other prolamins from less common cereals deserve further investigation as potential substrates for cell envelope proteinases.

The ability of cell envelope proteinases to hydrolyse globular proteins has also been studied, mainly from soy. Proteolytic activity in soy milk was shown to be correlated to bacterial growth rate in soy milk for eight different LAB species (Donkor *et al.*, 2007), and cell envelope proteinases from several LAB species were able to

Table 3. Structure and level of hydrolysis by select plant and dairy proteins.

Source	Protein	Protein type	% Unstructured	% β -sheet structure	Molecular weight (kDa)	% Proline residues	Hydrolysis by LAB
Dairy	α_{S1} -casein	Flexible	33 ^a	20 ^a	23.6 ^b	8.5 ^c	Some hydrolysis in hydrophilic regions ^d
	α_{S2} -casein	Flexible	9–34 ^a	23–32 ^a	25.2 ^b	4.8 ^c	P _{III} hydrolysis only ^d
	β -casein	Flexible	4–80 ^{±a}	0–34 ^a	24 ^b	16.7 ^c	Extensive P _I and P _{III} hydrolysis ^d
	κ -casein	Flexible	23–24 ^a	35–40 ^a	19 ^b	11.8 ^c	Some hydrolysis by P _I , P _{III} and PrtH enzymes ^d
	β -lacto globulin (whey)	Globular	18–35 ^e	49–57 ^e	18.4 ^b	4.9 ^c	Little hydrolysis ^d
Soy	β -conglycinin	Globular	14.4 ^f	45.6 ^f	150–200 ^b	5.4 ^g	Reasonable hydrolysis of α_S and α_S' subunits, some hydrolysis of β subunit ^h
	Glycinin	Globular	16.5 ^f	47.3 ^f	300–380 ^b	4.3 ^g	Some hydrolysis of A subunit, very little hydrolysis of B subunit ^h
Pea	Vicilin	Globular	-	30 ^f	150 ^b	4.4 ^g	Not tested
Maize	Zein	Prolamin	-	-	19–24 ^b	10.1 ^g	Not tested
Wheat	α/β -Gliadin	Prolamin	-	-	30–45 ⁱ	15–20 ⁱ	Some hydrolysis by 8 selected LAB species ^j , extensive hydrolysis by sourdough starters ^k .

a. Home (2002)

b. McClements and Grossmann (2021)

c. O'Regan *et al.* (2009)d. Ji *et al.* (2021)e. Bhattacharjee *et al.* (2005)f. Shevkani *et al.* (2019)

g. Fukushima (1991)

h. Aguirre *et al.* (2008)

i. Shewry (2019)

j. Pescuma *et al.* (2013)k. Shan *et al.* (2002).

hydrolyse low-molecular-weight proteins from soy to varying extents (Pescuma *et al.*, 2013). Emkani *et al.* (2021) demonstrated the evidence of proteolysis in pea extract after fermentation with LAB. Comparison of proteolysis by wild-type LAB *Streptococcus thermophilus* compared with a mutant *S. thermophilus* in which the PrtS enzyme had been removed showed much more extensive proteolysis by the wild-type, and population growth of the wild type was threefold greater (2.7×10^8 CFU/mL for the mutant compared with 7.6×10^8 CFU/mL for the wild type) (Boulay *et al.*, 2020). The main proteins in soy are β -conglycinin, a trimer formed of α -, α' - and β -sub units, and glycinin, a hexamer with each subunit comprising an acidic and basic polypeptide, linked by a disulphide bond (Nishinari *et al.*, 2014). LAB can hydrolyse these proteins to varying degrees depending on the strain. For most LAB strains, β -conglycinin is more effectively broken down than glycinin, with the α - and α' -subunits generally being the most preferred substrate for the majority of strains tested, with an average of 46% and 38% of these subunits being hydrolysed, respectively, and the basic subunit of glycinin being the least degraded fraction for all strains with only an average of 13% of this subunit being hydrolysed (Aguirre *et al.*, 2008). Identification of the peptides produced by different LAB strains showed that the specificity for soy proteins

differs between LAB strains (Aguirre *et al.*, 2014). The possible cleavage site in soybean proglycinin has been elucidated by X-ray crystallography, showing that the protein subunits are mainly broken down via cleavage sites on the surface of the protein, likely due to accessibility (Shirovani *et al.*, 2021). Further breakdown of peptides into amino acids and volatile organic compounds has been shown to occur in soy, with compounds related to leucine, isoleucine and phenylalanine/tyrosine being able to be detected after LAB fermentation of soy (Shirovani *et al.*, 2021). These are known to be associated with the flavours of cheese (Yvon and Rijnen, 2001).

Milk proteins have been well studied as a source of bioactive peptides, which are often found in dairy products fermented with LAB (Rafiq *et al.*, 2021). Bioactive peptides are peptides, generally 3–20 amino acids long, which have defined health benefits, such as positive impacts on the nervous, digestive, immune and cardiovascular systems or anticancer properties. Fermentation of plant proteins has also been demonstrated to produce bioactive peptides [for review, see Singh *et al.* (2014); Rizzello *et al.* (2016)]. For example, fermentation of soy with *Lactocaseibacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus Bulgaricus* and *S. thermophilus* was found to produce antihypertensive peptides (Tsai *et al.*, 2006); concentrations of lunasin, a peptide with anticancer, anti-

inflammatory and antioxidative properties, increased when wholemeal wheat, soybean, barley, amaranth and rye flours were fermented with lactic acid bacteria (Rizzello *et al.*, 2012); and antioxidative peptides were produced from various cereal flours after fermentation with lactic acid bacteria (Coda *et al.*, 2012b).

While the studies referenced above have shown that plant proteins can be broken down by LAB proteolytic systems, this has largely been demonstrated in soy and the understanding of the mechanistic basis for proteolysis is limited. Given that proteolysis is one of the key flavour and texture-forming processes in fermentation, a better understanding of the process on a wider range of substrates would provide key insight into how to improve the fermentation of plant-based foods into more commercially viable plant-based dairy alternatives. β -Conglycinin, the soy protein most effectively hydrolysed by LAB (Aguirre *et al.*, 2008) is a 7S globulin protein. Other 7S globulin proteins such as vicilins in pea may therefore be promising candidates for successful proteolysis by LAB, as they have similar structure and solubility. Prolamins from cereals are also an interesting candidate for which the proteolysis by LAB has not been investigated in the context of plant-based dairy alternatives. Significant research into LAB proteolysis of gliadin proteins has been undertaken in sourdough research (Di Cagno *et al.*, 2002; El Mecherfi *et al.*, 2021; Reale *et al.*, 2021). However, this research has often focused on screening sourdough strains for gluten degradation and will often not investigate the underlying hydrolytic mechanism. Notably, the degradation of prolamins by sourdough bacteria/fungi significantly increases the solubility of prolamins, overcoming the insolubility barrier of prolamins and improving their potential functionality (Ogilvie *et al.*, 2021).

Understanding the specificity and accessibility of cell envelope proteinases towards plant proteins would be a valuable way to improve the structure of fermented products, while more research on the downstream metabolites, such as the volatile organic compounds produced, would help to improve the flavour of plant-based dairy alternatives. Utilising LAB isolated from existing fermented vegetable sources, such as olives, sauerkraut, kimchi and tempeh, may also be a useful strategy to better understand the action of LAB proteolytic systems towards vegetable sources, as these proteolytic systems may be better adapted to plant proteins. For example, LAB strains isolated from pickled vegetables (Sáez *et al.*, 2018) and tempeh (Lim *et al.*, 2019) have demonstrated extracellular proteolytic activity. Use of these strains, or strains isolated from similar sources, to ferment plant-based milk products may result in more effective proteolysis of plant proteins than the use of strains isolated from dairy sources as has commonly been used thus far.

Sugar metabolism

An important aspect of fermentation is the production of lactic acid from sugars. Lactic acid is produced from glucose, which in dairy milk comes from lactose. In the first step of lactic acid production, the sugar molecule is transported across the membrane and phosphorylated, then, in the case of disaccharides, hydrolysed into monosaccharide units. The glucose monomer is then broken down into lactic acid; in some LAB species the other monosaccharide (e.g., galactose from lactose) can also be metabolised (Stanley, 2003). The isomer of lactate produced varies between LAB species, depending on the levels of L-lactate and D-lactate dehydrogenase expressed by the LAB (Wang *et al.*, 2021).

LAB that produce primarily lactic acid are described as being homofermentative, while those that also produce ethanol, acetic acid and/or carbon dioxide are described as heterofermentative. Homofermentative LAB can theoretically produce two moles of lactate for every mole of glucose. Most starter cultures in the dairy industry are homofermentative LAB, as the other by-products from heterofermentative LAB can introduce undesirable flavours to the product. In homofermentative pathways, sugars are converted to pyruvate via the Embden–Meyerhoff–Parnas pathway, which is then converted to lactic acid by lactate dehydrogenase. Heterofermentative LAB produce only one lactic acid per glucose, as well as other by-products via the phosphoketolase pathway (Mayo *et al.*, 2010). This process is particularly important for the acidification of the product, as the production of lactic acid is responsible for the drop in pH seen during fermentation. This is important for the characteristic tart flavour associated with yoghurt and fermented drink products.

In a comparison of commercial plant-based yoghurts (soy, coconut, cashew, almond and hemp) and commercial dairy yoghurt, Grasso *et al.* (2020) found that none of the plant-based yoghurts had as high concentration of lactic acid as the dairy control did. The highest concentration was in soy yoghurt at 0.43/100 g, compared with 1.11/100 g in the dairy yoghurt. This suggests that the fermentation was more effective with the lactose substrate than the plant-based substrates in these products. The plant-based products mostly had acidity regulators added to decrease the pH to a similar level as was in the dairy control. The ability of different LAB species to convert sugars to lactic acid in plant substrates varies. Four strains of *Lactobacillus acidophilus* were able to grow with significant levels of lactic acid production in soy milk, and another four strains were able to grow after supplementation with additional sugar, while four strains of *Lactobacillus delbrueckii* subsp. *bulgaricus* were unable to effectively utilise sucrose and so

exhibited poor growth in soy milk, even when supplemented with additional sugar (Wang *et al.*, 1974).

Plant milks tend to contain high levels of α -galactosides such as raffinose and stachyose, which are indigestible in the gut and are therefore passed into the lower intestine where they are digested by bacteria which contain the α -galactosidase enzyme. This can result in gas which causes discomfort and flatulence, which can be a deterrent to consumption of plant-based products. Some LAB contain the α -galactosidase enzyme that is able to break these sugars down, so fermentation of plant-based products can lead to a reduction α -galactosides. A wide range of LAB species have been shown to have α -galactosidase activity and the ability to break down raffinose and stachyose in soy (Mital and Steinkraus, 1975; Donkor *et al.*, 2007; Hati *et al.*, 2014) as well as in pea and bean flour (Duszkiewicz-Reinhard *et al.*, 1994). Much of the sugar present in plant-based milks is in the form of raffinose and stachyose, so the presence of α -galactosidases in LAB allows them to grow more effectively in a plant-based medium. In the development of fermented plant-based dairy alternatives, it is important to select LAB which can utilise the sugars present in plant-based sources, and which express α -galactosidase, for both consumer acceptance of the product and viability of the LAB growth.

Exopolysaccharide formation

Exopolysaccharides are generated by some LAB during the fermentation process and have been identified as key components to developing a desirable flavour and texture in fermented dairy products (Korc and Varga, 2021). Exopolysaccharides may improve the texture of dairy products by interacting with other components of milk, such as proteins and fats, to form a large network throughout the substrate, acting as texturisers and thickeners to reduce syneresis and increase viscosity and stiffness (Mende *et al.*, 2016). Exopolysaccharides impart desirable qualities onto dairy products at much lower concentrations than commercial polysaccharides that are commonly added during production (Ruas-Madiedo *et al.*, 2002). They are responsible for firm body, creaminess and a shiny surface in fermented dairy products, due to water-binding and protein interaction (Korc and Varga, 2021).

The structure and properties of exopolysaccharides can vary widely. Exopolysaccharides can be homopolysaccharides, consisting of only one type of monosaccharide unit, or heteropolysaccharides, consisting of two or more different monosaccharide units. Homopolysaccharides are relatively simple to synthesise and require little energy expenditure. They are usually synthesised from

extracellular starch and sucrose, are generally large ($>10^6$ Da) and produced in large quantities (up to 10 g.L^{-1}). Heteropolysaccharides require a more complex and energy-intensive synthesis, which can include charged groups, where phosphate or sulphate groups are bound to the monosaccharide subunits, or glucuronic acid is part of the subunit (Mende *et al.*, 2016). They are synthesised from intracellular nucleotide precursors, are usually in the range of 10^4 – 10^6 Da and are produced in mg.L^{-1} quantities (Abarquero *et al.*, 2021). Exopolysaccharides can vary in the composition of the monosaccharide building blocks and in the degree of branching present. The majority of LAB in a dairy context synthesise uncharged heteropolysaccharides, usually consisting of some combination of glucose, galactose, N-acetylgalactosamine and rhamnose (Mende *et al.*, 2016).

Exopolysaccharides in plant-based milk

Exopolysaccharide-producing LAB strains have been used effectively in both low-fat yoghurt and low-fat cheese production, as the mouthfeel and creaminess imparted by the exopolysaccharides compensates for the loss of these characteristics due to the removal of fat (Güler-Akin *et al.*, 2009). Plant-based milks tend to be much lower in fat than dairy milk (Chalupa-Krebdak *et al.*, 2018), so the use of exopolysaccharide-producing LAB strains is important in producing plant-based products with a desirable texture profile.

Exopolysaccharide-producing LAB strains are able to produce exopolysaccharides in plant-based milks and in doing so increase the viscosity and decrease the syneresis of the product. *Lactiplantibacillus plantarum* 70810 and *Lacticaseibacillus rhamnosus* 6005 increased the exopolysaccharide content of fermented soy milk, resulting in an increase in viscosity compared with the control (Li *et al.*, 2014). Fermentation of lupin milk was able to produce a yoghurt-like product with an exopolysaccharide concentration comparable with dairy milk products. When the fermented lupin milk was ultra-high temperature heated, it had a higher exopolysaccharide concentration, increased viscosity and lower susceptibility to syneresis compared with a pasteurised product (Hickisch *et al.*, 2016a). Oat milk fermented with exopolysaccharide-producing LAB strains showed greater viscosity in the resulting product than when fermented with non-exopolysaccharide-producing strains. The greatest viscosity was seen from oat milk containing glucose, which is able to be utilised in exopolysaccharide synthesis (Mårtensson *et al.*, 2002).

Evidence suggests that many of the effects of exopolysaccharides on dairy products are caused by their interaction with proteins (Ayala-Hernández *et al.*, 2008, 2009). Exopolysaccharides increase the firmness of the

casein network by interacting with the proteins and micelles. As the pH of milk drops during fermentation, neutrally and negatively charged exopolysaccharides interfere with coagulation of casein proteins, which change from negative to neutral or positively charged by the end of fermentation. This leads to a protein network with greater water retention ability and, therefore, decreased syneresis and increased viscosity (Ayala-Hernández *et al.*, 2009; Gentès *et al.*, 2013; Zeidan *et al.*, 2017). Fermentation of broad bean protein concentrate with two species of exopolysaccharide-producing LAB increased the viscosity when enriched with sucrose, in a way that could not be mimicked by simply combining the protein concentrate with dextran and organic acids. A lack of proteolysis as determined by SDS-PAGE and the requirement for sucrose confirmed that the texture relies on the *in situ* production of exopolysaccharides, and the interaction of these with the protein network (Xu *et al.*, 2019). No other studies to our knowledge have investigated how the textures of fermented plant-based dairy products are affected by the interaction between exopolysaccharides and plant proteins. Understanding how these interactions work would allow manipulation of composition to improve the texture of fermented plant-based milk products.

Few studies have investigated the mechanism of exopolysaccharide production or their structure. While the carbohydrate content of dairy milk is made up almost exclusively of lactose, plant sources provide a much wider range of carbohydrate substrates that vary in their monosaccharide units, size, structure, digestibility, fermentability and solubility. They can exist as simple mono- or disaccharides (*e.g.*, fructose, galactose, glucose, sucrose or maltose), as oligosaccharides (*e.g.*, inulin or oligofructose) or as larger polysaccharides (*e.g.*, dietary fibre) (McClements and Grossmann, 2021). Understanding how LAB utilise this wide range of carbohydrates would help to understand which sugar sources are best able to create exopolysaccharides to give the best improvement in texture for plant-based products. The lack of research in this area is understandable, given that the knowledge of how exopolysaccharides contribute to texture in dairy products is also limited, due to the difficulties in separating, purifying and analysing these complex compounds (Mende *et al.*, 2016), but this is a clear area where gains could be made in improving the texture of plant-based dairy alternatives.

Applications

Fermentation of plant-based milks with LAB can be used to make products analogous to fermented dairy products, such as cheese, yoghurt and fermented drinks. These can be produced from a wide range of plant-

based milks. Table 4 illustrates a detailed breakdown of types of plant-based milks that have been used for fermented products that are either available commercially or have been investigated in the scientific literature. Plant-based yoghurts tend to be made from a defined plant-based milk or combination of milks and are almost always fermented. It is important that plant-based yoghurts are fermented, as yoghurt is seen as an important source of probiotics, so the presence of live cultures in plant-based yoghurt is a key aspect that makes them a 'dairy yoghurt-like' product. Plant-based cheeses, on the other hand, are not seen as an important source of probiotics, and cheese-like products are very often made without cultures. It is also more difficult to define them as being made with a specific plant-based milk, as the base ingredients are often plant oils and/or starches. This makes the plant-based cheese making process less analogous to the dairy equivalent than yoghurt. Table 4 highlights the difference in prevalence of fermentation in the production of plant-based cheese compared with yoghurt. There are many different fermented plant-based yoghurts, based on a wide range of different plant-based milks, both commercially sold and in the literature. In contrast, fermented cheese alternative products are scarce. Only a handful of fermented plant-based cheese products can be found commercially, mostly based on nut milks, and a few more have been developed experimentally. The number of plant-based cheese products that do not utilise fermentation is much greater, so there is clearly a large gap in current knowledge of how to successfully utilise fermentation of plant-based milk for development of dairy-free cheese alternatives.

Current trends are moving consumers towards 'clean-label' products. That is, products with fewer ingredients and ingredients that are perceived as 'more natural'. Fermentation with LAB provides an advantage in this respect, as it can impart desirable properties into fermented products without the need to add ingredients to the label (Perpetuini *et al.*, 2021). Breakdown of proteins into smaller peptides, free amino acids and volatile organic compounds can impart desirable flavours and potential health benefits into a product without the need for any additives, and exopolysaccharide formation by LAB can introduce thickening properties.

Yoghurt

There has been considerable development in plant-based yoghurt production in recent years [for review see Boeck *et al.* (2021); Montemurro *et al.* (2021)]. As yoghurts are important sources of probiotics, much of the evaluation of plant-based yoghurts has focused on the ability of LAB to grow to probiotic concentrations, and their viability in a plant-based media, as well as the

Table 4. Plant-based milk types which have been used for experimental and commercial fermented yoghurts and cheeses.

Plant	Commercial yoghurt brands	Experimental yoghurt references	Commercial cheese brands	Experimental cheese references
Coconut	Oui by Yoplait, Cocoyo, So Delicious	-	-	-
Barley	Coda <i>et al.</i> (2012a)	-	-	-
Emmer	-	Coda <i>et al.</i> (2012a)	-	-
Maize	-	-	-	-
Millet	-	Song <i>et al.</i> (2020)	-	-
Oat	Oatghurt, So delicious	Coda <i>et al.</i> (2012); Bernat <i>et al.</i> (2014); Brückner-Gühmann <i>et al.</i> (2019b)	Miyoko's (cheddar)	-
Rice	Ricera (discontinued)	Cáceres <i>et al.</i> (2019); Magala <i>et al.</i> (2015); Uzuner <i>et al.</i> (2016)	-	Nakamura <i>et al.</i> (2016)
Lupin	Luve	Jiménez-Martínez <i>et al.</i> (2003); Hickisch <i>et al.</i> (2016a); Laaksonen <i>et al.</i> (2021)	-	-
Pea	Ripple (in development)	Klost and Drusch (2019)	-	Sharma <i>et al.</i> (2018)
Peanut	-	Isanga and Zhang (2009); Santos <i>et al.</i> (2014)	-	-
Chickpea	-	-	Miyoko's (cheddar)	-
Soy	Alpro, Silk, Sojade	Cheng <i>et al.</i> (1990); Ferragut <i>et al.</i> (2009); Yang <i>et al.</i> (2012)	-	Li <i>et al.</i> (2013); Lorrungruang <i>et al.</i> (2014); Matias <i>et al.</i> (2014); Li <i>et al.</i> (2020)
Almond	Silk, Activia	Bernat <i>et al.</i> (2015)	Kite hill (spreadable cheese)	-
Cashew	Forager project	-	Savour (feta, ricotta), Vtopian (aged cheddar, brie, camembert), Treeline (aged cheddar), RIND (French-style cheese)	Chen <i>et al.</i> (2020)
Quinoa	-	Lorusso <i>et al.</i> (2018); Zannini <i>et al.</i> (2018)	-	-
Hemp	Sojade	-	-	-
Potato	-	Levy <i>et al.</i> (2021)	-	-
Tigernut	-	Ogundipe <i>et al.</i> (2021)	-	Mutiat <i>et al.</i> (2019)

development of desirable flavours and textures. Most probiotic LAB are isolated from dairy milk, so the differences in available nutrients, pH and presence of antinutritional factors in plant-based milk can all affect the viability of LAB species. Despite this, a wide range of plant-based milks have been able to be successfully utilised as probiotic carriers (Rasika *et al.*, 2021).

The most common strains of LAB for yoghurt production are a combination of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, which exhibit a mutualistic relationship. *Streptococcus thermophilus* grows rapidly, with fast acid and carbon dioxide production that stimulates growth, but most strains exhibit low proteolytic activity. *Lactobacillus delbrueckii* subsp. *bulgaricus* strains tend to have much higher proteolytic activity, so produce peptides and free amino acids that *S. thermophilus* can utilise as a nitrogen source (Tian *et al.*, 2018). This combination of LAB species has been utilised in the production of several experimental plant-based yoghurts using oat (Brückner-Gühmann *et al.*,

2019b), potato (Levy *et al.*, 2021), soy (Grygorczyk and Corredig, 2013), lupin (Jiménez-Martínez *et al.*, 2003; Laaksonen *et al.*, 2021), pea (Klost and Drusch, 2019) and tigernut (Ogundipe *et al.*, 2021) as a substrate, and in commercial yoghurts using soy, cashew, coconut and almond (Grasso *et al.*, 2020) as a substrate. Brückner-Gühmann *et al.* (2019b) reported a change in structure of oat protein concentrate, which they attributed to aggregation of proteins when fermented with *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, and some proteolysis, but no significant accumulation of free amino acids. These species were also used to produce an oat-based yoghurt product with desirable flavour and texture attributes (Brückner-Gühmann *et al.*, 2019a). Jiménez-Martínez *et al.* (2003) reported an acceptable sensory evaluation of lupin-based products fermented with this combination of LAB species, while sensory evaluation of lupin-based products by Laaksonen *et al.* (2021) showed that the product fermented with this combination of LAB species was the 'most liked' out of those tested.

The lower protein content and the difference in coagulation properties of the proteins means it can be difficult to obtain a similar consistency in plant-based yoghurt compared with dairy yoghurt without the addition of thickening agents, especially at low pH (Sim *et al.*, 2021), which affects the clean-label image of these products. The use of exopolysaccharide-producing LAB is one way that a similar texture can be achieved. However, in an analysis of 78 commercial plant-based yoghurt products, 80% used additional thickening agents (Boeck *et al.*, 2021). Exopolysaccharide-producing LAB can impart similar properties to plant-based yoghurt alternatives as added hydrocolloids, which improves the clean-label image of the product (Korc and Varga, 2021). Recent studies have investigated fermentation of quinoa milk with exopolysaccharide-producing species of LAB. Fermentation with an EPS-producing strain of *Weissella confusa* increased the viscosity of the product from 0.06 to 0.7 Pa/s and the water-holding capacity from 63% to 98%, compared with fermentation with non-EPS producing LAB strains which actually decreased both the viscosity and water-holding capacity (Lorusso *et al.*, 2018). Fermentation with *Weissella cibaria* resulted in a yoghurt product as viscous as dairy yoghurt (Zannini *et al.*, 2018). The former attributed the textural properties to the formation of an exopolysaccharide-protein network, whereas the latter observed extensive proteolysis of the protein matrix and instead attributed the increased viscosity to the production of high-molecular-weight exopolysaccharides.

The heat treatment method can affect the textural qualities of plant-based yoghurt products. A more intensive heat treatment (UHT compared with pasteurisation) of a lupin-based yoghurt reduced the time needed for LAB strains to ferment the product, improved the rheological properties and resulted in a greater concentration of exopolysaccharides (Hickisch *et al.*, 2016a). Further research confirmed that the improved textural properties were due to denaturing of the proteins, allowing improved aggregation and network formation (Hickisch *et al.*, 2016b). Almond milk treated with heat and pressure caused the proteins and oil droplets to aggregate into a continuous protein matrix, which promoted weak gel formation (Bernat *et al.*, 2015).

Particle size also affects the quality of yoghurt products. Reducing particle size can reduce the grittiness and improve mouthfeel. Particle size is particularly relevant for plant-based yoghurts, due to the tendency of plant proteins to aggregate, which has a negative impact on the syneresis and mouthfeel of the product. High-pressure processing, where food is treated with a high pressure for a set period of time, was used to create plant-based yoghurts from mung bean, chickpea, pea, lentil and broad bean and to produce gels with comparable viscosity and viscoelastic properties with commercial

dairy yoghurts, without fermentation (Sim *et al.*, 2020). High-pressure homogenisation produces a uniform particle size by forcing the substrate through a narrow orifice. This technique was used in broad bean samples to cause a change in secondary structure of proteins and dissociated all protein aggregates $>1 \mu\text{m}$, leading to an increase in solubility from 35 to 99% (Yang *et al.*, 2018).

Cheese

The range of plant-based cheeses reported in the literature, especially those produced through fermentation by LAB, is considerably smaller than that of yoghurt. This is likely due to the greater challenges of producing a product from plant sources that can imitate the texture and flavour of cheese compared with yoghurt, and the fact that technology development has not focused on this area until recently.

Most dairy cheese is manufactured through curdling of milk, often with the use of proteases such as chymosin from animal or microbial-derived rennet, or acidification and then subsequent fermentation of the curds by LAB (Visser, 1993). The structure of plant proteins is very different to that of caseins, and rennet does not induce curdling in plant-based milks, so a range of other curdling and coagulation methods, or alternative methods moving away from those used in dairy, must be employed (Jeewanthi and Paik, 2018). Plant-based cheeses can broadly be split into those that utilise fermentation and those that do not. Those that do not utilise fermentation are usually not based on plant milks, due to their low fat and protein content, but are instead based on oils (e.g., coconut or palm oil) and starches (e.g., potato or tapioca starch). These bases impart a cheese-like fat content and structure to the product and are able to somewhat mimic the texture and meltability of cheese. The oil ingredients help the product to mimic the meltability of cheese but not the stretch and flow, while the starches provide some amount of stretch to the product (Mattice and Marangoni, 2020). This type of plant-based cheese is the most readily available commercially, with 80% of plant-based cheeses in the UK being based on coconut or palm oil (Saraco and Blaxland, 2020). However, very little evaluation of these types of cheeses can be found in the literature. Fermented plant-based cheeses use LAB to ferment the plant base until a desired texture is achieved. Among fermented plant-based cheese products, many commercial products are cashew nut-based, while soy is the base of most literature-reported fermented cheeses, and many studies have been undertaken to attempt to improve the quality attributes of soy cheeses (Jeewanthi and Paik, 2018). It is possible that manufacturers avoid soy due to allergies and negative perceptions of soy

due to issues around genetic modification, presence of phytoestrogens and perception of unpleasant taste (Schyver and Smith, 2005).

The meltability and stretchability of cheese is caused by the weakening of non-covalent casein-casein interactions, which is particularly difficult to mimic with plant-based proteins (Mattice and Marangoni, 2020), and commercial cheeses that are currently available do not score well in sensory studies or have comparable meltability (Saraco and Blaxland, 2020). Advances in improving the meltability and stretchability of plant-based cheeses have been made using zein as the plant protein, which has a much higher proline content and a much less tightly packed structure than other plant proteins and forms a non-covalently linked network in aqueous solutions. A 30% zein content cheese-like product displayed comparable rheological, textural and melt-stretch properties with a dairy cheddar control. The sensory properties of this product have not yet been tested to determine whether this is a viable product, but early work looks promising (Mattice and Marangoni, 2020).

The literature on sensory evaluation has been very limited for different plant-based cheese products. In all studies that utilised fermentation, the likeability of the fermented product was better than that of the unfermented product, mostly attributed to the reduction of gritty mouthfeel and beany flavours. However, none had a likeability rating equal to or better than the dairy control, where this was tested (Short *et al.*, 2021). This clearly demonstrates that further research is needed to develop plant-based cheese products that can achieve similar or better likeability scores to dairy-based products. Until this is achieved, plant-based products will be limited in their potential market. Many different techniques have been employed to improve the acceptability rating of soy-based cheeses. Adejuyitan *et al.* (2014) reported a Nigerian cheese product based on soy and coconut milk and found that increasing the percentage of coconut milk improved the overall acceptability of the product. In a study by Li *et al.* (2013), the soy cheese product that combined fermentation with the addition of glucono- δ -lactone received the best score. Combined fermentation by LAB and *Geotrichum candidum* also increased positive texture attributes of a soy cheese product, although this was not compared with a dairy control or an LAB-only soy product (Li *et al.*, 2020). Chumchuere *et al.* (2000) developed a fermented soy cheese product of which the hedonic score was only acceptable once the product was fried. Research into development of a cream cheese-like product from soy found that fermentation produced diacetyl, to which the cream cheese flavour was attributed. Diacetyl concentration increased upon the addition of sodium acetate or sodium citrate to the fermentation (Hofmann and Marshall, 1985).

Despite the wide range of fermented cashew nut cheese products commercially available, there is a clear gap in the literature analysing these products. Chen *et al.* (2020) developed a cashew-based cheese and analysed the microbial and nutritional content but did not describe the sensory perception or textural qualities. Oyeyinka *et al.* (2019) measured the consumer acceptability of cheeses with different percentages of soy and cashew milk and found that increasing the percentage of cashew milk decreased the acceptability rating. However, this was not a fermented product. It would also be valuable to understand how the sensory rating of fermented commercially available products compares with non-fermented products, as they are very different products attempting to fill the same market niche.

There is limited research into cheeses produced with other plant milk bases. Nakamura *et al.* (2016) developed a cheese based on rice milk. However, none of the products achieved a very high overall acceptability rating (29–42%). The products that obtained the most similar consistency to dairy cheese all used gelatine as a coagulating agent that, as an animal-derived ingredient, is unlikely to be appropriate for many of the target market for this product. Sharma *et al.* (2018) developed a fermented peanut-based cheese spread but did not analyse the sensory or textural properties of this product.

Proteolysis and the eventual breakdown of proteins into free amino acids and volatile organic compounds is one of the most important processes for flavour production in cheese manufacture (Yvon and Rijnen, 2001; Smit *et al.*, 2005). Analysis of compounds produced during plant-based cheese production in the literature is limited. Shiro-tani *et al.* (2021) observed leucine, isoleucine and phenylalanine or tyrosine catabolised into 3-methylbutanal, 2-methylbutanal and benzaldehyde, respectively, which are known cheese flavour compounds, in fermentation of soy protein isolate with *L. helveticus* strains. Further study into flavour compounds released during the fermentation of plant-based milks would contribute to the understanding of how to improve the flavour of plant-based cheeses.

Unlike yoghurt, which necessarily contains two microbial species for fermentation (*S. thermophilus* and *L. delbrueckii*) and potentially a small number of additional species for enhanced flavour, texture or nutritional qualities, cheese can utilise a wide range of microbial biodiversity. This becomes particularly important in aged, artisanal and traditional cheese types, where a wide range of varying microbial species is integral to the development of unique and interesting flavours. For example, analysis of Livanjski cheese, a traditional cheese from Bosnia and Herzegovina identified a total of 159 viable isolates across 11 cheese samples (Vladimír *et al.*, 2020). Very little is known about the importance of microbial biodiversity in plant-based cheeses, especially

since many products currently available do not even utilise fermentation (Grossmann and McClements, 2021). An understanding of the role played by different starter cultures in plant-based cheese production will help to produce cheese alternatives with more complex and developed flavour profiles.

Although there have been numerous studies on the development of plant-based cheeses and there are a wide range of products available commercially, it is clear that there is still a lot of work to be done in developing products that will be viable alternatives for the majority of consumers. Understanding the molecular processes occurring during fermentation in plant-based food may hold the key to developing new and improved plant-based cheese products.

Conclusions and future focus

Although the systems that LAB use to ferment dairy products have been well studied, there has been very little research to date on how these processes work in plant-based foods. There is evidence that LAB do effectively ferment plant-based milks, as has been shown many times in the development of various plant-based cheeses and yoghurts, but this has for the most part been demonstrated only through the acidification, bacterial count and textural and sensory changes of the substrate. That is, the outcome of the fermentation has been well documented but what is occurring on a molecular level has not. The downstream metabolites of proteolysis are responsible for much of the flavour formation in fermented dairy products, especially cheese, and bioactive peptides produced can result in health benefits. Carbohydrate metabolism allows for the characteristic acidification of the product through production of lactic acid, and the formation of exopolysaccharides that through their interaction with the protein network helps to develop desirable textures. These mechanisms are complex and interconnected. How they work in dairy milk systems is very different to how they work in plant-based systems, due to the inherent differences in the structure of the substrates. Future work needs to focus on better understanding the underlying processes occurring during fermentation in plant-based foods and, in particular, how the proteins and sugars specific to different types of plant-based milk serve as substrates for the proteolytic and glycolytic systems within LAB. Better understanding of the interactions between networks and the breakdown products from different plant-based milks will help to develop improvements in the texture and flavour in dairy alternative products, through developing processes to help optimise desirable outcomes.

In addition, studying other plant-based milk types in addition to soy, where most studies to date have

focused, would be advantageous to determine which plant substrates undergo fermentation in a way that results in a product most acceptable to consumers. This review has focused on plant-based dairy alternatives, but better understanding fermentation in plant-based products more generally could help to develop a whole range of new plant-based foods, whether these are designed to mimic animal products or not. Sensory attributes are the most important factor when buying food, more so than price or perceived healthiness (Glanz *et al.*, 1998), so developing plant-based products with desirable sensory attributes is one of the biggest barriers to the acceptance of these products on the market. Achieving an acceptable sensory profile may be possible through either developing products that highlight natural flavours of the plants that consumers approve of or through developing products that most closely resemble the animal products with which consumers are more familiar. For either approach to be successful, improving our understanding of LAB fermentation processes on a molecular level will be one of the key factors for success. Overcoming the barriers for non-users to adopt plant-based dairy products is likely to be challenging, as resistance can come from a range of sources and is not necessarily based on the actual sensory properties of the product. Emotional, conceptual, situational, attitudinal and behavioural aspects can also play a role, as can neophobia (Jaeger and Giacalone, 2021). It will be important to understand all the factors that need to be overcome to encourage adoption of plant-based products into the mainstream, but having a product with desirable sensory qualities is undeniably an important first step.

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Conflict of interest

The authors declare no conflict of interest.

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