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Nutritional properties and health aspects of pulses and their use in plant-based yogurt alternatives

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Abstract

Plant-based yogurt alternatives are increasing in market value, while dairy yogurt sales are stagnating or even declining. The plant-based yogurt alternatives market is currently dominated by products based on coconut or soy. Coconut-based products especially are often low in protein and high in saturated fat, while soy products raise consumer concerns regarding genetically modified soybeans, and soy allergies are common. Pulses are ideally suited as a base for plant-based yogurt alternatives due to their high protein content and beneficial amino acid composition. This review provides an overview of pulse nutrients, pro-nutritional and anti-nutritional compounds, how their composition can be altered by fermentation, and the chemistry behind pulse protein coagulation by acid or salt denaturation. An extensive market review on plant-based yogurt alternatives provides an overview of the current worldwide market situation. It shows that pulses are ideal base ingredients for yogurt alternatives due to their high protein content, amino acid composition, and gelling behavior when fermented with lactic acid bacteria. Additionally, fermentation can be used to reduce anti-nutrients such as α-galactosides and vicine or trypsin inhibitors, further increasing the nutritional value of pulse-based yogurt alternatives.

KEYWORDS
Fermentation, lactic acid bacteria, legumes, market review, dairy alternatives

1 | INTRODUCTION

The reduction of the emission of greenhouse gases has been declared an imperative goal to stop global warming and the threat it poses to public health, food and water security, and biodiversity (Haines & Ebi, 2019; IPCC, 2018). Livestock production is responsible for 14.5% of man-made greenhouse gas emission, with dairy production being responsible for 20% of greenhouse gas produced by livestock. Minimizing dairy consumption and exchanging dairy with plant-based dairy alternatives could therefore provide a viable way to reduce greenhouse gas emissions (Gerber et al., 2013). These environmental concerns are also reflected in consumer behavior, and while only 8% of surveyed US-Americans report to follow a diet excluding all animal-derived foods, 27% think that eating more plant-derived protein contributes to an environmentally sustainable and healthy diet. While taste remains the main driver for food purchasing choices, 27% of Americans choose their food considering environmental sustainability. Other important drivers behind consumer choice toward plant-based dairy alternatives are cow milk protein allergies, and health concerns spread mainly by popular media (International Food Information Council, 2019).

While sales of dairy yogurt stagnate or even decreased, the market for plant-based yogurt alternatives is growing

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strongly, with sales having surpassed US$110 million in the United States in 2018. Their popularity is especially high among young consumer groups (i.e., millennials and iGen). Plant-based yogurt alternatives are being perceived as healthy, natural, and low in calories by consumers. However, the low protein content and the less appealing texture are aspects that increase interest in future product formulation. There is a large gap in the numbers of consumers minimizing their dairy intake and customers of plant-based dairy alternatives, therefore, plant-based yogurt alternatives have great economic potential (Mintel, 2018).

Pulse consumption is being promoted by the Food and Agriculture Organization (FAO) due to favorable nutritional composition, economic accessibility, and benefits for soil health maintenance (Calles et al., 2019), and plays a prominent role as a protein source in the EAT-Lancet Commission on healthy diets from sustainable food systems (Willett et al., 2019). As pulses are technologically similar to soybeans, they are ideally suited for the dry extraction and fermentation methods commonly used in soy-based yogurt-alternative production. Hence, an increased focus on pulse-based yogurt alternatives can result in additional dairy alternatives providing a larger range of products and increasing pulse consumption worldwide.

This review provides an overview of the nutritional benefits and drawbacks of pulse constituents, the impact of fermentation on pulse nutrients and anti-nutritive factors, and the behavior of pulse protein gelation in curd formation. To give a perspective on the current market situation of dairy-free yogurt alternatives, a market review and evaluation of plant-based yogurt alternatives was included.

2 | PULSES: CURRENT GLOBAL SITUATION AND AGRICULTURAL ASPECTS

The FAO defines pulses as dry grains harvested from legumes, and excludes legumes harvested in their green state, such as green beans or peas. Oil crops, like soybeans and peanuts, and legumes, like alfalfa and clover, which are grown for sowing and feed purposes only, are also excluded from the definition of pulses (FAO, 2019).

The eight most grown pulses worldwide in the year 2018 were dry beans (*Phaseolus spp.*), dry peas (*Pisum spp.*), chickpeas (*Cicer arietinum*), cowpeas (*Vigna unguiculata*), lentils (*Lens culinaris*), pigeon peas (*Cajanus cajan*), broad beans (also known as faba beans, *Vicia faba*), and lupins (*Lupinus spp.*) (FAOSTAT, 2020). Dry beans include common beans (*Phaseolus vulgaris*, e.g., Kidney, Pinto, and White beans), lima beans (*Phaseolus lunatus*), runner beans (*Phaseolus coccineus*), tepary beans (*Phaseolus acutifolius*), and year beans (*Phaseolus dumosus*). The vast majority of dry peas are yellow and green varieties of the garden or field pea (*Pisum sativum)*, while the other two pea species, *Pisum abyssinicum* and *Pisum fulvum*, are rarely cultivated for food purposes. Of the *Vigna* species, only cowpeas (*Vigna unguiculata*, with the Black-eyed pea being the most widely prevalent variety) and Bambara beans (*Vigna subterranea*) are counted separately by the FAO, while other *Vigna* species, like adzuki beans (*Vigna angularis*), mung beans (*Vigna radiata*), or black gram beans (*Vigna mungo*) are listed in the "pulses not elsewhere specified" category. The category of lupins includes the Andean lupin (*Lupinus mutabilis*), white lupin (*Lupinus albus*), narrow-leafed lupin (*Lupinus angustifolius*), and blue lupin (*Lupinus hirsutus*) (FAO, 2020; Sparvoli et al., 2015). An overview of the FAO categories of pulses, their genera, and common names are given in Table 1.

Pulses can be grown in a wide range of soils and climates, with the biggest producers being India, Canada, Myanmar, China, and the European Union (EU). Globally, pulses play only a minor agricultural role, with close to eight times more arable land being used for cereal than for pulse cultivation (FAOSTAT, 2020)

3 | NUTRITIONAL AND HEALTH ASPECTS OF PULSES

The following section provides an overview over the most important macro- and micronutrients as well as antinutritive substances in pulses (see Figure 1 for a summary of pulse constituents). In Section 4, the impact of fermentation on these pulse constituents is presented.

3.1 | Pulse proteins

One of the major criticisms of most plant-based dairy alternatives is their low protein content and the unfavorable amino acid composition (Sethi et al., 2016). While plant-based dairy alternatives from tree nuts or cereals often contain less than 1% protein, the protein content of soy-based dairy alternatives is closer to that of bovine milk products (Jeske et al., 2017). This is due to firstly, the naturally lower protein content of cereals and tree nuts compared to legumes, and secondly, the reduced amount of raw material because of their higher costs (Vanga & Raghavan, 2018). To mimic the mouthfeel and structure provided by native or acid-gelled protein in bovine products, bulking agents like maltodextrin, fibers such as inulin, and thickeners, such as gellan or locust bean gum, are frequently used (Chalupa-Krebzdak et al., 2018). The consumption of dairy alternatives as a substitute for bovine milk can pose the risk of protein deficiencies if consumers are not aware of the differences in protein content of these

TABLE 1 Pulses according to the definition of the FAO. As vetches are only commonly grown as animal feed only, they have not been included in this table

FAO category	Genera cultivated for food	Common name [names of popular varieties in brackets]
Beans, dry	*Phaseolus vulgaris*	Common bean [Kidney, White, Pinto, Navy, Great Northern, Cranberry]
	Phaseolus lunatus	Lima bean, Butter bean
	Phaseolus coccineus	Runner bean, Scarlet Runner bean
	Phaseolus acutifolius	Tepary bean
	Phaseolus dumosus	Year bean
	Vigna angularis	Adzuki bean
	Vigna radiata	Mung bean, Green gram bean
	Vigna mungo	Black gram bean
	Vigna aconitifolia	Mat bean, Moth bean
Peas, dry	*Pisum sativum*	Field pea
Chickpeas	*Cicer arietinum*	Chickpea [Kabuli, Desi, Garbanzo]
Cow peas, dry	*Vigna unguiculata*	Cow pea [Black-eyed pea]
Lentils	*Lens culinaris*	Lentil
Pigeon peas	*Cajanus cajan*	Pigeon pea, Red gram bean
Bambara bean	*Vigna subterranea*	Bambara groundnut, Earth pea
Lupins	*Lupinus mutabilis*	Andean lupin
	Lupinus albus	White lupin Blue/Narrow-leafed lupin
	Lupinus angustifolia	Yellow lupin
	Lupinus luteus	
Pulses not elsewhere specified (selection)	*Lablab purpureus*	Lablab bean, Hyacinth bean
	Canavalia gladiate	Sword bean
	Psophocarpus tetragonolobus	Winged bean
		Guar bean
	Cyamopsis tetragonoloba	Velvet bean
	Mucuna pruriens	Yam bean
	Pachyrhizus spp.	Horse gram bean
	Macrotyloma uniflorum	

products (Mäkinen et al., 2016). Without adequate compensation, severe malnutritional diseases, like kwashiorkor in young children, can occur (Carvalho et al., 2001).

Pulses are high in protein, with contents ranging from 20% to 36% depending on the species and variety, and are relatively inexpensive. Hence, by using pulses or pulse-derived protein concentrates, dairy alternatives with protein contents comparable to those of bovine milk can be achieved (Boye et al., 2010)

To evaluate the nutritional quality of protein and its ability to provide nitrogen and indispensable amino acids for the human metabolism, the protein digestibility-corrected amino acid score (PDCAAS) has been introduced by the FAO and World Health Organization (WHO) in 1989. In the PDCAAS, proteins are graded by the amino acid score (AAS), which is calculated by the first limiting essential amino acid divided by the corresponding amino acid of a reference protein, multiplied by the true fecal N-digestibility (TFND). The digestibility is established via rat fecal-balance method, in which nitrogen intake, fecal nitrogen, and metabolic nitrogen loss in rats fed a protein-free diet are measured. The reference protein is based on the amino acid pattern of a hypothetical protein meeting the safe protein requirements of pre-school age children (Nosworthy et al., 2018; Schaafsma, 2012).

$$AAS = \frac{\text{First limiting amino acid in test protein [mg/g]}}{\text{Corresponding amino acid of reference protein [mg/g]}}$$

$$\text{TFND [\%]} = 100 * \frac{(N \text{ intake} - (\text{faecal } N \text{ loss} - \text{metabolic } N \text{ loss}))}{N \text{ intake}}$$

$$\text{PDCAAS [\%]} = \text{AAS} \times \text{true fecal N digestibility [\%]}$$

Several points of concerns about the PDCAAS have been raised since its introduction. PDCAAS values over 100% are truncated to 100, so the benefits of two synergistic proteins with high contents of two different amino acids can

Pulses	Total [%]	Insoluble [%]	Soluble [%]
Pea	3-20[1]	10-20[1]	2-9[1]
Lentil	7-23[1]	11-19[1]	1-7[1]
Phaseolus spp.	14-30[1]	11-29[1]	1-6[1]
Lupin	14-55[1]	31-34[1]	4-5[1]
Faba Bean	34.9[2]	30.3[2]	4.6[2]
Chickpea	6–15[1]	10–18[1]	1–8[1]

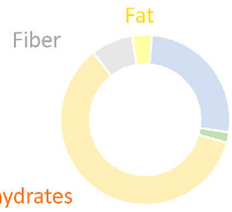
[1]Hall, Hillen, & Robinson, (2017),[2]Njoumi et al. (2019)

Pulses	Total [%]
Pea	2.8[1]
Lentil	2.6-2.8[1]
Phaseolus spp.	2.1-6.4[1]
Lupin	9-14[2]
Faba Bean	3.1-3.2[1]
Chickpea	6.1-6.8[1]

[1]Caprioli, Giusti, & Ballini (2016), [2]Boschin et al. (2008)

Pulses	Total [%]	Albumin [%]	Globulin [%]	Glutelin [%]	Prolamin [%]
Pea	14-31	15-25	49-70	11	5
Lentil	23-31	17	51	11	4
Phaseolus spp.	17-28	15-37	35-70	<1-22	<1-2
Lupin	32-44	9	44-60	6-23	NR
Faba Bean	36	2	74	18	4
Chickpea	19-27	8-12	53-60	18-24	3-7

Hall, Hillen, & Robinson (2017)



Pulses	Total [%]	Mono-, Di- & Oligosaccharides [%]	Starch [%]	Of which Amylose [%]
Pea	55-72[1]	5-12[1]	30-49[1]	23-49[1]
Lentil	42-72[1]	5-6[1]	37-59[1]	19-25[1]
Phaseolus spp.	56-76[1]	5-14[1]	18-43[1]	26-42[1]
Lupin	47[1]	10-16[1]	1-9[1]	0[1]
Faba Bean	44.1[2]	6.23[3]	34.57[4]	31.8[4]
Chickpea	52–71[1]	3–5 [1]	30–56[1]	23–28[1]

[1]Hall, Hillen, & Robinson (2017), [2]Khalil & Mansour (1995), [3]Njoumi et al. (2019), [4]Ambigaipalan et al. (2011)

Pulses	Trypsin inhibitor activity[U/mg]	Phytic acid [%]	Saponins A and B [mg/100g]
Pea	2.2[1]	0.22-1.22[3]	100-250[4]
Lentil	7.4[1]	0.27-1.51[3]	110-700[5]
Phaseolus spp.	3.2-18[1]	0.61-2.38[3]	940-1180[5]
Lupin	1.5-1.7[1]	0.23-0.38[3]	5.67-46.95[5]
Faba Bean	8.13[2]	0.51-1.77[3]	1330-1370[6]
Chickpea	8.1-15.7[1]	0.28-1.60[3]	651-860[5]

[1]Avilés-Gaxiola et al. (2018), [2]Khalil & Mansour (1995), [3]Schlemmer et al. (2009), [4]Heng et al. (2006), [5]Singh et al. (2017), [6]Sharma & Sehgal (1992) (total saponins)

FIGURE 1 Overview of macronutrients and three secondary plant metabolites of six typical pulses

be overlooked. Protein reference scoring patterns based on safe protein intake instead of estimates of average amino acid, and therefore amino acid requirements might be underestimated. The fecal-balance method might not be truly reflecting amino acid digestibility, as amino acids that have not been absorbed in the ileum are being metabolized by the microflora of the large intestine (Moughan, 2003; Schaafsma, 2005).

On account of these concerns, the FAO suggested to replace the PDCAAS with the digestible indispensable amino acid score (DIAAS), which is based on ileal amino acid digestibility determined at the terminal ileus in humans, growing pigs or rats, not truncated and based on the amino acid requirements of a 0.5–3 years old child (FAO Expert Consultation, 2013; Rutherfurd et al., 2015).

$$\text{IAA} = \frac{\text{Digestible dietary indespensable amino acid in test protein [mg/g]}}{\text{Corresponding amino acid in reference protein [mg/g]}}$$

$$\text{DIAAS [\%]} = 100 * \text{Lowest value of AAS}$$

Protein quality varies within different pulse species, but generally, pulse protein is relatively high in lysine, and the limiting amino acids are either tryptophan or the sulfur-containing amino acids methionine and cysteine. Lysine uptake through plant protein is very low in countries in which cereals play a major role in protein supply, as lysine is the limiting amino acid in almost all cereal proteins. With a combination of pulse and cereal protein in human nutrition, adequate supply with both lysine and sulfur-containing amino acids can be accomplished (Leinonen et al., 2019). PDCAAS values in raw pulses vary widely, ranging from 44 (red lentil) to 77 (chickpea). PDCAAS values of pulses are affected by processing, and pulse extrusion or baking results in higher values compared to no processing or cooking (see Table S1).

Salt-soluble globulins and water-soluble albumins make up the majority of pulse proteins. Enzymes and lectins comprise the albumin group, while the legumin- and vicilin-like storage proteins are globulins and make up the majority pulse protein. *Phaseolus spp*. protein contains 15%–37% albumins and 53%–70% globulins; cow pea, faba bean, and chickpea protein contain 2%–17% albumins and 58%–80% globulins; lentil, pea, and lupin protein contain 9%–25 % albumins and 44%–70% globulins (Boye et al., 2010; Hall et al., 2017). Pulse protein composition depends not only on species and cultivar, but also on the extraction conditions of the protein fractions (Liu et al., 2008). A standardized method for the fractionation of pulse protein would achieve better comparability between values.

Pulse legumins (also called 11S globulins) have a hexameric quaternary structure of disulfide-bonded basic (MM

~20 kDa) and acidic (MM ~40–50 kDa) subunits, called α- and β-subunits. Legumins are not usually glycosylated, with the exception of lupin legumin, which contains covalently linked carbohydrates of about 1% v/v (Duranti et al., 1995). The trimeric vicilin-like protein (7S globulin) also consists of acidic and basic α- and β-subunits and has a total molecular weight of approximately 175 kDa, though sizes of subunits of legumins and vicilins and their subunits vary between pulse species and cultivars. Vicilins are not typically bonded via disulfide bonds as they only contain low amounts of sulfur-containing amino acids and are usually glycosylated (Boye et al., 2010; Durante et al., 1989; Sparvoli et al., 2015). Nomenclature of pulse legumins and vicilins varies in some pulses, and legumins are called glycinin in *Glycine spp*. and α-conglutin in lupins, while vicilins are called phaseolin in *Phaseolus spp*., conglycinin in *Glycine spp*. and β-conglutin in lupins (Mills et al., 2004). A third group of storage proteins called convicilins was first discovered in pea and later identified in more *fabaceae spp*. Convicilins are mostly nonglycosylated proteins of a size of about 70 kDa. Some argue, however, that convicilin is simply a polypeptide subunit of vicilin (Boye et al., 2010; O'Kane et al., 2004). Legumins make up the majority of storage proteins in most legumes, with the exception of *P. vulgaris* and *P. lunatus*, where legumin is only a minor component and the storage protein consists mainly of phaseolin (Durante et al., 1989). Vicilins and convicilins are the major allergens in peas, lentils, and lupins, while in chickpeas the major allergen is a legumin. These proteins can cause IgE-mediated allergic reactions, and their IgE-immunoreactivity is not decreased by boiling. (Sanchez-Monge et al., 2004; Verma et al., 2013). Allergic cross-reactivity is common among pulses, as their protein structure is highly homologous. In vivo studies have shown cross-reactivity is especially common among lentils, chickpeas, and peas (Martínez San Ireneo et al., 2008).

3.2 | Pulse carbohydrates

Carbohydrates make up between 60% and 70% of pulse dry weight, except for lupins, which have the lowest carbohydrate content of pulses with just 47%. *Phaseolus spp*. typically have the highest total carbohydrate contents (Hall et al., 2017). Starch content varies widely in pulses, with lupins containing only 2%–3%, *Phaseolus spp*. and Adzuki beans containing 20%–40%, and mung beans, cow peas, lentils, peas, chickpeas containing between 30% and 60% starch. Amylose makes up between 12% and 50% and is low in adzuki bean, faba bean, and yam bean (Ambigaipalan et al., 2011; Hoover et al., 2010; C. Martínez-Villaluenga et al., 2006). Pulses contain a high amount of slowly digestible and resistant starch. A study on in vitro starch digestibility of pea, lentil, and chickpea flours showed contents of slowly digestible starch between 22% and 30% of flour weight, and contents of resistant starch between 3% and 6% in chickpea flour and 23% and 27% in pea and lentil flour. Only 8%–12% of lentil, pea, and chickpea flours are rapidly digestible starch (Chung, Liu, Hoover, et al., 2008). An analysis of flours of 3 *Phaseolus vulgaris* cultivars showed that the flours only consisted of 0.2% to 1% readily digestible starch, 3%–4% slowly digestible starch, and 32%–36% resistant starch (Chung, Liu, Peter Pauls, et al., 2008).

Total dietary fiber contents in pulses can vary between 4% and 5% in chickpeas and mung beans and 39% in lupins. The cultivar can have large effect on dietary fiber content, for example, in chickpeas, the kabuli type has a much lower fiber content (5%–15%) than desi chickpeas (10%–25%). Insoluble dietary fiber makes up 85%–93% of total pulse dietary fiber and consists mostly of cellulose, hemicellulose, and lignin. Soluble dietary fiber is mainly found in the cotyledon and entails pectins, oligosaccharides, and low molecular weight polysaccharides. Galacturonic acid levels are higher in pea and lentil than in chickpea (15.6%–18.4% vs. 19% to 12%), which comprises the backbone structure of pectic polysaccharides. In navy and pinto beans, arabinose and xylose contents are higher than those of galacturonic acids, indicating a higher content of hemicellulose than pectic polysaccharides (Brummer et al., 2015; Hall et al., 2017; Njoumi et al., 2019). Dehulling significantly decreases the total content of fiber in pulses, and especially removes insoluble dietary fiber, of which there is a high amount in seed coats. In dehulled lentils, reductions of 21%–41% and 40%–50% of insoluble and soluble dietary fiber, respectively, were reported (N. Wang et al., 2009), while pea dehulling reduced soluble and insoluble fiber by 13%−27% and 38%−46%, respectively (N. Wang et al., 2008).

Monosaccharides usually make up less than 1% of pulse dry weight, whereas oligosaccharides contents lie between 6% and 10%. Sucrose makes up 3%–4% of pulse dry weight, with only *Vigna spp*. and lupins showing lower contents of 1%–2%. The α-galactosides stachyose, raffinose, and verbascose are important pulse oligosaccharides. Stachyose contents range between 1% in faba beans and 6% and 7% in cow peas and lupins, respectively. Raffinose contents are highest in peas where it makes up 1% of the dry weight. Verbascose is common in peas, faba beans, mung beans, and lentils, which contain around 2% verbascose but low (<0.5%) in other pulses. Melibiose makes up no more than 0.15% of pulse dry weight (Fan et al., 2015; Ispiryan et al., 2020; C. Martínez-Villaluenga et al., 2006). While α-galactosides confer protection against frost and desiccation damage and improve storability of seeds, they cannot be metabolized by humans and are microbially fermented in the hindgut. In this fermentation, carbon

dioxide, hydrogen, and, in small amounts, methane and short-chain fatty acids are produced, which can cause flatulence and abdominal discomfort and should especially be avoided by subjects affected by irritable bowel syndrome. On the other hand, α-galactosides also pose as a prebiotic and can promote the growth of beneficial bifidobacteria and lactobacilli (Martínez-Villaluenga et al., 2008).

3.3 | Antinutritional factors in pulses

While pulses are a highly beneficial food source due to their high protein content and beneficial fatty acid composition (Boschin et al., 2008; Caprioli et al., 2016), they also contain various factors which can lower their nutritional value, such as lectins, enzyme inhibitors, tannins, oxalates, or phytates. Plant lectins, also called phytohemagglutinins, are a class of carbohydrate-binding proteins and glycoproteins present in a wide variety of plant food, with cereals, legumes, and tubers showing the highest lectin contents. Upon ingestion, lectins can bind to intestinal villi, thus inhibiting nutrient uptake, and weaken the tight junctions of intestinal mucosal cells, thereby causing enhanced permeability. Upon absorption into the bloodstream, lectins bind to carbohydrate-moieties on the surface of proteins, causing agglutination of cells, for example, red blood cells (Thompson, 2019). Lectins also show anti-microbial, anti-fungal, anti-viral, and anti-tumor activities (Lagarda-Diaz et al., 2017). The content of lectins varies widely between species and cultivars. In legumes, soybeans show exceptionally high levels of lectins, and beans contain moderate amounts, while peas, chickpeas, lentils, and faba bean are relatively low in hemagglutinating activity (Muramoto, 2017; L. Shi et al., 2018). Lectins are susceptible to thermal inactivation, and cooking of pulses was shown to reduce the hemagglutinating activity by 93% to 99% (L. Shi et al., 2018).

Pulses contain proteins which inhibit the activity of digestive enzymes α-amylase, trypsin, and chymotrypsin, thus affecting the digestibility and absorption of starch and protein by the formation of indigestible complexes (Avilés-Gaxiola et al., 2018). α-amylase inhibition is only present in *Phaseolus* and *Vigna spp*. but not detectable in peas, lentils, kidney beans, and faba beans (El-Hady & Habiba, 2003; Melo et al., 1999; L. Shi et al., 2017). When gut trypsin is inactivated by legume inhibitors, the pancreas is stimulated to produce more trypsin, chymotrypsin, and amylase. This causes a loss of sulfur-containing amino acids and can lead to pancreatic hypertrophy and have carcinogenic effects (Savelkoul et al., 1992). Pulse trypsin inhibitors are divided into two classes, the approximately 20 kDa Kunitz inhibitors, which show mainly trypsin inhibitory activity, and the 6–10 kDa Bowman-Birk inhibitors, which can inhibit both trypsin and chymotrypsin at separate binding sites. Pulses contain either both Kunitz and Bowman-Birk inhibitors, or only one of the two types (Gilani et al., 2005). An unusual Bowman–Birk inhibitor has been found in lupin, which only shows activity against trypsin but not chymotrypsin (Scarafoni et al., 2008). As proteinase inhibitors are synthesized as protectants against herbivores, they are common in a wide range of plant tissues (Ryan, 1989). Soy is the food with the highest trypsin inhibitor content (Gilani et al., 2012), and pulses generally contain much lower amounts. Soybean seeds contain 16.7–48.2 mg/g, while field beans and peas show trypsin inhibitor levels between 1.4–12.5 mg/g (Gilani et al., 2005). In a review by Avilés-Gaxiola et al. (2018), soy trypsin inhibitor activity is given as 94.1 U/mg, while pulses such as kidney beans, lentils, peas, cowpeas, and lupins have activities below 10 U/mg, and the highest pulse trypsin inhibitor activities are those of chickpea (8.1–15.7 U/mg) and tepary bean (11.5–18.0 U/mg). Like lectins, pulse protease inhibitors are usually not heat stable, and activity has been shown to be reduced by 78% to 100% by boiling for 1 hr (L. Shi et al., 2017). Heat stable trypsin inhibitors have been reported however (Rayas-Duarte et al., 1992), and research on whether the standard heating processes in pulse milk-alternative manufacture are sufficient to significantly lower trypsin inhibitors activity is still lacking.

Phytic acid, the dihydrogen phosphate ester of inositol, and its salts are the major storage molecules for phosphorus and cations in pulse seeds. The main sources of phytic acids in the human diets are pulses, oilseeds, nuts, and cereals. Pulse phytic acid content ranges from 0.27%−2.90% in pulses, 0.18%−3.35% in cereals, 1.0%−5.36% in oilseeds, and 0.15%–9.42% in nuts (Schlemmer et al., 2009). The antinutritional effect of phytic acid is based on its ability to form strong chelates with cations such as Fe^{2+}, Zn^{2+}, Ca^{2+}, and Mg^{2+} through its six highly reactive phosphate groups. It binds not only to cations in the seed, but also to chelates minerals in the gut from other food sources. Monogastric species lack phytases in their digestive tract and can therefore not sufficiently hydrolyze these chelates, so neither the phosphorus nor the minerals can be absorbed (Urbano et al., 2000). Phytates can also lower protein digestibility by binding to proteins, either directly or via a cation bridge. The direct binding occurs at a pH below the isoelectric point of proteins, such as in the stomach, where the basic amino acid residues of the protein form electrostatic bonds with phytic acid. These phytate-protein complexes are insoluble and resistant to pepsin proteolysis. At pH values higher than the isoelectric point, dominant in the small intestine, proteins are negatively charged and can bind to the cations of the phytates (Gilani et al., 2012). In vitro inhibition of phytic acid by

formation of ternary complexes has also been discussed and needs to be further investigated (Selle et al., 2000; M. Singh & Krikorian, 1982). Phytate content in a variety of Spanish legumes ranged from 0.4% in a common bean variety to 1.2% in a lentil variety, and it was shown that phytate content varies between species and cultivars (Muzquiz et al., 2012). Phytates are heat stable, and small losses of phytate in cooking processes can mainly be attributed to leaching into the cooking water (El-Adawy, 2002). Plant seeds also contain phytases, however, and soaking of seeds can reduce phytate contents through enzymatic degradation. While Lestienne et al. (2005) could show a 23% reduction in phytate levels in whole soybeans by soaking in water for 24 hr at 30°C, the same treatment did not have an effect on cow peas and mung beans. pH conditions and temperature are influential in phytate degradation. The optimum for bean phytase activity has been reported to be at pH 7 and 55°C, which differs from cereal phytases which have higher activity in acidic environments. When ground beans were soaked at these conditions for 17 hr in buffer to prevent a pH drop, a phytate reduction of 98% could be observed (Gustafsson & Sandberg, 1995).

Antinutritive compounds specific to faba beans are vicine and convicine, which cause favism upon ingestion in susceptible individuals. They are glycosidic aminopyrimidine derivatives that are hydrolyzed by β-glycosidase of the microflora in the intestinal tract at the β-glycosidic bond between glucose and the hydroxyl group at the C-5 on the pyrimidine ring, generating the aglycones divicine (2,6-diamino-4,5-dihydroxypyrimidine) and isouramil (6- amino-2,4,5-trihydroxypyrimidine) (Crépon et al., 2010). Upon intestinal uptake of divicine and isouramil into the bloodstream, they generate reactive oxygen species (ROS) that rapidly oxidize glutathione and nicotinamide adenine dinucleotide phosphate (NADPH). While normal red blood cells can counteract these ROS with NADPH-dependent production of catalase and glutathione peroxidase, NADPH levels in glucose-6-phosphate dehydrogenase (G6PD)-deficient red blood cells are too low to facilitate this process and suffer oxidative damage. The red blood cells are then subjected to phagocytosis, causing acute hemolytic anemia (Luzzatto & Arese, 2018). G6PD deficiency is the most common enzyme defect in humans, affecting 4.9% of the global population, with prevalence as high as 8%–20% in tropical regions, as G6PD deficiency also confers resistance against malaria (Khazaei et al., 2019). Vicine and convicine are relatively thermostable but can be removed entirely from seeds by continuous flow soaking in water for 72 hr (Jamalian & Ghorbani, 2005). Boiling and roasting also decrease vicine and convicine contents, with boiling being the more effective method. Cardador-Martínez et al. (2012) found vicine and convicine levels in boiled, de-hulled faba bean seeds lowered by 18.9% and 22.5%, respectively, while roasting resulted in reductions of 6.06% and 22.53%, respectively. Vicine and convicine levels cannot be lowered by air classification, as it has been found that this method vastly increases the concentration in the protein-rich fraction (Khazaei et al., 2019). Khalil and Mansour (1995) found that boiling, autoclaving, and germination of soaked faba beans equally reduced vicine and convicine content by about 34%. Luzzatto and Arese (2018) report that a meal of low vicine faba beans did not induce favism in G6PD-deficient human subjects; however, further research is needed to determine which levels of vicine are safe for consumption by at-risk individuals.

3.4 | Pulse phenolic compounds and saponins

Pulses contain phenolic compounds such as phenolic acids, polyphenols, lignans, and flavonoids and are of great interest due to their many health benefits, such as antimicrobial, antioxidant, and phytoestrogenic properties. In plants, phenolic compounds play a role in protection against environmental stresses, ultraviolet (UV) light and pathogens, and their synthesis is upregulated with stress (Treutter, 2005). The most abundant classes of flavonoids in pulses are flavanols, flavanones, flavones, isoflavones, and anthocyanidins. In the plant, flavonoids play a crucial role in host-symbiont signaling to nitrogen-fixing rhizobia, as well as during germination processes. Isoflavones also function as phytoalexins (Aoki et al., 2000). Isoflavones are the most common flavonoids in pulses and exhibit the strongest estrogenic activities, while other pulse flavonoids, such as kaempferol and quercetin, show no or low estrogenic or even antiestrogenic activity (Boué et al., 2011; Collins-Burow et al., 2000). Common phytoestrogens in pulses are genistein, daidzein, and coumestrol, the latter being present at lower concentrations, but having an estrogenic activity 30–100 times higher than other isoflavones (Sukanya & Gayathri, 2014; Verdeal & Ryan, 1979). Studies have shown that a higher dietary intake of phytoestrogens correlates with lower rates of breast and uterine cancer as well as cardiovascular diseases in women (Keinan-Boker et al., 2004; Park et al., 2005).

Another important group of physiologically active compounds found in pulses are saponins. Saponins consist of a hydrophobic aglycone glycosidically linked to chains of two to five hydrophilic saccharides, resulting in amphiphilic compounds with good emulsifying and foaming properties. D-glucose, D-galactose, L-arabinose, D-xylose, L- rhamnose, and D-glucuronic acid are the most common constituents of the oligosaccharide chain.

The aglycone in legume saponins commonly is a 30-carbon triterpenoid and is linked to either one or two oligosaccharide chains. Depending on the structure of the triterpenoid moiety, these saponins are categorized into A, B, and E saponins and are then sub-categorized depending on their oligosaccharide moieties, which can also be acetylated. Group A saponins have sapogenol A as the aglycone moiety and are linked to two oligosaccharide chains at C-3 and C-22. Group B saponins differ from group A in that sapogenol B is only linked to one glycosyl chain at C-3 and has a hydrogen moiety instead of a hydroxyl group at C-21. Soyasaponins I, II, III, and IV belong to the B group of saponins. Group DDMP saponins are group B saponins with a 2,3-dihydro-2,5- dihydroxy-6-methyl-4H-pyran-4-one (DDMP) group at C-22 of sapogenol B and entail soyasaponin αa, αg, βa, βg, γa, and γg. Group E saponins differ from group B in that their aglycone, sapogenol E, has a carbonyl instead of a hydroxyl group at C-22. Soyasaponins Bd, Be, Bf, and Bg belong to group E (B. Singh et al., 2017). Legumes are one of the major dietary sources for saponins in the human diet, but saponins are also common in other plant foods such as leaf vegetables, pseudocereals, and solanaceous vegetables such as potatoes, tomatoes, and eggplant (Price et al., 1987). Saponin content varies among pulse species and cultivars but is usually lower than that of soybean. Reported saponin contents in legumes vary widely. In soy flour, contents between 0.35% and 4.3% have been reported (Curl et al., 1985; Fenwick & Oakenfull, 1983; Gurfinkel & Rao, 2002; Ridout et al., 1988), while in chickpeas, saponin levels in literature range between 0.21% and 5.6% (Fenwick & Oakenfull, 1983; Jood et al., 1986; Ridout et al., 1988). In dry peas, relatively low levels between 0.07% and 0.19% have been found (Daveby et al., 1997; Heng et al., 2006). Legume protein isolates, obtained by isoelectric precipitation, contain saponin levels that can be higher than those of whole flour, while the carbohydrate fractions have no low levels of saponins (Fenwick & Oakenfull, 1983; Ireland et al., 1986). Saponins are relatively heat stable, but dehulling, soaking and, cooking can reduce pulse saponins levels, possibly due to leaching effects of the saponins. Pressure cooking of soaked seeds has been found to be the most efficient method, reducing pulse saponins by 20%–39% (Duhan et al., 2001; Jood et al., 1986; Sharma & Sehgal, 1992). Germination also reduces pulse saponins levels between 10% and 66%, depending on species and germination time (Duhan et al., 2001; Kataria et al., 1989; Khokhar & Chauhan, 1986). Saponins were considered antinutritive factors in the 1950s and 1960s, as they were found to cause growth impairment and ruminant bloat in monogastric animals, show hemolytic activity when injected, and can form complexes with minerals such as iron in the digestive tract, lowering these mineral's bioavailability (Milgate & Roberts, 1995). However, since numerous positive health effects of dietary saponins have been discovered, they are now considered important pro-nutritive factors in pulses. As saponins are a chemically diverse group of compounds, not all deleterious or nondeleterious effects can be attributed to all saponins (B. Singh et al., 2017). Pulse saponins have shown hypocholesterolemic activity by forming insoluble complexes with cholesterol, thus inhibiting cholesterol uptake in the small intestine. Saponins can also lower blood cholesterol levels indirectly, as they bind to bile acids and increase fecal bile acid excretion, increasing de novo synthesis of bile acids from cholesterol in the liver (Sidhu & Oakenfull, 1986). Cholesterol lowering activity of pulse saponins has been shown in rats, rabbits, and humans (J. Shi et al., 2004). The binding of saponins to cholesterol also prevents cholesterol oxidation into atherogenic and cancerogenic oxystyrols (J. Shi et al., 2004; Valenzuela et al., 2003). Saponin use in higher concentration is limited, however, as they impart a bitter taste and astringency (Heng et al., 2006).

4 | IMPACT OF FERMENTATION ON PULSE NUTRIENTS AND ANTINUTRIENTS

While pulse-based yogurt alternatives are novel products, fermented pulse products have long been included in many cuisines around the world. Traditional fermented pulse dishes include *dhokla*, an Indian fermented pancake from rice and chickpea or bean, *siljo*, an Indian gruel made from fermented faba beans and safflower, *ugba*, a Nigerian condiment made from fermented locus beans or other pulses, and *idli*, a steamed Indian breakfast food made from fermented black gram beans and rice. All these dishes are fermented with the natural pulse microbiota. Southeast Asian *tempeh*, a protein-rich staple source of protein, is commonly made from fermenting cooked whole soybeans with *Rhizopus* ssp., but chickpeas and local bean varieties are also being used. Natto, a Japanese breakfast food made from cooked whole beans inoculated with *Bacillus subitilis* var *natto*, is most commonly made from soybeans, however, varieties using chickpeas or black beans exist (Frias et al., 2017).

Fermentation of pulses can cause a wide range of changes in the composition of legumes: degradation of antinutritional macro- and micro-constituents by microbial metabolism or activity of endogenous enzymes, formation of substances inhibiting the growth of spoilage-related microbiota, biological fortification with microbial metabolites, changes in flavor, and a probiotic effect (Marco et al., 2017).

4.1 | Impact of fermentation on protein digestibility and protein quality

As mentioned in Section 3.3, pulse protein utilization depends not only on the amino acid composition, but also influenced by enzymes inhibiting digestive enzymes. Fermentation can affect levels of trypsin and chymotrypsin inhibitors via hydrolytic processes, and alter amino acid composition by amino acid synthesis (Filannino et al., 2018). *Lactobacillus plantarum* fermentation of pea protein lowered trypsin inhibitor activity from 2.3 to 1.1 TIU/ mg after 9 hr fermentation time, but activity increased on further fermentation, and trypsin inhibitor activity was not significantly different from to after 11 hr of fermentation. Chymotrypsin inhibitor activity decreased steadily over the fermentation and was reduced from 3.7 to 1.1 CIU/mg after 11 hr. Fermentation unfavorably altered amino acid composition by lowering the amount of the limiting amino acids, cysteine + methionine. IVPDCAAS (in vitro protein digestibility-corrected amino acid score) was lowered from 67.0% to approximately 54.6% after 11 hr of fermentation (Çabuk et al., 2018). In a study on chickpea and faba bean flours fermented with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, trypsin-inhibitor activity did not significantly change by fermentation. In vitro protein digestibility was not significantly altered by fermentation in kabuli and faba bean samples. However, fermentation of desi chickpea flour increased the in vitro digestibility by 9.5% (Chandra-Hioe et al., 2016). In grass pea flours fermented in liquid state with *L. plantarum*, the activity of trypsin inhibitor decreased by 40% after 24 hr fermentation (Starzyńska-Janiszewska & Stodolak, 2011). Furthermore, solid-state fermentation of cooked cow peas using either *Rhizopus oligosporus* or *L. plantarum* for 36 hr reduced trypsin inhibitor activity by 100% and 96.9%, respectively (Ibrahim et al., 2002). Neither liquid nor solid-state fermentation of whole cooked black beans with *L. plantarum* or *R. oligosporus* significantly altered amino acid composition. However, in vitro protein bioavailability was increased strongly in beans fermented with either strain. The strongest increase was found in solid state mixed fermentation with both *L. plantarum* and *R. oligosporus*, with bioavailability rising from 26% to 41%. This is especially surprising as only the solid-state fermentation with *L. plantarum* resulted in a slight reduction in trypsin inhibitor activity, and trypsin inhibitor activity even more than doubled in the mixed solid-state fermentation samples (Starzyńska-Janiszewska et al., 2014). Coda et al. (2015) fermented doughs made from faba bean flours and the protein-rich fraction of faba bean flour with *L. plantarum* and found trypsin inhibitor activity increasing by 56% and 86.5%, while IVPD (in vitro enzyme protein digestion) increased by 2% and 1.6%, respectively. A 7-day solid-state fermentation of lupin flour with *Aspergillus sojae*, *Aspergillus ficuum*, and a combination of both strains resulted in a lowered IVPD by 23%, 16%, and 32%, respectively, compared to unfermented lupin flour. The authors hypothesized that this is due to proteins being enclosed in the fiber matrix, which reduces the activity of proteolytic enzymes (Olukomaiya et al., 2020).

4.2 | Impact of fermentation on α-galactosides

The lowering of α-galactoside levels via fermentation to reduce the induction of abdominal discomfort upon pulse ingestion has also been of wide interest to research. Shimelis and Rakshit (2008) found that a natural fermentation of common bean flour slurry for 96 hr eliminated stachyose and raffinose almost entirely. A controlled fermentation with *Lactobacillus acidophilus* LA-5, *Bifidobacterium* BB-12, and *Streptococcus thermophilus*, however, did not result in a significant reduction of α-galactoside levels. Note that 24 hr fermentation of various pulse flour sourdoughs with *L. plantarum* C48 and *Lactobacillus brevis* AM7 reduced α-galactoside concentrations by 7%–64%, depending on pulse species (Curiel et al., 2015). Tempeh made from common beans by fermentation with *R. oligosporus* var. *chinensis* decreased raffinose, stachyose, and verbascose to 7%, 16%, and 27% of their initial concentrations, respectively. Co-fermentation with *L. plantarum* DSM 20174 caused an even larger decrease in α-galactoside levels. Interestingly, a pre-fermentation of the soaked beans with *L. plantarum* prior to fermentation with *R. oligosporus* did not cause a stronger decrease in α-galactoside levels compared to tempeh fermented with *Rhizopus* only (Starzyńska-Janiszewska et al., 2014). Another study, however, found fermentation with *L. plantarum* DSM 20205 completely eliminated raffinose and stachyose from cooked cowpeas after 24 h, and the same effect was observed by fermentation with *R. oligosporus* (Ibrahim et al., 2002).

4.3 | Impact of fermentation on phenolic compounds and antioxidant capacity

As pointed out in Section 3.4, high levels of phenolic compounds are desirable due to their various health effects. Fermentation can positively influence polyphenol levels in pulses. Total polyphenols in cowpea flour fermented in submerged state more than doubled after fermentation with both the microorganisms native to cow pea seeds and *L. plantarum*. A following heating step to

121°C further increased polyphenol content. Rats fed these fermented flours showed significantly increased plasma ABTS antioxidant activity, demonstrating the bioactivity of the polyphenols (Kapravelou et al., 2015). Gan et al. (2016) performed submerged-state fermentation of various pulse flours with either the naturally occurring microbiota, *L. paracasei* ASCC 279 or *L. plantarum* WCSF1, and studied changes in antioxidant capacity and total phenolic compounds in the bound and soluble fractions. The effect of fermentation by the lactic acid bacteria (LAB) on the antioxidant capacity varied strongly between pulses, with strong increases in mottled cowpea and speckled kidney bean and lowered antioxidant capacity in black and yellow soybean. Natural fermentation increased antioxidant capacity in mottled cowpea, speckled kidney bean and small rice bean samples, decreased in small runner bean and yellow soybean, and remained relatively unaffected in black cow gram, lentil, and black soybean. These differences are due to the varying species of microorganisms that make up the individual microflora of the pulses and also depend on the polyphenol composition of the individual pulses. Both the LAB mediated and the natural fermentations increased total phenolic content in all pulse varieties (Gan et al., 2016). In a study with mung bean and soybean milk-alternatives fermented with *L. plantarum* WCFS1, it was found that total phenolic content increased with fermentation in both the lipophilic and the hydrophilic fraction of the samples. The effect on the antioxidant capacity varied between methods. The reducing capacity was increased by fermentation in both milk alternatives, while the results of scavenging activity varied between methods of testing. It is important to mention that both the total phenolic content and the antioxidant capacity were significantly higher in the lipophilic than the hydrophilic fraction, and it is therefore imperative to assess both fractions to avoid underestimation of these factors (Gan, Shah, et al., 2017).

4.4 | Impact of fermentation on vicine and convicine

Fermentation of lupin bean flour doughs for 48 hr with *L. plantarum* VTT E-133328 (also known as DPPMAB24W) lowered vicine and convicine concentration by 90% and 95%, respectively, while natural fermentation decreased vicine and convicine levels to a much lower extent. In vitro blood hemolysis tests showed that extracts from controlled fermented doughs had less than half the hemolytic activity than extracts from spontaneously fermented doughs (Coda et al., 2015; Rizzello et al., 2016). To be able to degrade vicine and convicine in fermentation, the microorganism must possess β-glucosidase activity to hydrolyze the aglycones vicine and convicine into divicine and isouramil. While these are the toxic forms, they are also very unstable due to oxidative degradation. Divicine and isouramil in faba bean extracts treated with β-glucosidase were almost completely degraded after 60 min at pH 5 and 37°C (Pulkkinen et al., 2016). Di Cagno et al. (2010) screened over 100 strains of LAB derived from food matrices for β-glucosidase activity. Note that 25% of the strains were found to have elevated β-glucosidase activity, and the strains with the highest activity were *L. plantarum* DPPMA24W and DPPMASL33, *L. fermentum* DPPMA114, and *L. rhamnosus* DPPMAAZ1.

4.5 | Impact of fermentation on isoflavones

Fermentation with strains possessing β-glucosidase activity also has an impact on isoflavone activity, as they hydrolyze conjugated isoflavones, such as daidzin and genistin, to their bioactive, unconjugated aglycone forms, daidzein and genistein (Di Cagno et al., 2010; Rekha & Vijayalakshmi, 2011). Contrary to vicine and convicine, the aglycone forms of isoflavones seem to be more stable than their conjugated forms (Otieno et al., 2007). While this topic has been well studied for soybean, research on pulse fermentation and isoflavones remains rare. Bartkiene et al. (2018) studied the effect of solid-state and submerged fermentation of wholemeal flours and protein isolates from different blue lupin hybrid lines using various *Pediococcus pentosaceus* strains on isoflavone content. From all tested isoflavones (daidzein, genistein, formononetin, and biochanin-A), only genistein was detectable, and levels in the protein isolate samples were generally lower than in the wholemeal flour samples. There was no clear trend in the effect of fermentation on genistein levels, however. *Pediococcus* strains lowered genistein levels in some hybrid lines but increased it in others. In a yogurt alternative based on a mixture of cow's milk and chickpea extract, fermentation with *L. bulgaricus* and *S. thermophilus* for 4 hr resulted in daidzein and genistein levels more than five times higher than prior to fermentation (Fu & Zhang, 2013).

4.6 | Formation of bioactive peptides by fermentation

Protein hydrolysis of food proteins by microbial enzymes has been shown to cause a formation of peptides with various bioactive properties, such as antioxidant, immunomodulating or antithrombotic activity (Chai et al., 2020). The most widely studied bioactive peptides (BAP) in fermented foods, however, are angiotensin-converting

enzyme (ACE) inhibitory peptides (Martinez-Villaluenga et al., 2015). The peptidase ACE is a central factor of the blood-pressure regulating renin-angiotensin system. It shows hypertensive activity by converting the hormone angiotensin I to the vasoconstrictory angiotensin II, as well as by its ability to deactivate the vasodilatory bradykinin (Schmieder et al., 2007). Dairy products fermented with LAB are a well-known source of BAP (Möller et al., 2008). Several studies have been performed that show that LAB fermentation of pulse extract can also lead to significant BAP formation, making them promising sources of food-derived BAP (Gan, Li, et al., 2017). The liquid-state fermentation of navy beans with *L. plantarum* and *L. bulgaricus* led to the degradation of mainly α and β type phaseolins and an ACE inhibition of around 70%, while the unfermented navy bean extract showed no ACE inhibitory activity (Rui et al., 2015). While both lentil and kidney bean extracts already show considerable ACE inhibitory activity before fermentation due to high levels of soluble phenolic compounds, the inhibitory activity was significantly increased from 68% to around 90% by fermentation with either the natural microbial flora or *L. plantarum* as a starter culture (Limón et al., 2015; Torino et al., 2013).

5 | PULSE-BASED YOGURT ALTERNATIVES

Plant-based yogurt alternatives, for example, based on soy and coconut, have been commercially available for years, while pulse-based yogurt alternatives only recently arrived on the market and remain relatively rare. Like pulse-based milk alternatives, yogurt alternatives can be manufactured from water extracts of whole pulses (S. Wang et al., 2018) or from a blend of pre-extracted pulse proteins, fat, water, and a sugar source for fermentation (Hickisch et al., 2016).

Traditional dairy yogurt derives its structure from acid-formed casein gels. Cow's milk protein consists of approximately 80% micellar casein and 20% whey proteins. κ-casein, a subunit of the casein proteins with a highly hydrophilic C-terminal, protrudes from the surface of the casein micelles like "brushes," and prohibit aggregation by steric stabilization. Upon acidification, the net charge of the protein decreases, the κ-casein "brushes" collapse, and electrostatic repulsion between casein micelles is lowered, while hydrophilic and electrostatic attraction between micelles increases. Thus, a three-dimensional network of casein chains and clusters is formed (de Kruif & Zhulina, 1996; Lee & Lucey, 2010).

While the acid gelation of casein has been extensively studied, research on the gelation of pulse protein received scientific attention relatively recent. It has been shown that pre-heating of protein in an aqueous solution at a pH far from the isoelectric point leads to unfolding and pre-aggregation of proteins into linear and percolated networks in pea and soybean. This pre-aggregation prior to acidification results in shorter gelation time and gels with a higher storage modulus (G') (Chen et al., 2016; F. Li et al., 2012). The degree of aggregation is dependent on heating time, temperature, and protein concentration. In pea protein isolate studies, heating steps of 30 min at 80°C and 1 hr at 60°C at a protein concentration of 10 g/L were sufficient to produce gels in a subsequent acidification via LAB fermentation (Klost et al., 2020; Klost & Drusch, 2019). In a study with lupin protein-based yogurt alternatives, ultra-high temperature treatment for 60 s at 140°C of the 2% lupin protein-isolate solution prior to fermentation resulted in much firmer yogurts with higher viscosity and lower syneresis than mere pasteurization at 80°C for 60 s (Hickisch et al., 2016).

To obtain yogurt from whole pulses with good sensory attributes, as in pulse milk production, lipoxygenase activity must be inhibited via heating steps early in the process. In addition, Rao et al. (1988) reported that roasting and subsequent dehulling of cow peas and mung beans prior to soaking resulted in a yogurt with far lower levels of beany flavors. For a yogurt alternative made from *Lupinus campestris*, the wild form of lupin that is usually too bitter for human consumption, soaking the seeds in boiling 0.5% $NaHCO_3$ solution prior to dehulling and grinding resulted in a product with acceptable sensorial properties, higher protein content, low beany flavors, and a total elimination of quinolizidinic alkaloids (Jiménez-Martínez et al., 2003).

For fermentation of pulse-based yogurt alternatives, the traditional combination of the LAB *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* has successfully been used (Aminigo et al., 2009; Jiménez-Martínez et al., 2003; Rao et al., 1988). As legume proteins generally produce softer gels than milk protein, the application of exopolysaccharide (EPS) producing LAB in yogurt alternatives has been tested. LAB EPS can be divided into homopolysaccharides (HoPS) and heteropolysaccharides (HePS). HoPS are polymers consisting of either glucose or fructose and are typically larger than 10^3 kDa. HePS typically contain between two and eight different monosaccharides, the most common of which are glucose, galactose, and rhamnose and can contain noncarbohydrate moieties. HePs are between 40 and 9×10^3 kDa in size and are usually synthesized in much lower quantities than HoPS (Lynch et al., 2018). In dairy yogurt model systems, fermentation with HePS producing LAB resulted in higher G' values and viscosity. It has been found that the flexibility of the HePS backbone as well as the type of protein (casein and whey protein) greatly influences the effect EPS have on dairy yogurt texture. HePS formation is very low in yogurt fermentation, but by using a culture mix

of HePS producing *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, HePS concentration can be increased (De Vuyst et al., 2003; Gentès et al., 2011, 2013). In soy yogurt alternatives, fermentation with HePS producing strains of *L. plantarum* and *L. rhamnosus* resulted in improved gel structure and a decrease in undesirable volatile compounds (C. Li et al., 2014). In a study by Hickisch et al. (2016), yogurt alternatives made from lupin protein isolate, coconut oil and glucose using HePS producing strains of *L. plantarum*, *P. pentosacaeus*, and *L. brevis*, it was shown that EPS production only played a minor role in determining yogurt viscosity, which was mainly determined by the heating temperature of the protein solution prior to fermentation. To the authors' knowledge, no studies have been performed on the effect of HoPS on pulse-based yogurt alternatives. In a study by Zannini et al. (2018), a quinoa-based yogurt alternative fermented with HoPS producing *Weissella cibaria* MG1 showed great potential for the application of HoPS producing LAB in plant-based yogurt alternatives.

6 | PULSE-BASED CURDS

An alternative to forming pulse protein gels by fermentation is the formation of protein curds via salt coagulation. Coagulating heat-denatured soy proteins with magnesium or calcium salts and subsequent pressing into firm blocks to produce tofu is a well-established technique. Due to their similar proteins, pulses can be used for curd production if the parameters are adjusted; however, pulse curds are not widely commercially available. A typical pulse curd production process is given in Cai et al. (2001), where six legume flours were water-extracted, the soluble fraction with various protein concentrations were boiled for 10 min, and either $CaSO_4$ or $MgSO_4$ solution was added at 85°C as the coagulant. After 20 min coagulation time at 80°C, the curds were pressed and analyzed. The pea, mung bean, and lentil curds showed much higher moisture content than faba, chickpea, and the soy control curds. All pulse curds had a lower springiness ratio than soy curds, and only the chickpea sample achieved hardness values comparable to soy curd, while pea and lentil resulted in the softest curds. The concentration of soluble protein and the dose of coagulants in chickpea curds was also studied. It was shown that curd hardness was highest in a 1% protein solution and decreased with increasing protein concentration. A coagulant dose of 0.5% resulted in very soft curds for both $CaSO_4$ and $MgSO_4$, but concentrations above 1% for $MgSO_4$ and 1.5 % for $CaSO_4$ did not further increase hardness or decrease moisture content in chickpea curd. Mohamed et al. (1989) report that high concentrations of $CaSO_4$ cause a bitter taste of the curds; however, they were able to produce chickpea, mung bean, and cowpea curds with satisfactory textural and sensory attributes at a $CaSO_4$ concentration of 0.3%.

In a study by DePalma et al. (2019) on yellow pea curd coagulated with $MgCl_2$, the influence of pasteurization and disruption processes on curds with and without the addition of corn oil were investigated and compared to commercial soy curds. Nonfat pea curds were significantly harder than curds with oil addition. Disruption of the curd into 1 mm pieces after the first curd pressing and re-pressing, as well as pasteurization of the curds in a 98°C water bath for 5 min, did not affect hardness of both no-fat and fat-added pea curds; however, the combination of disruption and subsequent pasteurization resulted in significantly harder pea curds with less water absorption.

7 | MARKET REVIEW ON PLANT-BASED YOGURT ALTERNATIVES

An online review of 78 plant-based, fermented yogurt alternatives currently on the market in 16 different countries was conducted by searching web presences of producers and retailers of plant-based dairy-alternatives (see Figure S2 for countries of origin of the reviewed yogurt alternatives). Only plain plant-based yogurt alternatives were included in the review. Just three of those are exclusively pulse-based, but six additional products include pulse proteins to enhance protein content. Of the reviewed yogurt alternatives, the main base ingredient of 32 products is coconut, 16 are soy based, 12 almond based, eight oat based, five cashew based, two lupin based, and one each is pea, flaxseed, and hemp based. Yogurt alternatives using two types of plant-milk alternatives were grouped according to their main base ingredient (Figure 2). Five whole milk dairy-based yogurts were included in the review for comparison.

7.1 | Macronutrients

The average nutritional composition of all types of yogurt alternatives was evaluated and compared to dairy yogurt. A total overview of nutrients per 100 g of product can be seen in Figure 3. The reviewed dairy yogurts contain total fat amounts between 3.5% and 5.3%, of which between 2.2% and 2.9% are saturated fats. Coconut yogurt alternatives have far higher total and saturated fat contents, averaging at 9.8% of total fat and 8.5% saturated fat. The surprisingly high saturated fat content in the lupin-based yogurt alternatives of 5.6% is due to the use of coconut fat as fat component. Almond- and cashew-based yogurt alternatives also have higher average fat contents than dairy-based whole

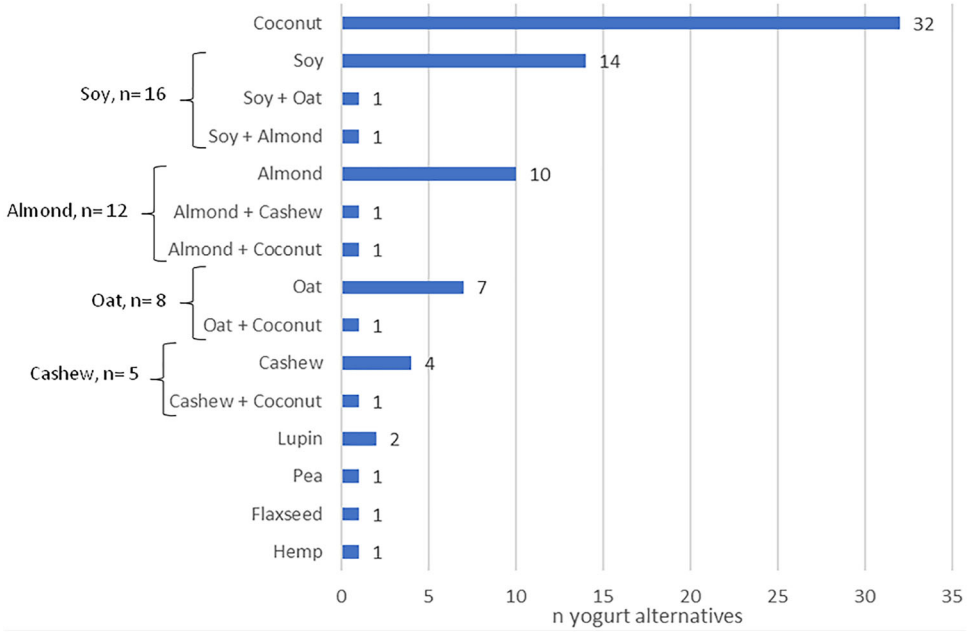


FIGURE 2 Base ingredients of the reviewed plant-based yogurt alternatives. Yogurt alternatives using two types of base ingredients are grouped according to their main base ingredient into the groups coconut, soy, almond, oat, cashew, lupin, pea, flaxseed, and hemp

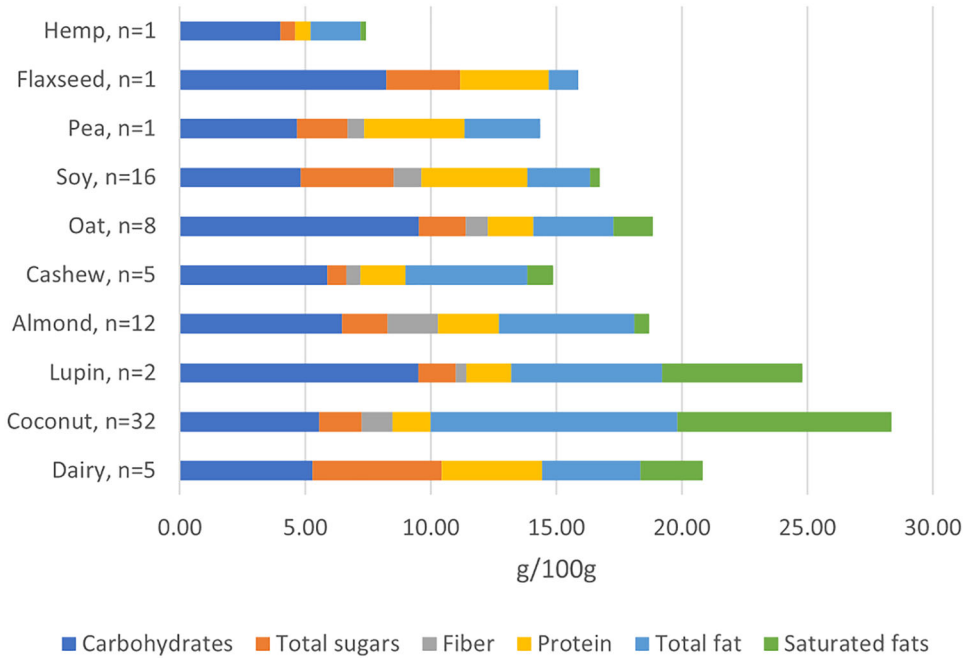


FIGURE 3 Average nutritional composition of different plant-based yogurt alternatives, and a whole milk yogurt for comparison. Error bars have been omitted for clarity. See supplementary Table S3 for values and standard deviations

milk yogurts (5.4 and 4.9%, respectively), while the other types of yogurt alternatives have fat contents between 1.2% and 3.2%.

Energy content, as well as the content of calories from protein per 100 kcal of the product, are given in Figure 4. Coconut yogurt and lupin yogurt have the highest energy content with 114 kcal and 101 kcal per 100 g, respectively. With only 6.2 and 7.3 kcal per 100 kcal provided by protein respectively, they are a relatively poor source of protein. To be permitted to use the EU nutrition claim "source of protein," at least 12% of the calories of a product have to be provided by protein (European

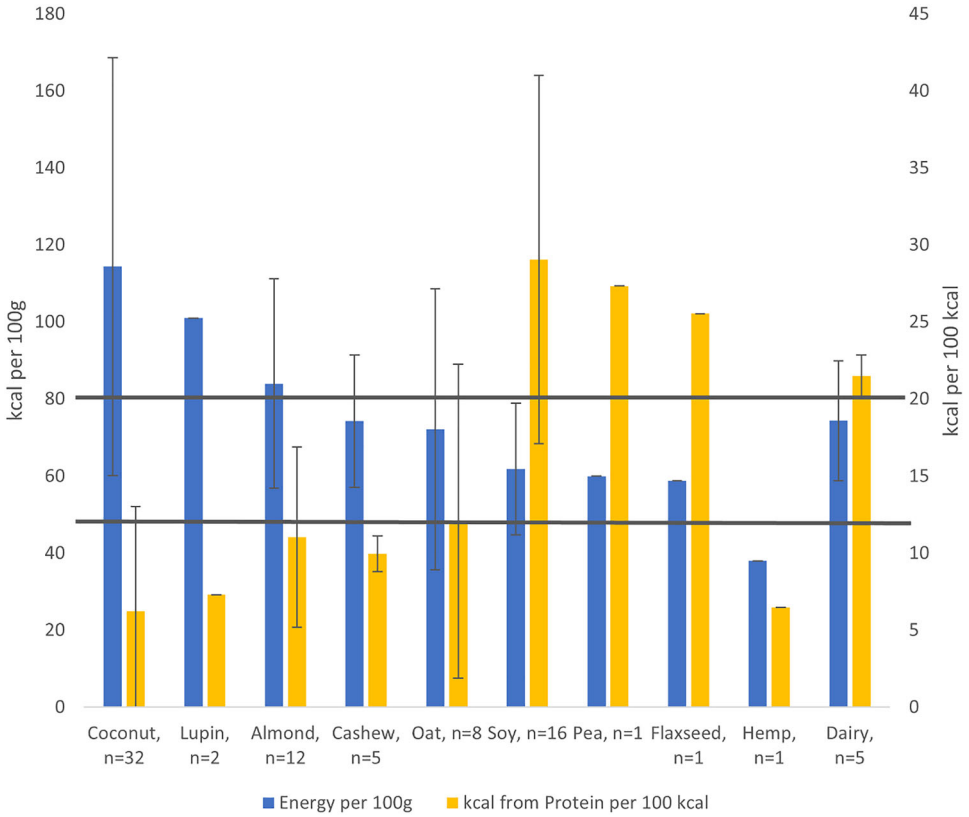


FIGURE 4 Energy content in kcal per 100 g, and kcal from protein per 100 kcal of reviewed plant-based yogurt alternatives. The gray horizontal lines signify the minimum requirements of kcal from protein per 100 kcal for the EU nutrition claim of "source of protein" and "high protein"

Parliament, Council of the European Union, 2006). Only three of the 32 reviewed coconut-based, one of 12 almond-based, and two of the eight oat-based products contain enough protein to be able to be labeled as a source of protein.

On the other hand, all soy-based as well as the pea- and the flaxseed-based products can be labeled as sources of protein. If a product derives at least 20% of calories from protein, it can be labeled "high protein" (European Parliament, Council of the European Union, 2006). One almond-based, one coconut-based, one oat-based, the pea- and the flaxseed-based, and 12 soy-based products meet the nutrition requirements for this claim. The reviewed dairy yogurts have protein contents ranging between 20 and 23.6 kcal from protein per 100 kcal, therefore all could be labeled "high protein."

7.2 | Ingredients

According to Codex Alimentarius, dairy yogurt typically only consists of milk and milk-derived products, incubated with the LAB *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, but alternative cultures (such as *Streptococcus thermophilus* or any *Lactobacillus* species) may also be used for fermentation. The Codex does not permit the addition of sweeteners or additives such as stabilizers or starch to plain yogurts, however, national legislation often digresses from this standard (FAO/WHO, 2018). For plant-based yogurt alternatives, no specific Codex Alimentarius standards exist, and the use of additives to alter texture, taste or nutrient content is common. Only six of the 78 reviewed yogurt alternatives contain no additives and consist only of the extract of their main ingredient and LAB.

Of all reviewed yogurt alternatives, eight products contain protein isolates to increase protein content. One product each contains almond or potato protein, the residual six contain either faba bean protein, pea protein, or a mixture of the two.

Approximately 80% of the reviewed products contain hydrocolloids as stabilizers. The most commonly used hydrocolloid is pectin, contained in 29 of the 78 products, either on its own or in combination with other stabilizers such as locust bean gum or agar-agar. Locust bean gum is added in 22 products. Other common stabilizers in plant-based yogurt alternatives are carrageenan, xanthan, gellan gum, and acacia gum (Figure 5). Note that 73% of the

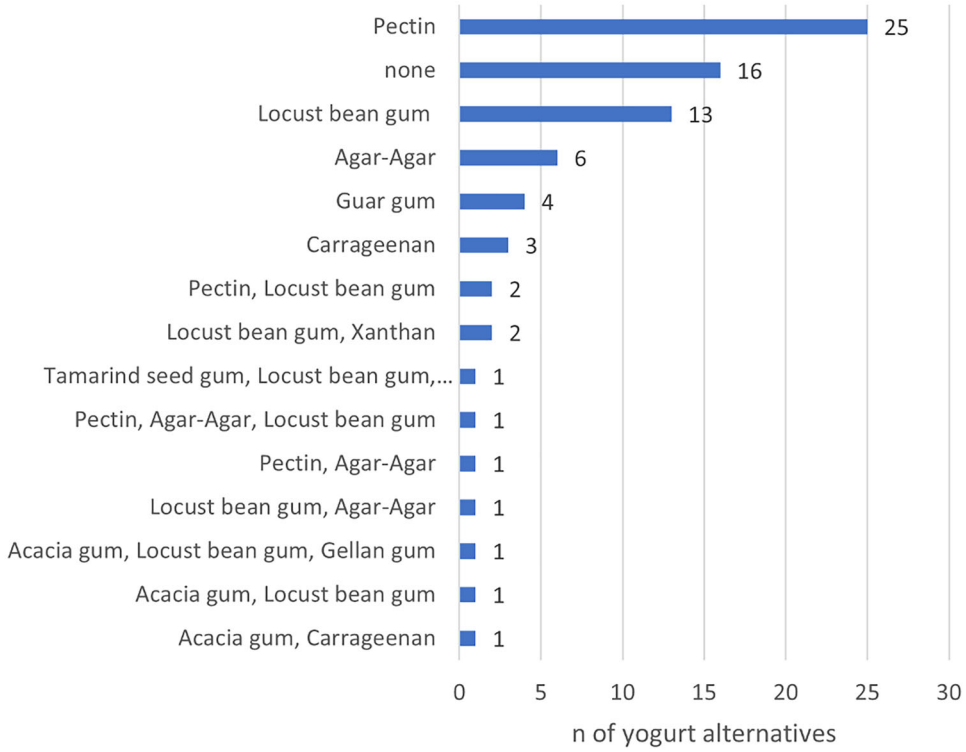


FIGURE 5 Stabilizers used in the reviewed plant-based yogurt alternatives

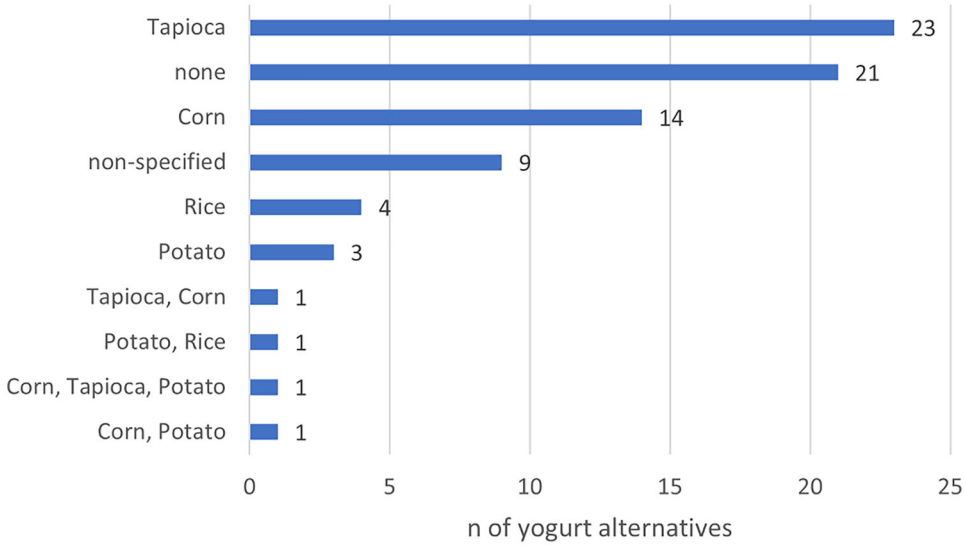


FIGURE 6 Starches contained in the reviewed plant-based yogurt alternatives

reviewed products also contain starches, tapioca and corn starch being the most frequently used (Figure 6). Only nine of the reviewed 78 products, all of which are either coconut- or soy-based, contain neither starches nor hydrocolloids. Emulsifiers and antioxidants are rarely used in yogurt alternatives. Only the flaxseed-based product and one coconut-based product contain an emulsifier (lecithin and citric acid esters of mono- and diglycerides of fatty acids, respectively), and two soy-based products contain antioxidants (tocopherol-rich extracts and fatty acid esters of ascorbic acid).

The majority of products rely solely on fermentation to obtain sufficient product acidity. Note that 26% of products use additional acidulants, most frequently citric acid, used in 14 of the products, but lactic acid, malic acid, glucono-δ-lactone, and lemon juice concentrate have also been used

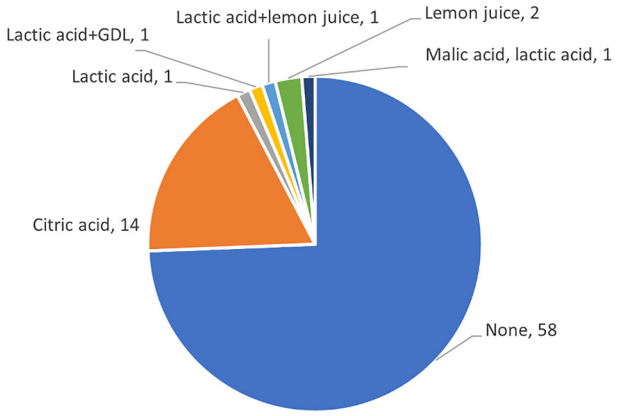


FIGURE 7 Acidulants used in the reviewed plant-based yogurt alternatives

(Figure 7). Some products also contain acidity regulators like sodium or calcium citrate, or calcium or potassium phosphate.

Thirty of the 78 reviewed yogurt alternatives are enriched with calcium, predominantly in the form of calcium phosphate and calcium citrate. Twelve of the calcium-enriched yogurt alternatives are additionally supplemented with vitamins to increase the nutritional value. Eleven of these products contain vitamin D, mostly in the form of vitamin D_2 (ergocalciferol), though the form of vitamin D is not specified in all products. As ergocalciferol is plant-derived, its use in dairy-free yogurt alternatives is plausible. However, the bioavailability of ergocalciferol is lower than that of the animal-derived cholecalciferol (vitamin D_3) (Houghton & Vieth, 2006). Ten products contain vitamin B12, and two products each are additionally enriched with vitamin A and B_2.

Eight of the reviewed yogurt alternatives used added oil from other plant sources than their main base. Six of these products are oat- or lupin-based yogurt alternatives containing coconut oil to enhance fat content. The pea protein-based yogurt alternative contains sunflower oil.

Only plain yogurt alternatives were being reviewed, nevertheless, 21 of the products contain flavoring, 17 of which natural flavoring. The pea-based yogurt alternative also contains yeast extract in addition to the natural flavoring which potentially was added as a natural flavor enhancer.

Fifty-three of the reviewed yogurt alternatives contain no added sweeteners. Only one, Icelandic product contains artificial sweeteners (acesulfam K and sucralose), the residual 24 products contain either sucrose, dextrose, fructose, glucose-fructose syrup, or concentrated fruit juices.

Eleven of the reviewed products contain added fiber ingredients, inulin being the most frequently used. Cellulose, citrus fiber, and oat fiber are used in one product each.

The most frequently used strains for fermentation are *S. thermophilus, L. bulgaricus*, and *L. acidophilus*, contained in 28, 27, and 27 of reviewed products, respectively. *Bifidobacteria spp*. are contained in 26 of the reviewed products, 12 of which contain *B. lactis*, two *B. bifidum*, and the residual 14 *Bifidobacteria* of nonspecified species. Other strains used are *L. paracasei*, *L. rhamnosus, L. casei L. plantarum*, and *B. bifidum*. Note that 46% of the reviewed products do not state which species of bacteria are used for fermentation (Figure 8). Most yogurt alternatives use a combination of two or more bacteria species, the combination of *S. thermophilus* and *L. bulgaricus* being the most common.

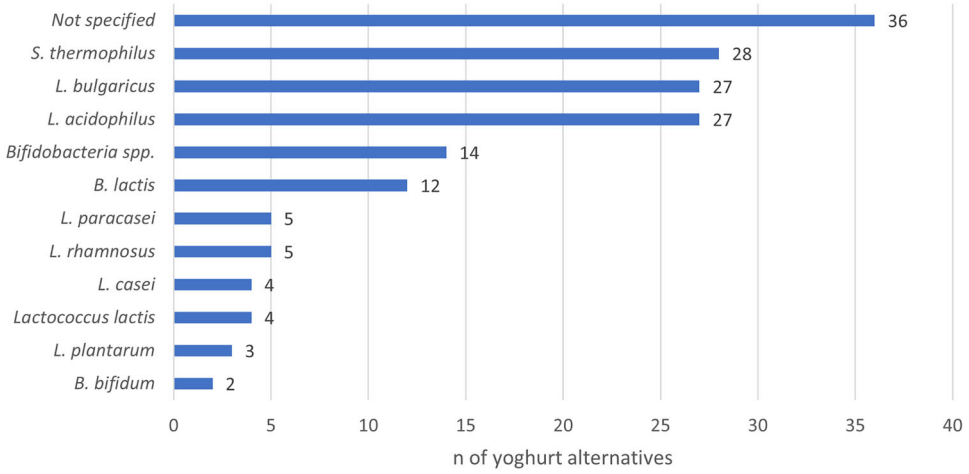


FIGURE 8 Species of bacteria used for the fermentation of the reviewed yogurt alternatives. Most products use a combination of two or more species for fermentation

8 | CONCLUSION

While not yet widely used in plant-based yogurt alternatives, pulses possess many traits that make them an excellent alternative to the currently predominant soy-, coconut-, and almond-based yogurt alternatives on the market. Pulses are high in protein, and while low in sulfur-containing amino acids, due to their high lysine content, they are complementary to a diet rich in cereals and therefore lacking in this amino acid. Pulses are also rich in phenolic acids, polyphenols, saponins, and flavonoids, which can be beneficial due to their antioxidant, anti-inflammatory, and anti-cancerogenic properties. On the other hand, pulses also contain anti-nutritional factors such as lectins, trypsin inhibitors, and phytates, which can result in decreased intestinal nutrient uptake. Vicine and convicine, glycosides present in faba bean, can cause hemolytic anemia in susceptible individuals. Some antinutritional factors, such as trypsin inhibitors, vicine, convicine, and α-galactosides, can be reduced or even almost eliminated by fermentation, while the level of beneficial compounds like isoflavones and antioxidants can be increased. The effect of fermentation is strongly dependent on strains, substrate, and fermentation conditions, however. More research is needed to be able to influence pulse constituents in a controlled way by fermentation. In experimental set-ups as well as in commercially available plant-based yogurt alternatives, pulses have proven to be well-suited substrates for lactic acid fermentation with traditional yogurt cultures. Current plant-based yogurts alternatives use a plethora of thickeners and stabilizers to attain satisfactory textures. More research into acid- and salt-induced gelling behavior of pulse proteins could result in "clean label" yogurt alternatives with promising market prospects.

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AUTHOR CONTRIBUTIONS

Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Validation; Visualization; Writing-original draft; Writing-review & editing: Theresa Boeck. Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-original draft; Writing-review & editing: Emanuele Zannini.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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