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COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

The modification of volatile and nonvolatile compounds in lupines and faba beans by substrate modulation and lactic acid fermentation to facilitate their use for legume-based beverages—A review

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Abstract

Lupines and faba beans are promising ingredients for the beverage industry. They contain high amounts of protein and can be grown in different climate zones and agricultural areas. Therefore, these legumes appear as ideal raw material for vegan, functional, and sustainable beverages. Nevertheless, the sensory characteristic of legumes is generally not accepted in beverages. Therefore, the market contribution of legume-based beverages is currently only marginal. This review highlights known major flavor aspects of lupines and faba beans and the possibilities to improve these by germination, heat treatment, enzymatic treatment, and subsequent lactic acid fermentation. First, the main aroma and taste compounds are described. Thereby, the "beany" aroma is identified as the most relevant off-flavor. Second, the nutrients and antinutrients of these legumes regarding to their use as food and as substrate for lactic acid fermentation are reviewed, and possibilities to modulate the substrate are summarized. Finally, the modification of the sensory profile by lactic acid fermentation is outlined. To conclude, it seems likely that the nutritional and flavor attributes in legume-based beverages can be improved by a combined process of substrate modulation and fermentation. In a first step, antinutrients should be decomposed and proteins solubilized while transforming the solid grains into a liquid substrate. Due to such substrate modulation, a broader variety of strains could be employed and the fermentation could be based exclusively on their impact on the flavor. By applying the concept of combining a substrate modulation with a subsequent fermentation, the use of legumes in beverages could be facilitated and new products like vegan, protein-rich, refreshing beverages could be marketed.

KEYWORDS
beany aroma, faba bean, hexanal, lactic acid bacteria, lupine

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1 | INTRODUCTION

The use of lupines (*Lupinus sp.*) and faba beans (*Vicia faba*) in the nutritional sector is a re-emerging market. Lupines are part of the traditional diets in South America and the Mediterranean region (Kinder & Knecht, 2011; Wolko et al., 2011) and faba beans are one of the oldest cultivated crops and very popular in the Middle East region (L'Hocine et al., 2020). Nevertheless, as the protein demand in Europe is mainly satisfied with animal proteins, both legumes show only a marginal presence in the alimentary sector in Europe and were mainly perceived as animal feed, catch crop, or substitution products (e.g., lupine as coffee substitute) until recently. Further obstacles are the antinutrients in both plants. Wild forms of lupines contain high amounts of alkaloids and those of faba beans contain tannins, vicine, and covicine. In order to prevent intoxication and their bitter impact on the food products, these compounds needed to be removed prior to consumption (e.g., by soaking in running water over many hours). Important breakthroughs were the introduction of sweet lupines low in alkaloids (Kinder & Knecht, 2011; Wolko et al., 2011) and of the low-tannin cultivars of faba beans (L'Hocine et al., 2020). With those new cultivars, the time-consuming traditional pretreatment to extract toxic alkaloids or bitter tannins was made redundant. A further breakthrough was the breeding of faba beans containing low or no vicine and covicine, as those β-glucosides cause illness or even life-threatening hemolytic anemia to persons with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Luzzatto & Arese, 2018). With the ongoing trend in the nutritional sector toward a healthier and environmentally conscious diet, lupines and faba beans are of interest as functional foods (Sweetingham & Kingwell, 2008; Wolko et al., 2011) and as an attractive alternative to soy (Arnoldi, 2011; Sweetingham & Kingwell, 2008). This is because they are not only vegan, rich in protein, gluten-free, and low in purine (Arnoldi, 2011), but can also be cultivated on the central European soil (Arnoldi, 2011; Sweetingham & Kingwell, 2008). In the European Union, a special focus is on the promotion of protein crops. The EU aims at replacing the imports of soy by domestic pulses like faba beans and promote the substitution of animal protein by plant proteins. In the European Union, the production of protein crops tripled from 2013 to 2018. Nevertheless, the self-sufficiency rate of soy in the EU accounts for only 5%, which resulted in imports of approximately 30 million tons of soy in 2018 (European Commission. Directorate General for Agricultural & Rural Development, 2018). This not only shows a strong dependency on soy imports but also an increasing production of pulses in Europe. General reasons for reducing imports are the reduction of long and energy-consuming transports, the exclusion of genetically modified soy products, and the promotion of legumes as catch crops in the crop rotation to increase biodiversification and to reduce the need for fertilizers. In the food sector, two trends boost the use of domestically grown pulses. First, an increasing number of people following a vegan, vegetarian, or flexitarian diet demands for high-quality plant proteins. Second, consumers tend to be interested in regional product, and are willing to pay higher prices for local products (European Commission. Directorate General for Agricultural & Rural Development, 2018, 2019). Therefore, the EU issued a number of policies to promote the cultivation of legumes in Europe; a number of research programs were issued to increase the competitiveness of domestic protein plants, and support was given to farmers to grow domestic legumes (European Commission. Directorate General for Agricultural & Rural Development, 2018). The increasing trend in the production of protein plants can be also be observed in the EU agricultural outlook. There, the EU estimated an increase in the production of protein crops of about 37% from 2020 to 2030, which was explained with a strong surplus in the human consumption of plant-based proteins (European Commission, 2020). While there is growing interest in plant-based protein in beverages and a trend toward a more functional and sustainable character, the current use of lupines in the beverage industry is still negligible and faba beans are not used at all (Nawaz et al., 2020). Lupines-based milk analogs and few beverages (Nawaz et al., 2020; Tangyu et al., 2019) are available, but no lupine- or faba bean-based soft drinks have been placed on the market so far. The "taste barrier" was named as one of the main obstacles for the acceptance of legumes in food (Lea et al., 2005; Oomah et al., 2013; Tangyu et al., 2019; Tesini, 2017). In order to increase the consumer acceptance of legume-based beverages, the distinctive beany aroma impression needs to be removed or altered (Oomah et al., 2013).

In recent years, aroma and taste active compounds in lupines were identified (Bader et al., 2009; Oomah et al., 2006; Schindler et al., 2011; Stephany et al., 2016) and altered by germination (Kaczmarska et al., 2018), storage (Jacobs et al., 2016; Schindler et al., 2011; Stephany et al., 2016), fermentation (Kaczmarska et al., 2018; Schindler et al., 2011; Schlegel et al., 2021, 2019) or different forms of heat treatments like pasteurization (80°C/10 min) and ultra-high-temperature treatment (140°C/10 s) (Jacobs et al., 2016). Exemplarily, lactic acid fermentation improved the overall aroma of lupine protein extract by reducing off-flavors (e.g., hexanal) as well as by introducing new pleasant aroma compounds (e.g., β-damascenone) and therefore masking unpleasant aroma impressions (Schindler et al., 2011). Kaczmerska et al. (2018) described fewer beany and green odors during

germination, while fermentation with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* introduced pleasant odors (mushroom, meaty, baked) but also led to an increase of beany and green odors. They concluded that germination is better suited to enhance the flavor acceptability of legume products. As only two different strains of lactobacteria were used in this study, the generality of the concluding statement needs to be questioned. The resulting aroma profile of fermentation with lactic acid bacteria (LAB) is substrate- and strain-depending. Therefore, only an experimental setup with a greater strain variation could support the statement that germination is a better means than LAB fermentation. Moreover, the aroma analysis was done by gas chromatography olfactometry (GC-O), which does not present interactive effects of aroma compounds on the overall aroma. In order to include such effects, a sensory analysis of the overall aroma impression would be required. Compared with lupines, less information is available regarding the aroma profile of faba beans. The aroma compounds were analyzed semiquantitatively (Akkad et al., 2019; Oomah et al., 2013; Tesini, 2017), but neither was a sensory analysis performed nor were key aroma compounds identified. Tesini (2017) combined individual aroma compounds (analyzed by GC-MS) with sensory impressions (like grassy, rancid, etc.) by using a statistical approach but did not identify the key aroma compounds by aroma recombination and omission experiments as required according to the sensomics approach (Granvogl & Schieberle, 2022). Changes in the aroma compounds of faba beans were studied for heat treatment and germination (Rami Akkad et al., 2021; Tesini, 2017). Tesini (2017) applied conventional oven heating and microwave heating and showed that both increase the beany aroma impression in faba bean extract. Rami Akkad et al. (2021) showed that distinctive changes in the aroma spectrum occur upon germination. They observed a reduction of the hexanal content in all studied cultivars of faba beans, while the hexanol content increased.

A major off-flavor in lupines and faba beans is often described as beany and green (Bader et al., 2009; Kaczmarska et al., 2018; Mittermeier, 2013; Schindler et al., 2011; Tesini, 2017). To date, the aroma compounds triggering the beany aroma impression in these two legumes have not identified. But in all likelihood, they seem to originate from the oxidation of unsaturated fatty acids in the legumes (Tesini, 2017). Resulting aldehydes like hexanal are often mentioned in connection with the beany aroma (Boatright & Lei, 1999; Bott & Chambers, 2007; Jacobs et al., 2016; Oomah et al., 2013; Roland et al., 2017; Tangyu et al., 2019; Tesini, 2017; Yang et al., 2016).

A further concern regarding the use of legumes are antinutrients like the flatulence-causing raffinose family oligosaccharides (RFOs) and phytic acid, which reduce the bioavailability of proteins and minerals (Granito et al., 2001; Schlemmer et al., 2009; Tangyu et al., 2019). Those antinutrients can be reduced by using lactic acid fermentation (Fritsch et al., 2015; Harlé et al., 2020) and especially by proper pretreatment prior to the fermentation (Chilomer et al., 2010; Dagnia et al., 1992; de la Cuadra, 1994; Khalil & Mansour, 1995).

The aim of this review is to evaluate the literature in order to answer the question as to how a combined process of modulating the legume-based substrate and subsequent lactic acid fermentation can be designed to improve the aroma and reduce the antinutrients. Therefore, the aroma compounds in lupines and faba beans will be outlined and the origin of the off-flavors will be discussed. The composition of carbohydrates, proteins, and lipids in lupines and faba beans will be evaluated regarding their value as substrate for the lactic acid fermentation as well as for the resulting beverage. While the availability of nitrogen, carbon, sulfur, phosphate, and trace elements is essential for the microbial growth during fermentation, other aspects as high protein content or low sugar content are of interest in the final beverage. The presence of antinutrients will be considered and their hazard potential will be examined. In the next step, the impact of different technological processes on the antinutrients will be explained. Finally, the possibilities of aroma improvement by using LAB will be discussed and reaction pathways for hexanal as main aroma compound will be reconsidered.

2 | MAJOR SENSORY ASPECTS OF LUPINES AND FABA BEANS FOR BEVERAGES

The origin of the beany odor in legumes cannot be traced back to a single aroma compound, but seems to result from the interaction of different substances. In the literature, hexanal is often mentioned in combination with the beany aroma. This leads to the assumption that a reduction of the hexanal content might be the key to reducing the beany overall impression. However, scientific proof is still pending. Lactic acid fermentation seems to be a feasible method to perform the reduction of hexanal in food products, as LAB possess the required enzymes and are widely accepted in the alimentary industry. Besides the beany aroma, a bitter taste impression is often observed in legume-based products. The advantage of showing a high protein content goes along with the disadvantage that bitter-tasting peptides might be formed by proteolysis during processing. Those peptides need to be further hydrolyzed either during substrate modulation or in the subsequent LAB fermentation to reduce the bitter taste impression.

2.1 | "Beany" aroma and further off-flavors

The overall aroma of lupines and faba beans was described as beany or legume-like and green, grassy (Bader et al., 2009; Kaczmarska et al., 2018; Mittermeier, 2013; Schindler et al., 2011; Tesini, 2017). Further described aroma impressions are fruity and hay-like or woody and mushroom-like (Mittermeier, 2013). Nevertheless, not a single aroma compound stated in the respective literature was described as beany (see Table 1). Therefore, the beany aroma impression cannot result from one single aroma compound, but seems to depend on a mixture of different aroma compounds. In the literature, hexanal is frequently named as one of the main components responsible for the distinctive beany aroma in lupines (Kaczmarska et al., 2018), in faba beans (Oomah et al., 2013; Tesini, 2017), in peas (Schindler et al., 2012), and in soy (Boatright & Lei, 1999; Bott & Chambers, 2007; Li et al., 2014). However, other compounds like hexanol and pentanol (Wang et al., 1998), 2-pentylfuran and heptanol (Kaczmarska et al., 2018), and methoxypyrazines (Bader et al., 2009) are named in connection with the beany aroma, too. Table 1 presents the aroma compounds in lupines, with aroma descriptors and their intensity according to flavor dilution analyses in different forms of processing (lupine protein extract and isolates, flour from dehulled lupines, and lupine kernel fiber). So far, there are no quantitative aroma compound evaluations or aroma dilution analysis for faba beans available in the literature. To understand the overall beany aroma impression of lupines and faba beans, the interactions causing the aroma need to be evaluated instead of simply considering the aroma impression of the individual compounds. Such perceptual interactions can be partial or complete masking (one component is perceived as primary and conceals further compounds), partial or complete blending (mixed compounds are perceived as a novel odor, which differs from the odors of the individual compounds), and synergy (increased perception of one aroma after mixing) (Thomas-Danguin et al., 2017). A blending effect is known for the mixture of ethyl isobutyrate (strawberry odor) and ethyl maltol (caramel odor), which is described as showing a pineapple odor after mixing (Le Berre et al, 2007). For a mixture of isoamyl acetate (fruity, banana-like odor) and β-methyl-γ-octalactone (woody, coconut-like odor), the resulting odor impression depends on the concentration of β-methyl-γ-octalactone. Ishii et al. (2008) reported a synergy effect for low concentrations, which supports the fruity character, whereas high concentrations lead to a masking effect that suppresses the fruity odor of isoamyl acetate.

In order to explain the beany aroma in soy, blending effects were assessed and hexanal was named as one of the main contributors (Ang & Boatright, 2003; Bott & Chambers, 2007). As the concept of blending effects implies that two or more aroma compounds result in a novel aroma impression after mixing (Thomas-Danguin et al., 2017), the total or partial extraction would negate the blending effect. Consequently, this novel aroma impression would disappear. This would explain why the reduction of hexanal correlated with the decrease in the green/beany flavor (Lee & Beuchat, 1991). The beany aroma was defined as a combination of musty/earthy, musty/dusty, green/pea pot, nutty and brown aroma impressions (Vara-Ubol, 2004). Bott and Chambers (2007) evaluated the odor impression of different aroma active compounds as pure substances and when interacting with other substances. Their key finding was that the beany aroma impression results out of the combination of different aroma compounds, even when those compounds show no beany impression as pure substances (e.g., hexanal in combination with (E,E)−2,4-decadienal or (E)−2-nonenal). Even as Bott and Chambers (2007) or Vara-Ubol (2004) do not describe it explicitly, this indicates that the beany aroma is due to a blending effect. Furthermore, they identified hexanal as the key substance for the appearance of the beany impression in combination with other substances, especially with 1-octen-3-one and 3-methyl-1-butanol (Bott & Chambers, 2007). Further important compounds for the overall beany impression in legumes are hexanol and pentanol (Wang et al., 1998), 2-pentylfuran and dimethyl trisulfide (Ang & Boatright, 2003), 2-pentyl pyridine (Boatright & Lei, 1999) and acetophenone, 2,4-nonadienal, and 2,4-decadienal (Boatright & Lei, 1999; Bott & Chambers, 2007). In soy milk, 2-isopropyl-3-methoxypyrazine was mentioned as a contributor to a pea-like and earthy aroma impact (S. Kaneko et al., 2011). Schlegel et al. (2021) named 2-isopropyl-3-methoxypyrazine, 3-sec-butyl-2-methoxypyrazine, and 3-isobutyl-2-methoxypyrazine in connection with a pea-like aroma impression in lupine protein isolate. The beany aroma in lupines and faba beans could be due to a similar blending effect and might be undone by removing hexanal, the main contributor. Nevertheless, further research is required to elucidate the role of hexanal in the interactive effect triggering the beany aroma impression. Moreover, the aroma of faba beans needs to be deciphered in more detail and the key aroma compounds should be identified.

The formation of hexanal happens by immediate enzymatic oxidation of linoleic acid (C18:2) and likewise by autoxidation of this fatty acid during storage. It was shown

TABLE 1 Aroma compounds evaluated in different processing of lupines by aroma dilution analysis (values as flavor dilution factor FD) and connections to the volatile profile of faba beans

			Lupinus angustifolius[B]					Mentioned for *Vicia faba*
Compound	**Molecular formula**	**Aroma descriptor**[a]	**Protein extract**[1]	**Protein extract**[2]	**Protein isolate**[3]	**Flour**[4]	**Fiber**[5]	
(Methyltrisulfanyl)methane (dimethyl trisulfide)	$C_2H_6S_3$	Sulfurous, fecal	200					
Acetic acid	$C_2H_4O_2$	Vinegar			9	32		
3,4-dihydro-2H-pyrrole (1-pyrroline)	C_4H_7N	Sperm, milky	200					
3-methylsulfanylpropanal (methional)	C_4H_8OS	Cooked potato			9		32	
Pentanal (valeraldehyde)	$C_5H_{10}O$	Milky, green	200					X[7]
2-/3-methylbutanoic acid	$C_5H_{10}O_2$	Cheese, sweaty		64	9	2048	16	X[8]
Pentanoic acid	$C_5H_{10}O_2$	Cheese, sweaty, fruity				32	4	
Hexanal	$C_6H_{12}O$	Grass, green	200	256	1		32	X[6,7,8,9]
3-hydroxy-2-methyl-pyran-4-one (maltol)	$C_6H_6O_3$	Caramel		8	3	256	4	
4-hydroxy-2,3-dimethyl-2H-furan-5-one (sotolone)	$C_6H_8O_3$	Spicy, savory			729		512	
4-hydroxy-2,5-dimethylfuran-3-one (furaneol)	$C_6H_8O_3$	Caramel			3			
1-(3,4-dihydro-2H-pyrrol-5-yl)ethanone (2-acetyl-1-pyrroline)	C_6H_9NO	popcorn, fatty			3	32	2	
2-phenylacetic acid	$C_8H_8O_2$	bee wax, honey			729	256		
4-hydroxy-3-methoxy-benzaldehyde (vanillin)	$C_8H_8O_3$	vanilla			729			
(5Z)-octa-1,5-dien-3-one	$C_8H_{12}O$	geranium, metallic			1	128		
2-isopropyl-3-methoxypyrazine (2-methoxy-3-propan-2-ylpyrazine)	$C_8H_{12}N_2O$	pea, green, metallic, green pepper		2	9	256	–	

(Continues)

TABLE 1 (Continued)

Compound	Molecular formula	Aroma descriptor[a]	*Lupinus angustifolius*[B]					Mentioned for *Vicia faba*
			Protein extract[1]	Protein extract[2]	Protein isolate[3]	Flour[4]	Fiber[5]	
Oct-1-en-3-one	$C_8H_{14}O$	Mushroom, metallic	50	16	9	32	N/A	
Octanal	$C_8H_{16}O$	Lemon, soapy					8	X[7,8,9]
Oct-1-en-3-ol	$C_8H_{16}O$	Fungi, earthy, cooked potato	50					X[6,7,8,9]
Octanoic acid	$C_8H_{16}O_2$	Musty, coriander, fatty			3			X[9]
5-butyloxolan-2-one (γ-octalactone)	$C_8H_{14}O_2$	Coconut, sweet				64		
3-ethoxy-4-hydroxybenzaldehyde (ethyl vanillin)	$C_9H_{10}O_3$	Vanilla				256		
(2E,4E,6Z)-nona-2,4,6-trienal	$C_9H_{12}O$	Nutty, oat flake		512		256		
2-methoxy-3-(2-methylpropyl)pyrazine	$C_9H_{14}N_2O$	Pea, green pepper			9			
2-butan-2-yl-3-methoxypyrazine	$C_9H_{14}N_2O$	Green, metallic; green pepper, earthy			3	32	32	
(E,Z)-non-2,4-dienal	$C_9H_{14}O$	Fatty, cardboard, cucumber			9			
(E,E)-non-2,4-dienal	$C_9H_{14}O$	Fatty, green, cucumber, rancid		512			32	
(E,Z)-non-2,6-dienal	$C_9H_{14}O$	Green, cucumber		32	9	256		
2-pentylfuran	$C_9H_{14}O$	N/A						X[6,7,8,9]
(E)-non-2-enal	$C_9H_{16}O$	Fatty, green, cucumber, cardboard		512	9	256	–	X[8]
(Z)-non-2-enal	$C_9H_{16}O$	Cardboard		64	9	32		
5-pentyloxolan-2-one (γ-nonalactone)	$C_9H_{16}O_2$	Coconut, peach, sweet			3	256	32	

(Continues)

TABLE 1 (Continued)

Compound	Molecular formula	Aroma descriptor[a]	*Lupinus angustifolius*[B] Protein extract[1]	Protein extract[2]	Protein isolate[3]	Flour[4]	Fiber[5]	Mentioned for *Vicia faba*
6-butyloxan-2-one (δ-nonalactone)	$C_9H_{16}O_2$	Coconut			729		32	
Nonanal	$C_9H_{18}O$	Fatty, lemon, green, soapy			1		8	X[6,7,8,9]
Nonanoic acid	$C_9H_{18}O_2$	Soapy			729			
(2E,4E)-deca-2,4-dienal	$C_{10}H_{16}O$	Fatty, fried, rancid		256			128	
(2E,4Z)-deca-2,4-dienal	$C_{10}H_{16}O$	Fatty, fried					32	
(E)−3-[(2R,3R)−3-pentyloxiran-2-yl]prop-2-enal (trans-4.5-epoxy-(E)-dec-2-enal)	$C_{10}H_{16}O_2$	Metallic		512	9	1024	128	
(E)-dec-2-enal	$C_{10}H_{18}O$	Cardboard		512				
(Z)-dec-2-enal	$C_{10}H_{18}O$	Cardboard		64				
6-pentyloxan-2-one (δ-decalactone)	$C_{10}H_{18}O_2$	Peach			729			
5-hexyloxolan-2-one (γ-decalactone)	$C_{10}H_{18}O_2$	Coconut			3			
5-octyloxolan-2-one (γ-dodecalactone)	$C_{12}H_{22}O_2$	Peach, flowery			9			
(E)−1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one (β-damascenone)	$C_{13}H_{18}O$	Sweet, floral, rose	50					
(E)−4-(2,6,6-trimethylcyclohexen-1-yl)but-3-en-2-one(β-ionone)	$C_{13}H_{20}O$	Flower, violet		128		512	256	X[8]

[1]data from (Schindler et al., 2011);
[2]data from (Mittermeier, 2013);
[3]data from (Schlegel et al., 2021);
[4]data from (Bader et al., 2009);
[5]data form (Stephany et al., 2016);
[6]data from (Tesini, 2017);
[7]data from (Oomah et al., 2013);
[8]data from (R. Akkad et al., 2019);
[9]data from (Lampi et al., 2020);
[A]All aroma descriptors for the respective compounds are reported according to the cited authors;
[B]The resulting FD values of the aroma dilution analysis depend highly on the extraction procedure, the temperature and degree of concentration in the rectification, and the individual sensitivities of the test participants toward the respective aroma compounds. Therefore, these semi-quantitative data should be compared within an experimental series only.; N/A…not available.

that no-LOX breeding can degrease the hexanal content in soy (Yang et al., 2016). As polyunsaturated fatty acids are prone to autoxidation during storage, the reduction or inactivation of LOX activity might be misleading. Stephany et al. (2016) investigated not only the aroma development in *Lupinus angustifolius* kernel fiber during storage, but also the impact of a thermal treatment in order to inactivate LOX prior to storage. It was shown that the concentration of aroma compounds related to the oxidation of fatty acids, like hexanal, increases during a 1-year storage period. Moreover, the final concentration was even higher in the heat-treated samples. Therefore, the reduction of the enzyme-catalyzed formation of off-flavors might not be sufficient, as the remaining fatty acids will oxidize eventually and form beany odor impressions during storage.

In order to mitigate the impact of the oxidation of fatty acids on the aroma profile, the focus should be on the aroma active compounds themselves or on their precursors. The first strategy is removing the off-flavor relevant precursors prior to the reaction. The beany off-flavor can be reduced strongly by generally de-oiling the legumes prior to further processing (Mittermeier, 2013) and therefore removing the specific precursors (unsaturated fatty acids) before they oxidize. However, as this will not eliminate the beany aroma impression entirely, the second strategy is to reduce the off-flavors or their impact by fermentation. By transforming the undesired aroma components into those with a higher aroma threshold like hexanal with an odor threshold value in water of 2.4 μg/kg (Grimm & Steinhaus, 2019) into hexanol (590 μg/kg (Dunkel et al., 2014)) or hexanoic acid (4800 μg/kg (Grimm & Steinhaus, 2019)), the off-flavor is not perceivable anymore (Schindler et al., 2011). Fermentation seems to be an applicable method to improve the overall aroma profile by reducing or masking off-flavors, as it is a commonly and widely accepted process in the food industry.

2.2 | Bitter components

The bitter impression is a negative attribute in legume-based products and needs to be considered thoroughly as the bitter taste threshold is much lower than the one of sweet, sour, and umami. During processing of legumes, the bitter impression can change. This is not only due to changes and reactions of the bitter substances themselves, but also because the perception of bitterness depends on the matrix of the food and its pH (Engel et al., 2001). In omission experiments, Engel et al. (2001) found that the presence of sodium chloride increases the perception of sourness, while it suppressed the bitter impression. They also observed in sensory experiments with bitter peptides, that only few bitter peptide fractions from camembert cheese were found bitter when tasted out of the food matrix.

Bitter and toxic alkaloids were a major drawback for the use of lupines in food and feed (Wolko et al., 2011). However, they were mostly removed from the so-called sweet lupines by breeding (von Sengbusch, 1938). Another source of bitter tastants results from the high protein content of lupines and faba beans. During food processing, proteins are enzymatically degraded and bitter peptides can be formed. The bitter impact of the different bitter peptides varies highly (Temussi, 2012). It depends on the molecular mass (Engel et al., 2001; M. R. Kim et al., 1999; Lovšin-Kukman et al., 1996), the number of their amino acids (H.-O. Kim & Li-Chan, 2006), their hydrophobic character (H.-O. Kim & Li-Chan, 2006; Maehashi & Huang, 2009), and the structure of the peptide and the involved amino acids (H.-O. Kim & Li-Chan, 2006). The bitter impression can be reduced by further enzymatic degradation to shorter peptides or even to amino acids (Meinlschmidt et al., 2016; Zhao et al., 2016). This is confirmed by the finding that the bitter peptides show a higher bitter impression than the sum of their amino acids (Maehashi & Huang, 2009). A different approach is masking or suppressing the bitter taste as Keast and Breslin (2002) reviewed by adding sweet, salty, sour, or umami taste components. Due to the high protein content in legumes, bitter peptides might be formed by enzymatic hydrolysis (Schlegel et al., 2019). Therefore, the bitterness needs to be reduced or masked in the further processing, which can be achieved by further enzymatic degradation to shorter peptides or even to the amino acids.

To conclude, the origin of the beany aroma impression in legumes like lupines and faba beans seems to be connected to hexanal via an interactive effect. However, the exact mechanism of this interactive effect regarding the beany aroma is not entirely understood and should be the aim of further scientific effort. This will not only prove that hexanal is the key to the beany aroma impression but would also facilitate the identification of strategies to remove this off-flavor. This would consequently lead to a higher consumer acceptance of legumes in beverages and meat analogs. Regarding both aroma and taste, LAB fermentation appears to be a possible solution to reduce off-flavors and bitter peptides at the same time. Especially as bitter peptides might be formed during processing, the proteolytic activity of LAB might prove to be valuable for refined legume-based products.

3 | COMPOSITION AND MODULATION OF LEGUME-BASED SUBSTRATES FOR THE LAB FERMENTATION

In order to use lupines or faba beans as raw material to produce lactic acid fermented beverages, the different demands for the microbial growth and for the nutritional value of the resulting beverage need to be considered. Firstly, LAB require sources for nitrogen, carbon, sulfur, phosphate, and trace elements. Only few LAB strains break down and metabolize RFOs (Harlé et al., 2020; C. Martínez-Villaluenga et al., 2005) or phytic acid (Fritsch et al., 2015). In order to avoid restrictions in the choice of strain that should be based on the resulting aroma profile only, these storage compounds need to be decomposed at least partially prior to fermentation. Proteins need to be hydrolyzed to increase their solubility and availability as nitrogen source for the bacteria. Secondly, a low carbohydrate and high protein content in the beverage should be achieved to advertise the product with claims on health and functionality. By modulating the legume-based substrate, in order to increase the availability of nutrients for the microorganism and decrease antinutrients, the subsequent lactic acid fermentation can be aimed on aroma and taste attributes exclusively.

3.1 | Composition of lupines and faba beans

The composition of the macronutrients varies highly between lupines and faba beans (see Figure 1). The main difference is the starch content of the legumes. While faba beans contain plenty of starch (Duc et al., 1999; Hood-Niefer et al., 2011; Mattila, Makinen, et al., 2018; Wei, 2019), in lupines it is only present in minor concentrations or not at all. This is compensated by higher contents of dietary fiber and RFOs in lupines. Moreover, lupines contain higher amounts of protein and fat than faba beans. Furthermore, it should to be mentioned that the composition of legumes varies highly with the choice of cultivar, location, and year of growth (Hood-Niefer et al., 2011; Khazaei & Vandenberg, 2020; Labba et al., 2021; Lizarazo et al., 2015).

3.1.1 | Carbohydrates

Lupines and faba beans contain RFOs as storage carbohydrates (compare Figure 2). These α-galactosides cannot be digested by humans and are therefore metabolized by bacteria in the intestine (Rumney & Rowland, 1995). Beside their prebiotic effects (Rumney & Rowland, 1995; Trindade et al., 2003), these carbohydrates cause flatulence and gastric disorders (Granito et al., 2001). In addition, Martinez-Villaluenga et al. (2008) reviewed this topic and also found that RFOs act as a laxative and reduce the absorption of nutrients (namely amino acids) due to the imbalances of the osmotic pressure that they cause in the small intestine. To avoid intestinal problems after consumption, they need to be reduced for the production of beverages.

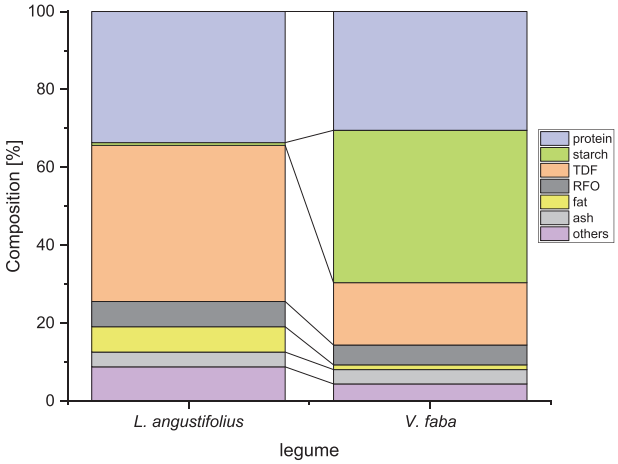

FIGURE 1 The comparison of the composition of *L. angustifolius* and *V. faba* shows that lupines contain higher amounts of protein, dietary fiber (TDF), RFOs and fat, while faba beans contain high amounts of starch (Bähr et al., 2014; Duc et al., 1999; Fan et al., 2014; Fritsch et al., 2015; Hood-Niefer et al., 2011; Labba et al., 2021; Landry et al., 2016; Lizarazo et al., 2015; Mattila, Makinen, et al., 2018; Musco et al., 2017; Siegert et al., 2021; Sobotka et al., 2013; Sujak et al., 2006; Torres et al., 2005; Wei, 2019) For better visualization, the RFOs are not included in the dietary fiber content

Among the RFOs, stachyose is the most abundant in lupines (de la Cuadra, 1994; Piotrowicz-Cieslak et al., 2003) and verbascose in faba beans (Duc et al., 1999; Fan et al., 2014) (Table 2). The origin of the RFOs is the anabolism of sucrose during ripening (J. Frias, C. Vidal-Valverde, et al., 1996; Martinez-Villaluenga et al., 2008) and their concentrations are influenced by the temperature and humidity during the ripening period (Zalewski et al., 2001), as RFOs are important for the drought resistance and frost tolerance of legumes (Muzquiz et al., 2012). In contrast to bifidobacteria, only few LAB can metabolize RFOs (C. Martínez-Villaluenga et al., 2005). Harlé et al. (2020) tested 276 strains of different LAB species for their ability to metabolize different carbohydrates. They showed that only 36% could grow on raffinose and only 6% on stachyose. While the ability to metabolize certain carbohydrates depends highly on the individual strain, a tendency can be seen among the species. It was shown that multiple strains of *Lactobacillus plantarum*, *Lactobacillus johnsonii*, *Lactobacillus acidophilus*, and *Lactobacillus helveticus* degrade

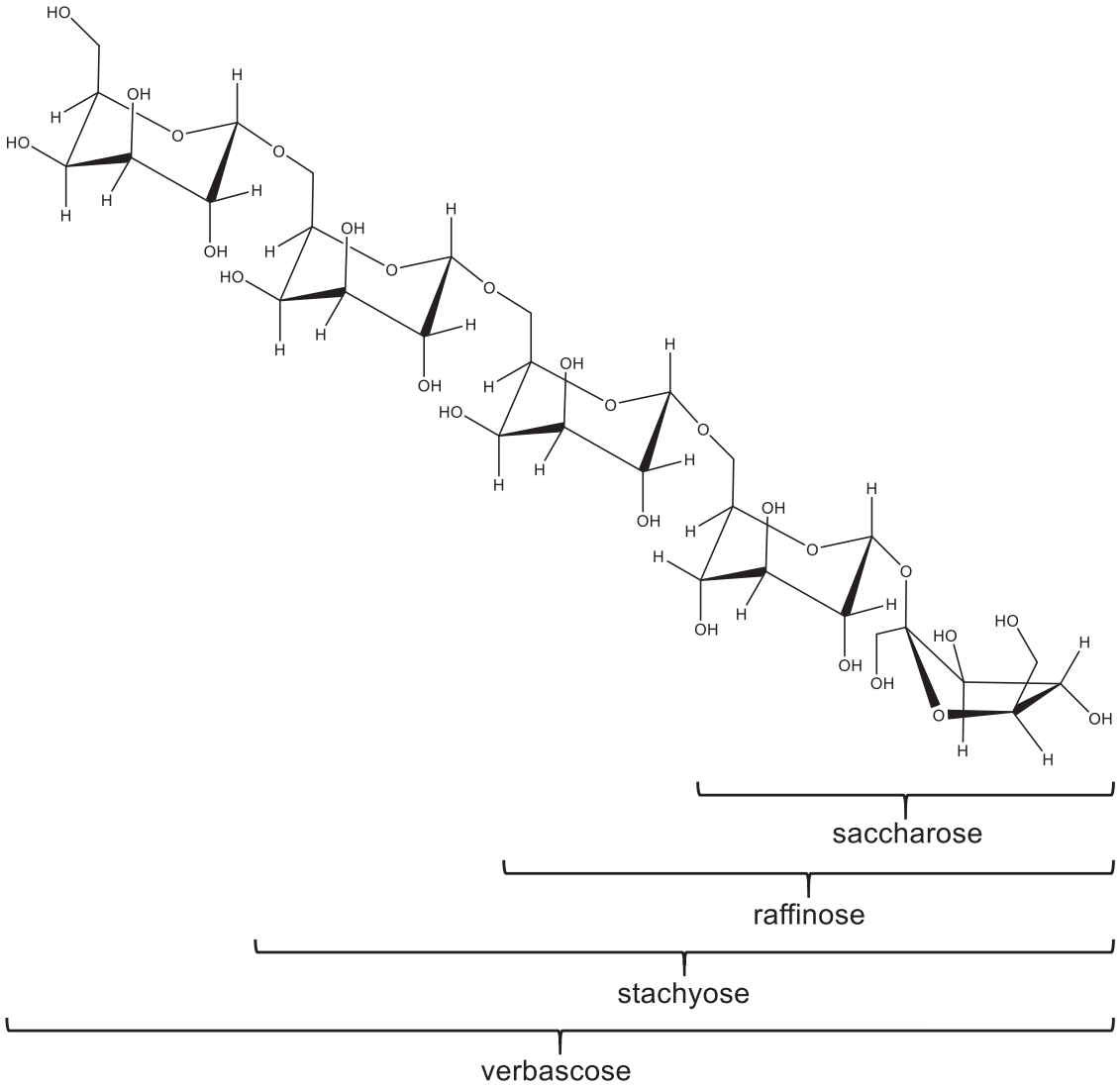

FIGURE 2 Chemical structure of the main RFOs in legumes. RFOs composed of galactose units linked to the glucose moiety of saccharose by 1,6-α-glycosidic bonds. Depending on the number of galactose units, they are denominated as raffinose (1 galactose unit), stachyose (2 galactose units), and verbascose (3 galactose units). (Based on Martinez-Villaluenga et al. (2008))

TABLE 2 RFOs and sucrose concentrations in lupines and faba beans (in g/100 g dry matter)

	L. albus[1]	*L. luteus*[2]	*L. angustifolius*[3]	*V. faba*[4]
Raffinose	0.33–0.62	0.54–1.23	0.23–2.02	0.06–1.45
Stachyose	4.98–7.26	4.89–8.61	2.99–7.54	0.27–3.41
Verbascose	0.19–1.19	2.75–3.54	0.71–2.48	0.67–8.97
Sucrose	2.16–4.12	1.01–1.56	1.4–5.05	0.37–3.8

[1]data from (Erbaş et al., 2005; C. Martínez-Villaluenga et al., 2006; Martıńez-Villaluenga et al., 2005).
[2]data from (Kaczmarek et al., 2016; C. Martínez-Villaluenga et al., 2006; Martıńez-Villaluenga et al., 2005; Sobotka et al., 2013).
[3]data from (Fritsch et al., 2015; Martıńez-Villaluenga et al., 2005; Mattila, Makinen, et al., 2018; Sobotka et al., 2013; Torres et al., 2005).
[4]data from (Duc et al., 1999; Fan et al., 2014; Labba et al., 2021; Landry et al., 2016; Mattila, Makinen, et al., 2018; Wei, 2019).

raffinose, while *L. acidophilus* seems to be efficient in using stachyose, too. Fritsch et al. (2015) observed the growth of 25 LAB strains on glucose, raffinose, and stachyose. They found that there is only a minor reduction of stachyose and raffinose with *Lactobacillus plantarum* and *Lactococcus lactis* in lupine-based substrates even when this LAB could grow in MRS broth with raffinose and stachyose as exclusive carbon sources. To avoid limitations in the choice of strain for the subsequent LAB fermentation and to assure the reduction of gastric disorders, RFOs should be decomposed to fermentable sugars like sucrose, galactose, glucose, and fructose in the substrate preparation.

Both lupines and faba beans are rich in dietary fiber. For lupines, values are in the range 33.7–51.3 g/100 g in *Lupinus luteus*, 34.4–52.1 g/100 g in *Lupinus albus* and 47.5–58.8 g/100 g in *Lupinus angustifolius*

(Mattila, Makinen, et al., 2018; Musco et al., 2017; Torres et al., 2005). The content in dehulled lupines is reduced to 21.0 g/100 g in *L. luteus*, 28.4–32.5 g/100 g in *L. albus*, and 36.7–40.1 g/100 g in *L. angustifolius* (Bähr et al., 2014). Information about the composition in lupines is contradictory. While some authors state that the major part of the fiber in lupines is insoluble in water (C. Martínez-Villaluenga et al., 2006; Mattila, Makinen, et al., 2018; Musco et al., 2017), Bähr et al. (2014) found that about 75% are water soluble. In *V. faba*, only 11.4–24.7 g/100 g of dietary fibers are reported (Labba et al., 2021; Lizarazo et al., 2015; Mattila, Makinen, et al., 2018), which can be reduced to 10.2 g/100 g by dehulling (Mattila, Makinen, et al., 2018). This difference between the two plants can be explained with starch being a major source of carbohydrates in faba beans.

Regarding the starch content in lupines, some authors mention traces (Lizarazo et al., 2015), or small amounts of about 3–4% (C. Martínez-Villaluenga et al., 2006; Mohamed & Rayas-Duarte, 1995a, 1995b), while others state that there is no starch at all in lupines (Jansen et al., 2006) or that no starch was found during analysis (Torres et al., 2005). This contradiction might be explained with findings stating that previously non-starch polymers were erroneously identified as starch (Jansen et al., 2006; Petterson et al., 1999). Another explanation is that starch seems to be formed only temporarily during ripening (Jansen et al., 2006). In contrast to lupines, starch concentrations of 21.5–45.3% are reported for faba beans (Duc et al., 1999; Hood-Niefer et al., 2011; Lizarazo et al., 2015; Siegert et al., 2021; Wei, 2019). By enzymatic decomposition of starch in faba beans, high amounts of maltose are set free and can be used as carbon source for the subsequent lactic acid fermentation.

To conclude, both legumes contain high amounts of RFOs, which can cause flatulence and need to be removed during the production of a beverage. As not all LAB strains are able to reduce the RFOs to a minimum, their concentration needs to be minimized during the substrate modulation. This would not only solve the problem with flatulence-causing substances in the final product, but also supplies the LAB with fermentable sugars. In order to use the starch in faba beans, it needs to be solubilized and degraded to fermentable mono-, di-, and oligomers, too. In the LAB fermentation, the fermentable carbohydrates are reduced by the metabolism of the microorganisms. Therefore, the resulting beverage would be low in sugar. Nevertheless, a certain sweetness is required for an appealing taste. Therefore, either the entire loss of sugars needs to be avoided upon fermentation (by the duration of fermentation) or sugars and/or sweetening agents need to be added in the final formulation of the beverage.

3.1.2 | Protein

Depending on cultivar, year of harvest, and location/weather during growth, lupines and faba beans contain protein contents of 24.4–55.3% and 20.6–39.9%, respectively (compare Table 3). Nevertheless, the protein needs to be decomposed in order to be solubilized and transferred into the substrate as nitrogen source and to claim a high protein content in the resulting beverage. Based on the daily amino acid requirements for adults (Joint WHO/FAO/UNU Expert Consultation, 2007), lupines and faba beans show a scarcity of methionine. Similar findings are reported by Sujak et al. (2006) while they also name lysine and valine as scarce in lupines. Other authors confirm the lack of the sulfurous amino acids methionine and cysteine but describe the content of lysine in faba beans as high (Duc et al., 1999; Mattila, Makinen, et al., 2018). Furthermore, tryptophan was mentioned as scarce in sweet lupines in general and isoleucine, especially in *L. luteus* (Sujak et al., 2006). The further loss of sulfurous amino acids during LAB fermentation due to the metabolic activity of the microorganisms should be monitored during the development of beverages. The addition of methionine and cysteine might be a strategy to counterbalance the scarcity and increase bioavailability.

In order to transfer the high protein content into a substrate for the production of a beverage, further treatment is required. By enzymatically degrading of the proteins' complex structure to smaller peptides and amino acids, solubility was increased in the acid pH range 4–5 from approximately 10% to more than 80% in lupine protein hydrolysate (Schlegel et al., 2019). Comparable results were reported for soy protein isolate, where the solubility at pH 4.0 was increased from 5.0% to 87.0% by proteolytic treatment (Meinlschmidt et al., 2016). Additionally, the released amino acids are available as nitrogen source for the cultivation of microorganisms. Therefore, by degrading the proteins into amino acids and short peptides, more protein can be transferred into the liquid substrate and sufficient amino acids are available for LAB fermentation. Moreover, the resulting protein content in the beverage can be increased.

3.1.3 | Lipids and fatty acids

Lupines and faba beans contain high amounts of oxidizable fatty acids. Depending on the species and cultivar (Straková et al., 2006), the oil content of lupines varies between 3.3% and 8.7%, which is higher than the oil content in faba beans with 1.1% to 4.7% (see Table 4). Some sources even report oil contents up to 20% for lupines (Borek et al.,

TABLE 3 Amino acid composition and protein content of lupines and faba beans (in g/100 g dry matter)

	L. albus[1]	*L. luteus*[2]	*L. angustifolius*[3]	*V. faba*[4]
Protein	25.8–52.9	33.6–55.3	24.4–44.2	20.6–39.9
Isoleucine (Ile)	0.93–1.69	1.04–1.82	0.88–1.50	0.50–1.50
Leucine (Leu)	1.93–2.88	2.46–3.44	1.25–2.69	1.25–2.89
Lysine (Lys)	1.28–1.80	1.76–2.33	1.24–1.70	1.12–2.37
Methionine (Met)	0.23–0.32	0.07–0.34	0.13–0.25	0.17–0.30
Cystine (Cys)	0.49–0.79	0.67–1.19	0.27–0.54	0.22–0.47
Phenylanine (Phe)	1.00–1.47	1.34–1.77	0.98–1.42	0.93–1.58
Tyrosine (Tyr)	0.53–1.30	0.45–1.55	0.46–1.39	0.79–1.15
Tryptophan (Trp)	0.19–0.25	0,27–0.29	0.21–0.31	N/A
Threonine (Thr)	1.01–1.50	1.08–1.49	0.88–1.21	0.65–1.34
Valine (Val)	0.24–1.64	1.11–1.70	0.90–1.45	0.80–1.67
Alanine (Ala)	0.92–1.20	1.03–1.49	0.89–1.18	0.90–1.58
Arginine (Arg)	2.18–4.40	3.85–5.23	2.28–4.03	1.22–4.33
Asparginine (Asn)	N/A	N/A	N/A	2.03–3.41
Aspartic acid (Asp)	2.67–4.17	3.41–4.69	2.32–3.54	2.12–3.49
Glutamine	N/A	N/A	N/A	N/A
Glutamic acid (Glu)	5.49–8.61	9.09–10.79	5.11–8.46	3.02–5.24
Glycine (Gly)	1.09–1.65	1.31–1.79	1.05–1.49	0.66–1.37
Histidine (His)	0.62–1.32	0.94–1.45	0.67–1.14	0.31–1.06
Proline (Pro)	1.17–1.61	0.77–2.92	0.82–1.39	0.74–1.50
Serine (Ser)	1.44–1.91	1.61–2.18	1.12–1.70	0.93–1.86

[1]data from (Bähr et al., 2014; Brenes et al., 2005; Sujak et al., 2006).
[2]data from (Bähr et al., 2014; Kaczmarek et al., 2016; Pastor-Cavada et al., 2009; Schumacher et al., 2011; Sujak et al., 2006).
[3]data from (Bähr et al., 2014; Lizarazo et al., 2015; Mattila, Makinen, et al., 2018; Pastor-Cavada et al., 2009; Schumacher et al., 2011; Sobotka et al., 2013; Sujak et al., 2006; Torres et al., 2005).
[4]data from (Khazaei & Vandenberg, 2020; Labba et al., 2021; Lizarazo et al., 2015; Mattila, Makinen, et al., 2018; Raikos et al., 2014; Schumacher et al., 2011; Siegert et al., 2021); N/A…not available.

TABLE 4 Total oil (in g/100 g dry matter) and fatty acid composition of different legumes (in % of total fatty acids)

	L. albus[1]	*L. luteus*[2]	*L. angustifolius*[3]	*V. faba*[4]
Total oil	1.4–12.7	3.3–8.2	3.3–9.8	1.0–4.7
Saturated FA	13.5–17.1	15.1–16.9	21.0–26.7	14.6–19.1
Monounsaturated FA	50.9–55.4	24.6–29.5	29.5–38.7	24.9–36.5
Polyunsaturated FA	28.7–32.0	54.3–60.3	34.7–49.5	48.8–61.7

[1]data from (Bähr et al., 2014; Chiofalo et al., 2012; Erbaş et al., 2005; Musco et al., 2017).
[2]data from(Bähr et al., 2014; Chiofalo et al., 2012; Musco et al., 2017; Sobotka et al., 2013).
[3]data from (Bähr et al., 2014; Chiofalo et al., 2012; Fritsch et al., 2015; Lizarazo et al., 2015; Mattila, Makinen, et al., 2018; Musco et al., 2017; Schindler et al., 2011; Sobotka et al., 2013; Torres et al., 2005).
[4]data from (Duc et al., 1999; Lizarazo et al., 2015; Mattila, Makinen, et al., 2018; Raikos et al., 2014; Siegert et al., 2021; Wei, 2019).

2015), which would be comparable with the oil content in soy (Straková et al., 2006). Especially for the formation of aroma active compounds like hexanal or 1-octen-3-ol, it is of interest that the fatty acids in both legumes are mainly mono- and polyunsaturated. Among the polyunsaturated fatty acids, linoleic acid and α-linolenic acid are the most dominant ones in *L. angustifolius* (33.5% and 6.6% of fatty acids), *L. luteus* (48.6% and 8.1% of fatty acids), and *L. albus* (19.2% and 9.3% of fatty acids) (Musco et al., 2017). The beany aroma impression was reported to appear in de-oiled lupine products, too (Stephany et al., 2016). Therefore, reducing the oil content in the substrate preparation seems not to be an adequate method to resolve the aroma deficit entirely.

3.1.4 | Phytic acid

Phytic acid (myo-inositol hexaphosphate, IP6) reduces the bioavailability of proteins and multivalent cations. The content of phytic acid varies with the species and 2.5–16.5 g/kg were reported for *L. albus* (de la Cuadra, 1994; Embaby, 2010; C. Martínez-Villaluenga et al., 2006; Trugo et al., 1993), 6.1–7.1 g/kg for *L. luteus* (de la Cuadra, 1994; C. Martínez-Villaluenga et al., 2006), and 3–11 g/kg for *L. angustifolius* (Mattila, Pihlava, et al., 2018; Torres et al., 2005; Trugo et al., 1993). For faba beans, amounts in a broad range from 1.1 to 20.3 g/kg were stated (Honke et al., 1998; Labba et al., 2021; Y. Luo et al., 2012; Mattila, Pihlava, et al., 2018; Oomah et al., 2011; Siegert et al., 2021; Wei, 2019). The high fluctuation in the reported concentrations of phytic acid might result from the different methods used for extraction, purification, and quantification. The choice of a proper method is highly linked to the need and/or intention of the experiment. On the one hand, a commonly used colorimetric method is based on the discoloration of an iron ions-containing solution by complexing them with phytic acid (Embaby, 2010; Oomah et al., 2011). This method represents the affinity toward iron ions and shows the potential to reduce the bioavailability. Nevertheless, no information is given about the lower phosphorylated myo-inositols, as they show a lower affinity toward iron ions. On the other hand, analytical methods using HPLC or ion chromatography (de la Cuadra, 1994; Honke et al., 1998; Labba et al., 2021; C. Martínez-Villaluenga et al., 2006; Siegert et al., 2021) can distinguish between the different forms of myo-inositols and the kinetics of the phytic acid degradation can be observed. The great variety of partially dephosphorylated myo-inositols requires a number of pure substances as references, which are not all commercially available. Other methods dephosphorylate the myo-inositol phosphates and calculate the phytic acid content based on the released phosphorus, without accounting for the original form (IP6, IP5, etc.) or their binding capacity. Phytic acid is generally considered an antinutrient because of its affinity toward forming complexes with minerals and proteins and the consequent impact on their bioavailability. Schlemmer et al. (2009) reviewed the topic comprehensively and depicted the affinity of phytic acid toward multivalent cations like Fe^{3+} or Ca^{2+} (compare Figure 3). Labba et al. (2021) analyzed 15 cultivars of *V. faba* and found that all but one cultivar showed a low bioavailability of zinc and iron due to the molar ratio of phytate to the respective mineral. Nevertheless, the binding capacity of phytic acid decreases with the number of phosphates bound to the molecule. Thus, the degradation releases not only phosphate but increases the bioavailability of minerals (Schlemmer et al., 2009). Between 66% and 88% of the phosphoinositols in lupines

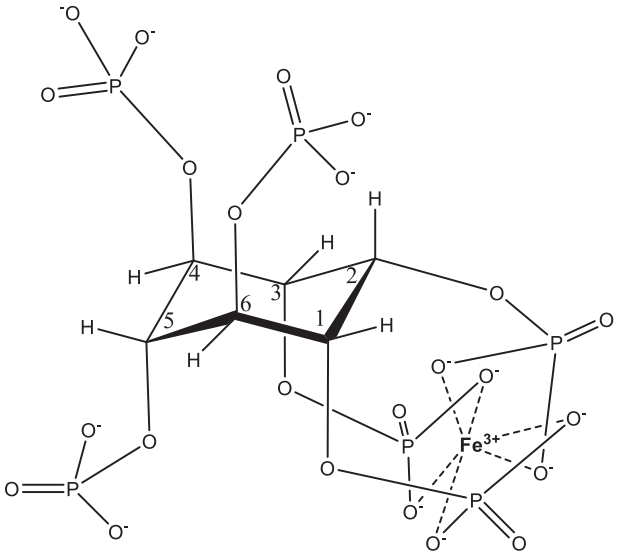

FIGURE 3 Chemical structure of phytic acid bound to iron (Fe^{3+}). The negative charges (O^-) are counterbalanced by the positive charge of the iron ion. (Redrawn from Schlemmer et al. (2009))

(Silva & Trugo, 1996; Torres et al., 2005) are phytic acid (IP6), while IP4 and IP5 form the remaining phosphoinositols with 13–16% and 8–17%, respectively (Torres et al., 2005). In ripe faba beans, 81–98% (Honke et al., 1998; Y. Luo et al., 2012; Siegert et al., 2021) are phytic acid (IP6), up to 19% are different forms of IP5 (Y. Luo et al., 2012; Siegert et al., 2021), and only traces are lower phosphorylated myo-inositols (IP1–4). Therefore, dephosphorylating the phytic acid seems to be applicable to increase the bioavailability of minerals in legume-based products. This can be accomplished either by using legume-borne enzymes or adding technical enzymes. While a comparison in MRS broth showed that phytases from LAB are more potent to degrade phytic acid than the lupine-borne enzyme, the effect was reduced in lupine-based substrate (Fritsch et al., 2015). This might be due to several plant substances, which hinder the enzymatic activity (Bohn et al., 2007; Fritsch et al., 2015). During substrate modulation, phytic acid might be decomposed to lower myo-inositols during substrate preparation to increase the bioavailability of proteins and cations and set phosphorus free for the lactic acid fermentation.

However, while generally considered as antinutrient, there are positive aspects connected to the intake of phytic acid, too. Champ (2002) reviewed the ambivalent bioactive effects of phytic acid and stated that the toxicity of heavy metal ions like cadmium or lead is reduced by forming insoluble complexes with phytic acid and consequently inhibiting their uptake in the intestine. Furthermore, preventive and ameliorative effects regarding heart diseases, cardiovascular disease, and colon cancer were reported.

As the effect of phytic acid is ambivalent, neither the reduction nor the preservation should be a major focus of the beverage production. Nevertheless, the dephosphorylating of phytic acid in the substrate modulation should be achieved up to a certain degree to assure the supply of available phosphorus for the LAB fermentation.

3.1.5 | Alkaloids

Alkaloids are secondary metabolites required to repel herbivores and pests. They trigger a bitter taste and can be toxic. Depending on the substance and the consumed amount, alkaloids can lead to respiratory failure, affect the liver and the central nervous system, and might be fatal in severe cases (Schrenk et al., 2019).

Only minor total alkaloid contents with 0.013% in the cotyledon and 0.003% in the hull were reported in faba beans (Vetter, 1995). But in contrast to faba beans, alkaloids need to be considered as health risk in lupines. As this problem was recognized long ago, the alkaloid content in lupines was reduced by breeding and for almost a century so-called sweet lupines have been commercially available. While sweet lupines contain less than 500 mg/kg quinolizidine alkaloids, bitter cultivars can contain 10,000 mg/kg and more (Pilegaard & Gry, 2008). Even as the alkaloid concentration was reduced by several magnitudes, a reduced weight gain was reported for rats fed with feed containing sweet lupines (Sobotka et al., 2013). In order to avoid poisoning and health impairments, only sweet lupines should be used in the production of food and beverages. Nevertheless, the quinolizidine alkaloids are water soluble and their concentration might be increased during beverage production. Therefore, the alkaloid concentrations in the final product need to be evaluated to avoid health risks and increase consumer acceptance.

Strict regulations regarding the alkaloid content exist only in few countries. In France, Great Britain, and Australia, the total alkaloid concentration is restricted to 200 mg/kg (BfR, 2017) and in Australia an upper limit of 5 mg/kg sparteine in alcoholic beverages is regulated (Schrenk et al., 2019). However, the main alkaloids in lupines have been identified (see Figure 4), their biosynthesis is understood, and toxic concentrations are known from reports of intoxications. With further information about the toxicity of the individual quinolizidine alkaloids, maximum values in food could be defined. The different species of lupines vary in the sort and concentration of different quinolizidine alkaloids (Wink et al., 1995). Lupanine was reported as the main quinolizidine alkaloid in *L. albus*, *L. angustifolius*, and *L. mutabilis*, whereas *L. luteus* contains mainly lupinine and spartein. The latter can also be found in *L. albus* and *L. angustifolius* but account only for approximately 1% of the total quinolizidine alkaloid content (Schrenk et al., 2019). Other quinolizidine alkaloids frequently named for different lupine species are tryptophol, gramine, angustifoline, isoangustifoline, tetraombifoline, α-isolupanine, 13-α-hydroxylupanine, albine, tetrahydrorhombifoline, 13-α-angeloyloxylupanine, 13-α-tigloyloxylupanine, multiflorine, and 11,12-deidrelupanine (Musco et al., 2017; Schrenk et al., 2019; Sobotka et al., 2013; Wolko et al., 2011). According to the Bunsupa et al. (2012) review, the biosynthesis of the different quinolizidine alkaloids starts with the decomposition of the amino acid lysine to cadaverine and further toward lupanine, lupinine, sparteine, and multiflorine. Consecutive reactions like hydroxylation, oxidation, or esterification lead to the great variety of quinolizidine alkaloids in the different lupines. Until now, the European Union has not issued regulations regarding the maximum values for the quinolizidine alkaloids in food (Schrenk et al., 2019). In a recent comprehensive study issued by the EU, a total alkaloid concentration of 10−50 mg/kg bodyweight was mentioned as critical value while children might be more sensitive to lupine alkaloids and even 10 mg/kg bodyweight might be lethal (Schrenk et al., 2019). Regarding the individual quinolizidine alkaloids, multiflorine and cytisine are named as connected to the "crooked calf syndrome" (de Cortes Sánchez et al., 2005; Schrenk et al., 2019) and spartein as being the most toxic lupine-borne alkaloid (Schrenk et al., 2019). To conclude, the total amount of alkaloids in a commercial product should not exceed 200 mg/kg and, in addition, critical individual quinolizidine alkaloids like sparteine, multiflorine, or cytisine ought to be monitored.

However, legislative action is required in the EU and many countries either to declare sweet lupines as generally safe to be used in food stuff or to define limit values in a legal regulation. First, this would give a reliable basis for evaluating test results of lupine-based products. Second, it would facilitate to convince potential consumers that the alkaloid content in lupine-based products does not impair their health.

3.1.6 | Vicine / Covicine

The alkaloid glycosides vicine and covicine and their aglycones divicine and isouramil (see Figure 5) trigger a life-threatening acute hemolytic anemia in persons suffering from glucose-6-phosphate dehydrogenase (G6PD) deficiency, which is known as "favism" (Luzzatto & Arese, 2018). They appear exclusively in faba beans and in recent years, the concentrations of vicine and covicine were reduced to 10% or less in so-called low- or no-vicine/covicine cultivars by breeding (Duc et al., 1999;

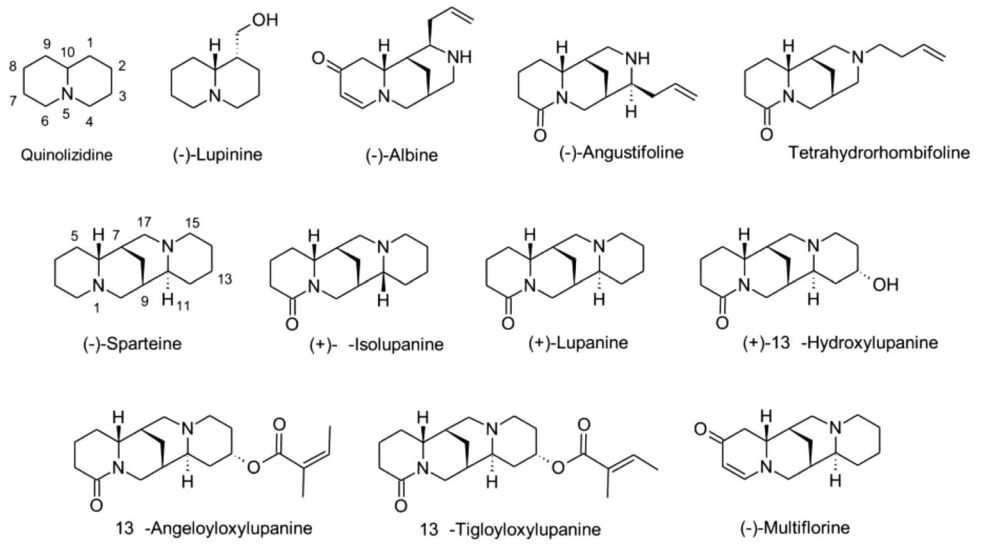

FIGURE 4 Quinolizidine alkaloids (and their core structure) relevant for human and animal consumption found in different lupine species. (Retrieved from Schrenk et al. (2019))

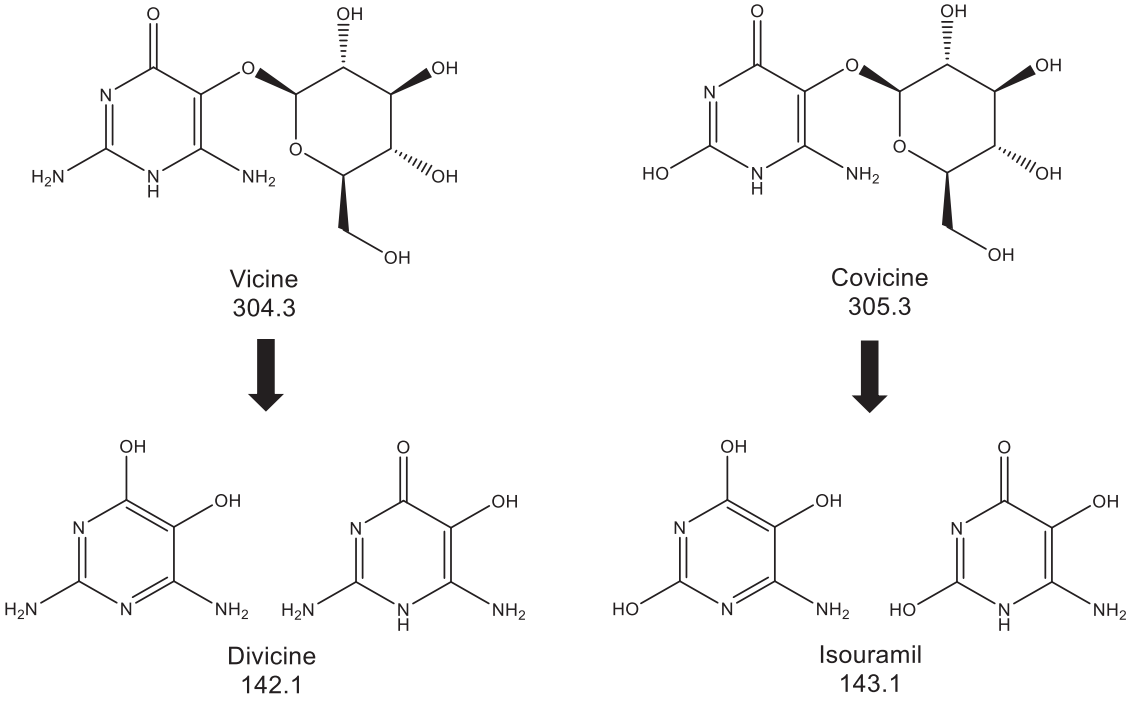

FIGURE 5 Chemical structure of the alkaloid glycosides vicine and covicine and their aglycones, divicine and isouramil. Monoisotropic molecular weights are given beneath the respective names. (Redrawn from Rizzello et al. (2016))

Khamassi et al., 2013). As the severity of favism symptoms is relative to the consumed amount of vicine and covicine (Luzzatto & Arese, 2018), the use of these new cultivars can attenuate favism attacks. However, no study exists which proves the new cultivars to be harmless for persons with G6PD deficiency and no maximum values have been legislated so far. Further research is required to assess the impact of the consumption of products made with low-vicine/covicine cultivars on the health of persons suffering from favism. Meanwhile, vicine and covicine can be lowered a great deal in faba bean products by using low or no vicine/covicine cultivars. However, to ensure that no accumulation happens during processing, those alkaloid glycosides should be monitored.

3.1.7 | Other antinutrients

Besides the antinutrients discussed extensively earlier, there are a number of further substances, which need to be addressed. Therefore, contents of tannins, lectins, trypsin inhibitors, and saponins in lupines and faba beans will be evaluated. Furthermore, it will be discussed whether they hinder the use of these two legumes in food products, if their concentrations are acceptable compared with the broadly accepted soybean, or if their impact can be mitigated by processing.

Tannins, perceived as bitter, form complexes with proteins, which lead to a decrease in protein bioavailability and to an inhibition of enzymes in the intestinal tract. In lupines, the amount of condensed tannins was described as not detectable (Mattila, Pihlava, et al., 2018; Ranilla et al., 2009) or as small as 31 mg/kg (Lampart-Szczapa et al., 2003). With 4.7–6.6 g/kg (Duc et al., 1999), the concentration of condensed tannins in faba beans is higher by several magnitudes. The tannin content can be reduced to approximately 50% by dehulling (Mattila, Pihlava, et al., 2018) or to 0.1 g/kg (Duc et al., 1999) by using new low-tannin cultivars.

In difference to the condensed tannins, literature covering the hydrolysable tannins is scarce, which might be explained due to two aspects. First, condensed tannins are the most common ones and are typically for legumes whereas hydrolysable tannins are rather being found in wood, fruits, and so on (K. Sharma et al., 2021). And second, hydrolysable tannins are degraded by gastric juice and therefor are of minor importance (Sinha & Amresh, 2018).

Lectins are proteins, which tend to aggregate erythrocytes but are also important for the symbiosis with nitrogen accumulating bacteria. Faba beans contain 0.6–1.9 g/kg lectins (Duc et al., 1999) while their hemagglutinating activity is almost negligible with 2% compared with soy (Valdebouze et al., 1980). In lupines, no lectins (Wolko et al., 2011) or hemagglutinating activity were detected (Valdebouze et al., 1980).

Trypsin inhibitors inhibit digestive enzymes, reduce protein digestion, and therefore protein bioavailability. Lupines contain only a negligible trypsin inhibitor activity of 1% compared with soy (Guillamón et al., 2008) or no activity at all (Mattila, Pihlava, et al., 2018; Torres et al., 2005; Valdebouze et al., 1980). For faba beans, trypsin inhibitory activities of 7–10% compared with soy were reported (Guillamón et al., 2008; Valdebouze et al., 1980), while other studies observed a strong inhibition of α-chymotrypsin (Mattila, Pihlava, et al., 2018). Labba et al. (2021) observed an almost 20-fold variation of the trypsin inhibitor activity in 15 different cultivars of *V. faba*. As the trypsin inhibitors are mainly located in the hull (Embaby, 2010; Mattila, Pihlava, et al., 2018), dehulling seems to be an applicable method to decrease the trypsin inhibitory activity in food processing.

Saponins can lead to a bitter taste and astringency. Moreover, they are surface active and therefore increase foaming. As they form complexes with proteins or minerals, bioavailability decreases. In high amounts, saponins can be toxic. Gulewicz et al. (2014) reviewed the topic and concluded that the saponin content in lupins and faba beans is by one magnitude less than the content in soy.

The antinutrients described earlier should not be seen as hindrance for the use of lupines and faba beans, as soy is widely accepted in the food sector and it contains those antinutrients in concentrations of at least one magnitude higher than lupines and faba beans (Guillamón et al., 2008; Gulewicz et al., 2014; Valdebouze et al., 1980).

In addition to the antinutrients discussed earlier, the allergic potential of lupine protein needs to be mentioned. As an allergen, it can trigger skin irritation, respiratory distress, and even life-threatening anaphylactic shock. Especially, consumers with a known allergy toward peanuts need to be aware, as cross-allergies between lupine and peanut protein occur in many cases. Within the European Union, it needs to be declared when used in food (BfR, 2011).

3.2 | Technological modulation of legume-based substrates

LAB are often used to reduce antinutrients like RFOs or to reduce certain aroma compounds (e.g., hexanal), which results in a more acceptable overall aroma of the legume products (J. Frias et al., 1996; Fritsch et al., 2015; Harlé et al., 2020; Kaczmarska et al., 2018; Rizzello et al., 2016). However, as only a limited number of strains are capable of metabolizing the specific antinutrients (e.g., RFOs) to a high degree, the strain selection is restricted. Regarding the RFO degradation, Harlé et al. (2020) found that out of 276 tested LAB strains, only 36% were able to grow on raffinose and only 6% to metabolize stachyose. Therefore, by pretreating legumes to reduce the concentrations of RFOs, phytic acid, and quinolizidine alkaloids (only lupines) in the obtained substrate, the strain selection can be based exclusively on the resulting aroma. A further advantage of a pretreatment is the hydrolyzing of proteins, which leads to a higher protein content in the beverage and the supply of fermentable nitrogen for the LAB. Consequently, a combination of the process steps such as germination, heat treatment, and enzymatic treatment can be applied to degrade the antinutrients and hydrolyze the protein while transforming the solid seeds into a liquid substrate (compare Figure 6).

In the production of beverages, a variety of basic technologies is usually combined to solubilize and modify ingredients, to reduce microbial contaminations, to stabilize the final product, or to let the product be more appealing to the customer. Such process steps can be soaking, blanching, grinding (wet or dry), extraction, heating, roasting, pasteurization, standardization, homogenization, separation of solids (filtering, sedimentation, centrifugation, etc.), flavoring, packaging, and many more (Cichońska & Ziarno, 2022; Veber et al., 2021). Furthermore, the impact of the receipt on the final product needs to be mentioned. The combination of different raw materials, the addition of flavorings, sugars, vitamins, or minerals, and so on highly affect the nutritional value and the aroma/taste of the final product. As this chapter is focused on the substrate modulation for a subsequent LAB fermentation, the most relevant technical processes germination, heat treatment, and enzymatic treatment will be discussed. The studies concerning germination and heat treatment of native lupines and faba beans are listed in Table 5. To our knowledge, no study exists regarding the enzymatic treatment of native lupines or faba beans.

3.2.1 | Germination

The germination of seeds is a widely used method to increase their enzymatic activity and leads to profound changes in the composition of nutrients and antinutrients. Germination combines the leaching out of antinutrients (quinolizidine alkaloids) with the increasing enzymatic activity, which mobilizes storage compounds (proteins) for the growth of the seedling.

In different species of lupines and faba beans, germination led to a strong decrease of 77–100% of the RFO content combined with an initial increase of sucrose (Chilomer et al., 2010; Dagnia et al., 1992; de la Cuadra, 1994; Khalil & Mansour, 1995; Wei, 2019). As the RFOs are degraded into mono- and disaccharides (Bellaio et al., 2013; Frias, Diaz-Pollan, et al., 1996; Martinez-Villaluenga et al., 2008), fermentable sugars are provided for a subsequent LAB fermentation. Additionally, the content of phytic acid was reduced by germination (Dagnia et al., 1992; de la Cuadra, 1994; Goyoaga et al., 2011; Honke et al., 1998; Vidal-Valverde et al., 1998). However, phytic acid was not degraded entirely by germination but up to 20–66% in lupines (Dagnia et al., 1992; de la Cuadra, 1994; Honke et al., 1998) and up to 37–81% in faba beans (Honke et al., 1998; Y.-W. Luo & Xie, 2013; Y. Luo et al., 2012). de la Cuadra (1994) showed that the phytase activity varies according to the lupine species and cultivar.

Furthermore, reductions are achievable in the concentrations of condensed tannins (Rami Akkad et al., 2021; Khalil & Mansour, 1995; A. Sharma & Sehgal, 1992), of trypsin inhibitors (A. Sharma & Sehgal, 1992) and of vicine/covicine in faba beans (Khalil & Mansour, 1995; Wei, 2019). Contradictive findings are reported from Goyoaga et al. (2008), as vicine/covicine degrade in cotyledons and in the embryo axis but not in the whole germs. As a negative aspect, germination affects protein bioavailability. This is generally due to a loss of about one-third of the total sulfurous amino acids (Dagnia et al., 1992). Cysteine, in particular, was depleted almost entirely in lupines (Chilomer et al., 2010). In addition to the germination itself, the improved enzymatic activity can be used to decompose antinutrients in a subsequent enzymatic treatment.

For cereals, Nsogning Dongmo et al. (2016) summarized that substrates from malted (germinated and dried) cereals promote the growth of LAB compared with substrates from raw cereals. This can be explained by the increased enzymatic activity during germination. Macromolecules like proteins, oligosaccharides, and so on are enzymatically decomposed to low molecular mass compounds like amino acids, sugar monomers, and so on, which are easily accessible to the metabolism of microorganisms.

Germination also changes the aroma and taste of legume seeds. For lupines, an increase of sweet (2,3-butanedione, methyl isobutyl ketone, guaiacol), meaty (2-methylbutanal), and sulfur-like aromas (DMS, DMTS) and reductions of compounds associated with the beany aroma impression (hexanal) was reported (Kaczmarska et al., 2018). Akkad et al. (2021) showed that compounds connected to the beany aroma (hexanal, nonanal, and 2-pentylfuran) decrease during germination of faba beans. However, both authors used only semiquantitative approaches. As the two fractions of the total peak area in a chromatogram of two different aroma compounds are not comparable in terms of absolute amounts or concentrations in the sample, measurements with a calibrated and validated method should substantiate those findings.

Roland et al. (2017) reviewed the topic generally for pulses and confirmed reductions in these aroma impressions. Nevertheless, they also pointed out that the content of saponins and phenolics increased during germination, and concluded that the possible reduction of the overall beany off-flavor might come along with an increase of bitterness and astringency. It should be mentioned that the fatty acid profile is also affected by germination. Comparing raw seeds with nonfermented bean-based milk substitute, Ziarno et al. (2020) observed a significant decrease of the polyunsaturated fatty acids (PUFA) linoleic acid and α-linolenic acid from 39.26% to 33.59% and 23.25% to 18.87%, respectively. At the same time, the production of saturated fatty acids increased. This is of special interest as both PUFAs are precursors for beany off-flavors and

TABLE 5 Changes identified upon substrate modulation for native lupines and faba beans[1]

Legume	Treatment	Observed changes	Reference
L. angustifolius cv. Graf *L. luteus* cv. Lord	Germinated at 15/24°C for 2–4 days in darkness; Seeds were sterilized for 30 min in sodium hypochlorite (0.25%), washed, and soaked in distilled water for 6 h	After 4 days of germination at the two temperatures, the RFOs decreased between 87–91% (*L. angustifolius*) and 83%–87% (*L. luteus*). Alkaloids decreased by 27–32% (*L. angustifolius*) and 17–31% (*L. luteus*) Nitrogen content increased by 3.5–3.9% (*L. angustifolius*) and 3.0–4.5% (*L. luteus*) A strong decrease of the sulphurous amino acid cysteine was observed.	(Chilomer et al., 2010)
L. angustifolius cv. Gungurru	Germinated at 20–25°C for 6 days under natural light; Seeds were initially soaked for 24 h in tap water and rinsed/drained daily.	After germination, the kernels (dehulled) showed negligible amounts of RFOs, the nitrogen content increased by 10%, phytic acid decreased by 66%, alkaloids decreased by 78%, and the total sulphurous amino acids decreased by 32%, which led also to a reduced protein bioavailability.	(Dagnia et al., 1992)
L. albus *L. luteus* (variety not mentioned)	Germinated at 20°C for 4 days with 8 h of light per day; Seeds were placed on moist paper	The alkaloid content increased in the first days and fell to a value slightly lower (*L. albus*) or higher (*L. luteus*) than the initial amount. The RFOs decreased to non-detectable values (*L. albus*) or by 95% (*L. luteus*). Phytic acid was reduced by 38% (*L. albus*) and 22% (*L. luteus*).	(de la Cuadra, 1994)
V. faba (variety not mentioned)	Germinated at ambient temperature for 3 days in darkness; Seeds were sterilized in ethanol for 1 min and soaked in distilled water (approx. 25°C) for 12 h.	The nitrogen content increased by 4%, stachyose was reduced to non-detectable amounts, tannins decreased by 29%, phytic acid decreased by 54%, vicine and covicine decreased by 28%, the activity of trypsin inhibitors decreased by 32% and the haemagglutinin activity decreased by 80%.	(Khalil & Mansour, 1995)
L. angustifolius var. zapaton	Germinated for 2–9 days at 20°C in darkness; Seeds were sterilized for 30 min in sodium hypochlorite (0.07%), washed, and soaked in distilled water for 5.5 h.	After 9 days of germination the free amino acids increased to the 26-fold, with asparagine accounting for 51% of the total free amino acids.	(Cristina Martínez-Villaluenga et al., 2006)
L. luteus var. Juno *V. faba* var. Tipo	Germinated for 1–8 days at 25°C in wet filter paper. A light regime was not specified.	The phytic acid content decreased by 42% in lupines and 78% in faba beans in 8 days of germination. An initial increase of the lower phosphorylated myo-inositols IP3–IP5 was observed, followed by a decrease.	(Honke et al., 1998)
V. faba var. major	Germinated for 6 days at 20°C in darkness; Seeds were soaked in distilled water for 6 h at ambient temperature and daily moistened.	At 6 days of germination the starch content decreased by 15%, the mono- and disaccharides by 27%, the phytic acid content by 55%, and the RFOs to non-detectable values.	(Vidal-Valverde et al., 1998)
L. luteus var. Juno *V. faba* var. Tipo	Germinated for 1–8 days at 25°C in wet filter paper. A light regime was not specified.	The phytic acid content decreased by 42% in lupines and 78% in faba beans in 8 days of germination. An initial increase of the lower phosphorylated myo-inositols IP3–IP5 was observed, followed by a decrease.	(Honke et al., 1998)

(Continues)

TABLE 5 (Continued)

Legume	Treatment	Observed changes	Reference
V. faba var. Brocal *V. faba* var. Alameda	Germinated for 0.5–9 days at 20°C with 8 h of light per day in wet sand; Seeds were rinsed daily. Cotyledons and embryo axis were analyzed separately.	In cotyledons, vicine and covicine decreased by 28% and 51% (var. Alameda) and by 40% and 51% (var. Brocal). In the embryo axis, vicine decreased stongly by 81% (var. Alameda) and 82% (var. Brocal), while covicine increased to 218% (var. Alameda) and 269% (var. Brocal) until day 5 and decreased afterwards to values of 133% (var. Alameda) and 120% (var. Brocal) of the initial concentration. For the whole seedling values were stated of vicine for 1% above (var. Alameda) and 17% beneath (var. Brocal) the initial concentration while for covicine increases of 133% (var. Alameda) and 120% (var. Brocal) were reported. They also observed increases of L-DOPA to the 38.7-fold and 27.4-fold in the embryo axis of var. Alameda and var. Brocal, respectively. The L-DOPA concentration in the whole seed increased to the 904-fold (var. Alameda) and 1019-fold (var. Brocal). No information is given for the L-DOPA content in the cotyledons.	(Goyoaga et al., 2008)
V. faba var. Brocal *V. faba* var. Alameda	Germinated for 0.5–9 days at 20°C with 8 h of light per day in wet sand; Seeds were rinsed daily. Cotyledons and embryo axis were analyzed separately.	During 9 days of germination the RFOs in the cotyledons decreased by 94% (var. Brocal) and 90% (var. Alameda), while the sum of mono- and disaccharides increased by 88% (var. Brocal) and 48% (var. Alameda). At the same time the RFOs in the embryo axis decreased by 79% (var. Brocal) and 94% (var. Alameda). The mono- and disaccharides increased by 2% (var. Brocal) and decreased by 24% (var. Alameda). Thereby, fructose (25-fold in var. Brocal; 12-fold in var. Alameda), glucose (7-fold in var. Brocal; 8-fold in var. Alameda), and sucrose (145% in var. Brocal; 78% in var. Alameda) increased while galactose decreased (13% in var. Brocal; 27% in var. Alameda) in both varieties. Only melibiose increased in var. Brocal (191%) and decreased in var. Alameda (57%). The phytic acid content showed a slight increase with a subsequent decrease to approximately the initial level in the cotyledons of both varieties. In the embryo axis phyic acid was almost entirely reduced within 5–6 days. In the total protein extract and the legumin fraction, a reduction of the molecular weight was observed	(Goyoaga et al., 2011)
L. luteus var. Juno *V. faba* var. Tipo	Germinated for 1–8 days at 25°C in wet filter paper. A light regime was not specified.	The phytic acid content decreased by 42% in lupines and 78% in faba beans in 8 days of germination. An initial increase of the lower phosphorylated myo-inositols IP3–IP5 was observed, followed by a decrease.	(Honke et al., 1998)
V. faba var. major	Germinated for 6 days at 20°C in darkness; Seeds were soaked in distilled water for 6 h at ambient temperature and daily moistened.	At 6 days of germination the starch content decreased by 15%, the mono- and disaccharides by 27%, the phytic acid content by 55%, and the RFOs to non-detectable values.	(Vidal-Valverde et al., 1998)

(Continues)

TABLE 5 (Continued)

Legume	Treatment	Observed changes	Reference
V. faba cv. Qidou 2	Germinated for 1–5 days at 30°C. Seeds were soaked for 24 h in demineralized water at 25°C. Different light regimes (red, yellow, orange, blue, white, ultraviolet, and dark) were applied.	The phytic acid content reached a minimum for all light regimes after 72 h of germination and increased slightly afterward. The reductions for the different light regimes at the 3rd day were 18% (dark), 35% (red), 21% (yellow), 21% (orange), 37% (blue), 21% (white), 19% (ultraviolet).	(Y.-W. Luo & Xie, 2013)
V. faba (variety not mentioned)	Germinated for 3 and 5 days at 25°C in darkness at moist filter paper. The seeds were soaked in a first with/without gibberellic acid for 12 or 24 h.	In general, the addition of gibberellic acid led to a less intense decrease of phytic acid in all variation of soaking or germination time. Without gibberellic acid the phytic acid content was reduced after 12 h of soaking from 38% (3 d) to 63% (5 d) and after 24 h of soaking from 38% (3 d) to 50% (5 d). With the addition of gibberellic acid the reductions were after 12 h of soaking form 25% (3 d) to 44% (5 d) and after 24 h of soaking from 25% (3 d) to 38% (5 d).	(Y. Luo et al., 2012)
V. faba var. Snowbird *V. faba* var. Snowdrop *V. faba* var. Fabelle *V. faba* var. FB9-4	Germinated at 25°C for 2–3 days; Seeds were rinsed and soaked in tab water for 16 h. Afterward, they were sterilized for 10 min in hydrogen peroxide (55 ppm), and rinsed before transferring into the germination trays where they were they were covered with moist cloth.	Upon 3 days of germination, the tannin content decreased from 6.5 to 1.2 catechin activity equivalents in the high-tannin variety Fabelle. The protein content increased significantly in the low-tannin variety Snowbird (24.0–25.8 %) and non-significantly in the high-tannin variety Fabelle (27.1–28.4 %). The aroma profile (HS-SPME GC-MS, semi-quantitative) changed during germination. Aromatic hydrocarbons, aldehydes, alkanes, and alkenes decreased in peak area while alcohols and ketones increased. For the aldehydes hexanal and nonanal related to a beany aroma impression, they observed a initial decrease (until 48 H) followed by an increase, which might result in an increasing off-flavor perception.	(Rami Akkad et al., 2021)
Australian sweet lupin (no further specified)	Germination at approx. 22°C for 72 h covered with germination paper. The light regime was 12 h of natural daylight and 12 h of darkness. Seeds were sterilized for 30 min in hydrogen peroxide (3%), rinsed in deionized water, and soaked for in purified water for 8 h	An increase of the aroma compounds 2-methylbutanal, dimethyl sulfide, dimethyl trisulfide, 2,3-butanedione, methyl isobutyl ketoe, (E,Z)–2,6-nonadienal, and guaiacol was measured upon germination. Also, the perception of compounds with a beany and/or green impression decreased. In sensory analysis, a decrease of beany impressions was observed, too.	(Kaczmarska et al., 2018)
Vicia faba (variety not mentioned)	Cooking: Seeds were soaked in distilled water for 12 h at 25°C, rinsed, and cooked in tap water for 45 min Autoclaving: Seeds were soaked in distilled water for 12 h at 25°C, rinsed, and autoclaved (121°C) in tap water for 30 min	Upon cooking the nitrogen content remained constant, stachyose was reduced by 47%, tannins decreased by 55%, phytic acid decreased by 31%, vicine and covicine decreased by 35%, the activity of trypsin inhibitors decreased by 72% and the haemagglutinin activity was reduced to non-detectable values. By applying autoclaving, the nitrogen content decreased by 6%, stachyose was reduced by 21%, tannins decreased by 60%, phytic acid decreased by 41%, vicine and covicine decreased by 40%, the activity of trypsin inhibitors decreased by 84% and the haemagglutinin activity was reduced to non-detectable values.	(Khalil & Mansour, 1995)

(Continues)

TABLE 5 (Continued)

Legume	Treatment	Observed changes	Reference
V. faba var. VH-131 *V. faba* var. WF	Germinated at 37°C for 24–48 h; Seeds were soaked and kept on moist filter paper.	The trypsin inhibitor activity decreased by 64–65% and the concentration of tannins by 90–91%, in 2 days of germination.	(A. Sharma & Sehgal, 1992)
Vicia faba (variety not mentioned)	Cooking: Seeds were soaked in distilled water for 12 h at 25°C, rinsed, and cooked in tap water for 45 min Autoclaving: Seeds were soaked in distilled water for 12 h at 25°C, rinsed, and autoclaved (121°C) in tap water for 30 min	Upon cooking the nitrogen content remained constant, stachyose was reduced by 47%, tannins decreased by 55%, phytic acid decreased by 31%, vicine and covicine decreased by 35%, the activity of trypsin inhibitors decreased by 72% and the haemagglutinin activity was reduced to non-detectable values. By applying autoclaving, the nitrogen content decreased by 6%, stachyose was reduced by 21%, tannins decreased by 60%, phytic acid decreased by 41%, vicine and covicine decreased by 40%, the activity of trypsin inhibitors decreased by 84% and the haemagglutinin activity was reduced to non-detectable values.	(Khalil & Mansour, 1995)
V. faba var. major	Cooking: Seeds were soaked in distilled water for 9 h and afterward cooked in distilled water for 35 min. Dry heating: Seeds were ground and heated at 120°C and 1 atm for 15 min.	Upon cooking, the starch content decreased by 7%, the mono- and disaccharides by 35%, the phytic acid remained unaffected, and the RFOs decreased by 25%. Due to dry-heating the starch content decreased by 5%, the mono- and disaccharides by 26%, the phytic acid content by 36%, and the RFOs decreased by 57%.	(Vidal-Valverde et al., 1998)
V. faba var. VH-131 *V. faba* var. WF	Cooking: First, seeds were soaked for 12 h at 37°C, dried in hot air (oven) at 70°C. Prior to drying, a fraction of soaked seeds were dehulled. Cooking was performed in water until they were soft. Autoclaving: First, seeds were soaked for 12 h at 37°C, dried in hot air (oven) at 70°C. Prior to drying, a fraction of soaked seeds were dehulled. The seeds were then autoclaved in water at 15 lbs/inch^2 (≈2 bar absolute pressure) for 15 and 25 min.	Cooking of the hulled seeds reduced the trypsin inhibitor activity by 50–56% and the tannin content by 58–65%. The dehulling prior to cooking increased the effect to 75–76% and 76–81%, respectively. It should be mentioned that soaking without cooking reduced the trypsin inhibitor activity by only 4–5% (10% when additionally dehulled) and the tannin content by 42–51% (70–73% when additionally dehulled). Autoclaving for 15 min reduced the trypsin inhibitor activity by 85–88% (92–94% when additionally dehulled) and the tannin content by 59–71% (80–82% when additionally dehulled). Increasing the time to 25 min increased the reduction to 95% (97–98% when additionally dehulled), and 68–76% (93–95% when additionally dehulled), respectively.	(A. Sharma & Sehgal, 1992)

(Continues)

TABLE 5 (Continued)

Legume	Treatment	Observed changes	Reference
V. faba var. major	Cooking: Seeds were soaked in distilled water, 0.1% citric acid solution, or 0.07% sodium bicarbonate solution, and cooked in bidistilled water for 35 min. Dry heating: Seeds were ground and heated at 120°C and 1 atm for 15 min.	According to the authors, the phytic acid content was reduced by dry heating (40%) and soaking in acid solution followed by cooking (30%), only. In addition to the cooking, the study also covered the impact of the three soaking methods without cooking. Hereby, the authors state no impact on the phytic acid content.	(Fernández et al., 1997)
Vicia faba (variety not mentioned)	Cooking: Seeds were soaked in distilled water at ambient temperature for 4 h, rinsed and cooked at 95°C in water for 1 h. This treatment was performed with whole and split seeds.	Due to soaking and cooking the hemagglutinin activity decreased strongly by 98% (split and whole), phytic acid by 19% (whole) and 38% (split), and the total oxalate content by 32% (whole) and 46% (split). The impact of soaking without cooking was observed, too. There the decreased were less intense and accounted for 1% (whole) and 5% (split) of the hemagglutinin activity, for 1% (whole) and 3% (split) of the phytic acid content, and for 17% (whole) and 37% (split) regarding the oxalate content.	(Shi et al., 2018)
V. faba var. Kontu	Microwave heating: For microwave heating, seeds were heated in 30 s intervals at 950 W followed by manual stiffing. The total microwave heating was for 1–4 min. Dry heating: Seeds were heated at 170°C for 30 min.	Both, microwave and dry heating inactivated LOX and peroxidase activity to non-measurable values. Protein solubility in water (87% in untreated sample) was slightly reduced by dry heating (to 80%). While in microwave heating, the solubility was unaffected for the first minute but declined strongly until minute 4 (< 20%). Microwave heating and to a lesser extend dry heating reduced the particle size after milling, which indicates a better milling result after heat treatment. Longer heat treatments (dry heating and > 1 min microwave heating) showed strong increases of hexanal, and increases in nonanal and 2-heptanon. Especially the changes in the hexanal content where connected to a rapid autoxidation of fatty acids.	(Jiang et al., 2016)

[1]To our knowledge, no study exists regarding the enzymatic treatment of native lupines or faba beans.

prone to autoxidation during storage. As the content of this precursor is reduced, the germinated seeds might be less affected by autoxidation, and the generation of off-flavors upon storage might be reduced.

Germination should be regarded as a highly valuable process step in the substrate modulation for the production of lactic acid fermented beverages as it increases the enzymatic activity, reduces antinutrients, and improves the fermentability of the substrate. However, the impact of germination on the sensory characteristics of legumes seems to be ambiguous and requires further research.

3.2.2 | Heat treatment

Thermal treatment needs to be considered in two steps of the substrate preparation. First, the drying of the germinated seeds is essential to prolong shelf-life after germination and to make the germinated seeds millable. Second, it is needed to reduce possible microbial contamination in the legume-based substrate by pasteurization prior to fermentation. Heat treatment did not reduce the beany aroma impression, as fatty acids are also prone to nonenzymatic autoxidation and hence, the sole inactivation of lipoxygenase is not sufficient (Stephany et al., 2016). Those findings are supported by Jiang et al. (2016), who reported that the activity of lipoxygenase was successfully reduced to non-detectable levels by heat treatment, but nevertheless the autoxidation of unsaturated fatty acids into aroma compounds like hexanal accelerated with increasing temperature or time of the heat treatment. Roasting at higher temperatures might contribute additional positive aroma compounds like pyrazines or β-damascenon (Vandecan et al., 2011). Nevertheless, it would decrease the enzymatic activity in the legume malt. This would affect the use of the legume-borne enzymes in a possible subsequent enzymatic treatment. Whether novel aroma components from roasting or a high enzymatic activity are of higher interest depend on the design of the overall process and on the producers or customers' attitude toward the use of technical enzymes in a possible enzymatic treatment. Moreover, the combination of legume malt roasted at higher temperatures with malt dried at lower temperatures to uphold high enzymatic acitivities should be considered. The further process would benefit from a higher enzymatic activity and the resulting beverage from additional positive aroma impressions.

Heat treatment of the liquid substrate (such as pasteurization) might be required to reduce microbial contamination and therefore facilitate monoseptic LAB fermentation with an aroma-specific selected strain. The choice of temperature and exposure time depends on the microbial contamination of the legume seeds, the loss of protein in the substrate by denaturating, and the aroma compounds formed by the Maillard reaction. Moreover, pasteurization led to a noticeable increase of hexanal in protein extracts (Schindler et al., 2012). In general, a reduction of antinutrients was reported for tannins (Khalil & Mansour, 1995), vicine and covicine (Khalil & Mansour, 1995), and phytic acid (Fernández et al., 1997; Khalil & Mansour, 1995; Shi et al., 2018). While no changes of phytic acid, vicine, and covicine were perceived during autoclaving (Wei, 2019), drying might affect the germinated seeds and pasteurization the legume-based substrate, but they are necessary for their storability and the exclusion of possible legume-borne contaminations.

3.2.3 | Enzymatic treatment

An enzymatic treatment can be applied to degrade antinutrients, specifically in legumes. The use of enzymes in order to modulate the substrate for the consecutive microbiological fermentation is an inherent part in the process of brewing beer and profoundly understood. In substrate production (so-called mashing), the basic idea is to create optimal temperature and pH conditions for the use of barley malt enzymes. For instance, amylolytic activity is increased by adjusting the temperature in order to degrade the starch to sugars fermentable by yeast (Kunze, 2016). Besides that, enzymatic hydrolysis increases the solubility of proteins, as shown for lupine protein isolate where the solubility in water at pH 5 was increased from 75 to 84.5% by treating with technical enzymes (Schlegel et al., 2019). Nevertheless, enzymatic hydrolysis in soy led to an increase in bitterness (Yoo & Chang, 2016). This is according to the commonly accepted theory that bitter peptides are first formed by protein hydrolysis and then degraded into less bitter amino acids and shorter peptides. Those findings are backed by Whitehurst et al. (2002), who recommended the proper selection of reaction parameters and enzymes in order to further degrade the peptides and thus reduce bitterness. The use of technical enzymes showed that bitterness can be predetermined by choosing the right enzyme (Schlegel et al., 2019).

Further research is required to understand whether a similar procedure might be adapted to reduce the contents of phytic acid and RFOs in the legumes and to increase the solubility of their proteins while producing a liquid substrate. For the enzymatic treatment, legume-borne enzymes could be used if they show sufficient activity and also technical enzymes might be added to increase the overall activity. The advantage could be that by varying the exposure time at a specific temperature, the substrate might be modulated as the activity of the different enzymes could be regulated. As a prerequisite,

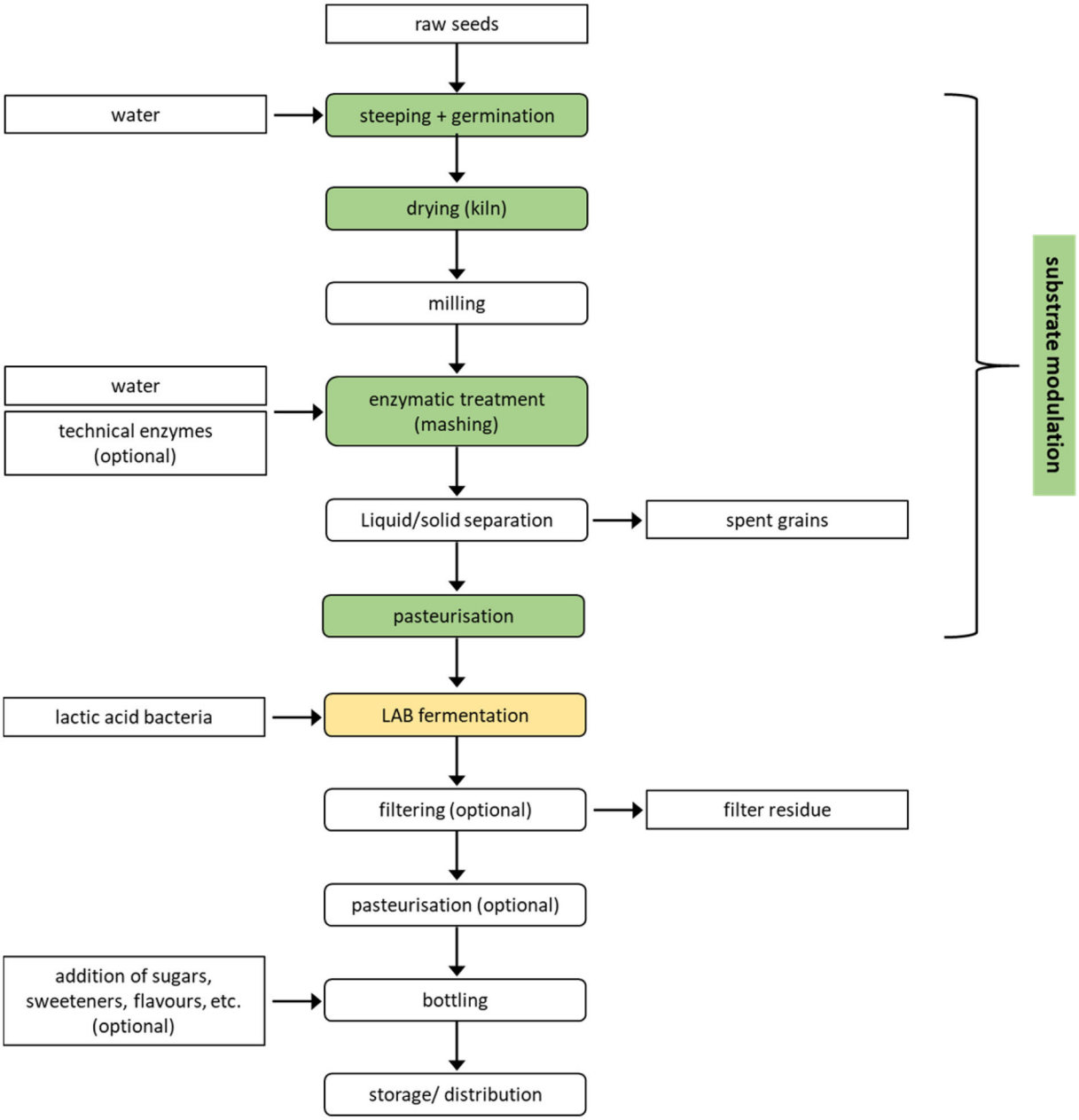

FIGURE 6 Flow chart showing the main process steps in the production of a LAB fermented refreshing beverage using lupines or faba beans. The raw seeds are steeped in water and germinated. Afterward, the seedlings are dried and milled. The resulting flour or grist is steeped in water. By adjusting the temperature and pH, the enzymatic activity can be regulated. Therefore, ingredients (proteins, starch, etc.) are solubilized and degree of degradation can be influenced. The enzymatic activity can be improved further by adding technical enzymes. The liquid substrate is separated from the solid particles and the remaining enzymatic activity as well as legume-borne microorganisms are removed by pasteurization. The subsequent LAB fermentation improves the aroma and taste of the beverage and adds a refreshing character. The microorganisms either remain as probiotic culture in the beverage or are removed in an optional filtration step, which might be followed by another pasteurization. Prior to bottling, the addition of flavors, sugars, and so on might be considered to customize the final beverage. The most relevant process steps of the substrate modulation (germination, heat treatment, and enzymatic treatment) are indicated in green and the subsequent LAB fermentation in orange

the temperature and pH optima of the particular enzymes need to be known. By modulating a schedule with specific temperatures, enzyme activities would be systematically influenced, antinutrients might be reduced, and proteins might be degraded to soluble and less bitter peptides and amino acids while producing a liquid substrate for a subsequent LAB fermentation (Kunze, 2016; Narziß & Back, 2009).

To conclude, the technological modification of the raw legumes should be regarded as a combined process rather

than individual modifications. By germination, the decomposition of RFOs, phytic acid, and other antinutrients can be achieved at least partially while increasing the solubility of proteins. A drying step is required afterward in order to prolong the shelf-life of the legume malt, which adds aroma compounds from the maillard reaction. As the resulting product is a beverage, the dry malt needs to be transformed into a liquid substrate prior to LAB fermentation. This transformation can be combined with an enzymatic treatment by applying a mashing process. Thereby, the legume-borne enzymes whose activity is increased during germination can be used and low or missing activities could be counterbalanced by adding technical enzymes. This would not only improve the degradation of antinutrients in general, but allows to degrade certain compounds in the malt specifically. According to the microbial contamination and remaining enzymatic activity in the liquid substrate, pasteurization is required before it is inoculated with LAB.

4 | MODIFICATION OF THE SENSORY PROFILE BY LACTIC ACID FERMENTATION

Lactic acid fermentation can improve two different aspects of the sensory profile. The aroma, which is perceived orthonasally or retronasally, can be changed in two ways. First, by reducing the beany off-flavors themselves beneath their odor threshold. Second, by creating new aroma compounds, which could not only mask the beany and green odor notes but also alter the overall aroma impression. Regarding the taste, the use of LAB can reduce a possible bitterness and sweetness in the substrate and increase acidic or umami impressions.

4.1 | Modification of the aroma

Lactic acid fermentation is a generally accepted process in the food industry and can improve the overall aroma impression of beverages based on lupines and faba beans. The aroma improvement by LAB fermentation was described for lupines (Schindler et al., 2011), soy (Harlé et al., 2020; Li et al., 2014), and peas (El Youssef et al., 2020; Schindler et al., 2012). The beany off-flavor was either reduced or masked by new aroma compounds created by the metabolism of the microorganism (2,3-butanedione, pentanoic acid, phenylethanol), and therefore the aroma was improved in general (Harlé et al., 2020; Schindler et al., 2011, 2012).

The modifications of the aroma profile differ highly with the substrate and the LAB strain employed for the fermentation. Firstly, this could be explained with the varying composition of aroma compound and precursors of the different substrates. Secondly, the different enzymatic properties of the different LAB lead to numerous metabolic pathways and therefore to a variety of resulting aroma compounds. Nevertheless, the fermentation parameters (temperature, pH, etc.) also influence the resulting aroma spectrum, which should be considered when comparing the results of different studies. For soy as substrate, a strong decrease of hexanal was reported for several LAB strains (Blagden & Gilliland, 2005; Zhu et al., 2019), whereas Harlé et al. (2020) pointed out that hexanal was reduced by *L. plantarum* and increased by *S. thermophilus*. A decrease was observed in hexanol and an increase of hexanoic acid with a mix of *L. paracasei* and *L. rhamnosus* (Zhu et al., 2019). Increasing concentrations in hexanoic acid were also reported for *L. plantarum* and *S. thermophilus* in soy (Harlé et al., 2020). In lupines and peas protein, decreases in hexanal were reported with *L. plantarum* (Schindler et al., 2011, 2012). Both increases of hexanal and the perception of a beany and green aroma were reported for a mixed culture of *L. delbrueckii ssp. bulgaricus* and *S. thermophilus* (Kaczmarska et al., 2018). For the fermentation of pea protein isolate with a mixed LAB culture, strong reductions in hexanal content and slight increases in hexanol concentration were observed (El Youssef et al., 2020). Using glucose-supplemented peanut milk, *S. salvivarius ssp. thermophilus* showed a strong capacity in reducing hexanal, while *L. delbrueckii ssp. bulgaricus* was less effective (Lee & Beuchat, 1991). In the LAB fermentation of sourdough, hexanal and hexanol decreased, while hexanoic acid increased strongly. Moreover, it was found that heterofermentative LAB strains show a higher ability to reduce aldehydes than homofermentative strains (Kaseleht et al., 2011). This indicates that first , the choice of the strain needs to be considered as it is highly important for achieving the desired aroma (Harlé et al., 2020) and second, the metabolic pathways of the hexanal reduction in LAB needs to be assessed further. Nevertheless, LAB fermentation proved to be an applicable method to improve the aroma of legume-based substrates.

While changes in the aroma compounds are described in the literature, the specific pathways and metabolic products of hexanal reduction remain partially uncertain. Hexanal may possibly react toward hexanol and hexanoic acid and successively to different esters (see Figure 7). Presečki and Vasić-Rački (2009) researched the reaction kinetics of the hexanol oxidation catalyzed by alcohol dehydrogenase in yeast. They concluded that the reduction of hexanal toward hexanol is a reversible reaction, while the reaction of hexanal toward hexanoic acid is nonreversible. The equilibrium of the reaction is directed toward the reduction of hexanal. As hexanal reacts reversibly

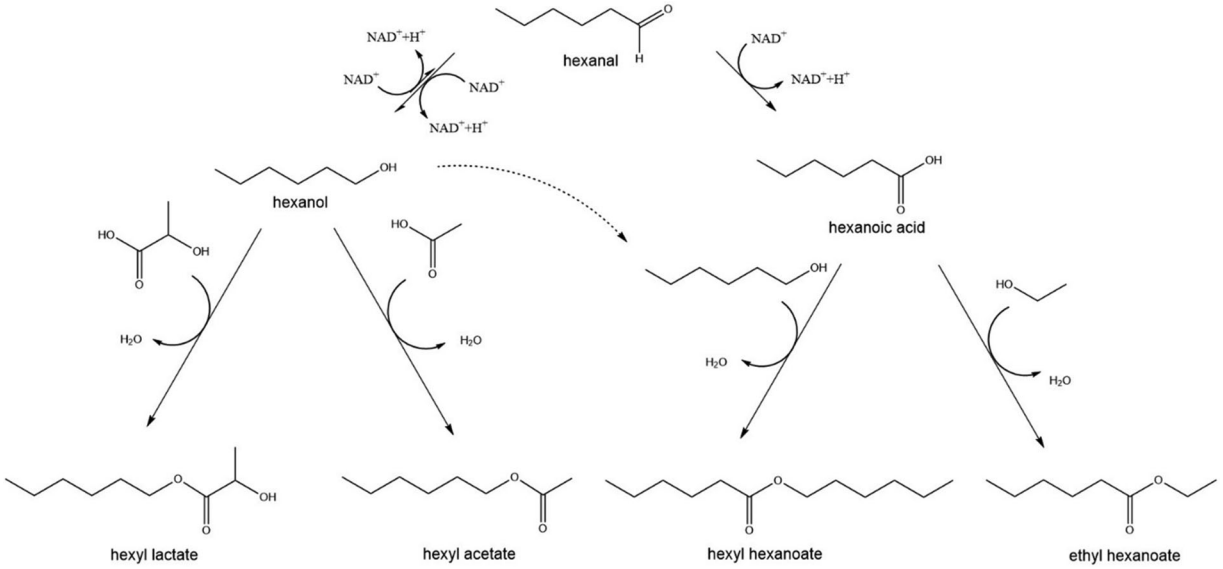

FIGURE 7 Hypothetical pathways of the degradation of hexanal in LAB. Hexanal reacts reversibly to hexanol and irreversibly to hexanoic acid (Presečki & Vasić-Rački, 2009). Further esterification might result with ethanol in ethyl hexanoate, with acetate to hexyl acetate, and with lactate to hexyl lactate. Both degradation products, hexanol and hexanoic acid, might react to hexyl hexanoate

toward hexanol but nonreversibly toward hexanoic acid, an accumulation of hexanoic acid occurred (Presečki & Vasić-Rački, 2009; Šalić et al., 2013). As decreases in hexanal together with strong increases in hexanoic acid and changes in the hexanol concentration were also reported for LAB (Harlé et al., 2020; Kaseleht et al., 2011; Li et al., 2014), a likewise enzymatic system should be present at least in some LAB strains, too. This is backed by the identification of five alcohol dehydrogenases in *L. reuteri*, which catalyze the interconversion between aldehydes and alcohols (Hu et al., 2019). The esters hexyl acetate and ethyl hexanoate (Kaseleht et al., 2011) were reported as further metabolic products in the lactic acid fermentation. As hexanol and hexanoic acid are formed during the degradation of hexanal, hexyl hexanoate should be considered as possible reaction product, too. Ethyl lactate was mentioned in connection with LAB fermentation (Nsogning Dongmo et al., 2016). Therefore, hexyl lactate might be considered as a possible product in the ester formation of LAB in legume-based substrates. The synthesis of short chain fatty acid esters (e.g. ethyl butanoate) by *L. lactis* was influenced strongly by reducing the water activity. This is as the esterases catalyze both directions of the reaction (hydrolyzing and synthesizing esters) depending on environmental conditions (Nardi et al., 2002). Further research is required to prove whether the reactions toward hexyl acetate, ethyl hexanoate, hexyl hexanoate, and other esters can be influenced by changing the fermentation parameters.

Besides the choice of strain, the variation of the fermentation parameters has a profound impact on the aroma compounds produced by LAB (compare Table 6). Due to the induced stress on the cell culture, shifts in the metabolic pathways can be triggered, which lead to a different resulting aroma profile. As Smid and Kleerebezem (2014) reviewed, changes in temperature and pH, but also of the nutrient composition, and the osmolarity or the salinity influence the use of metabolic pathways and consequently lead to a different aroma profile. An investigation of LAB in sourdough proved that a change of the pH from 5.8 to 3.6 led to a metabolic rerouting from carbohydrate to amino acid catabolism. Consequently, the amino acid leucine was metabolized into 2- and 3-methylbutonic acid. The resulting amounts of 2- and 3-methylbutonic acid were even higher when fructose as electron acceptor was in the substrate. The author concluded that LAB minimize the acid stress by the reduced production of lactate and acetate. Moreover, by using labeled leucine, they showed that less 3-methyl butanol and 3-methyl butanal are produced in favor of 3-methyl butanoic acid under acid stress conditions (Serrazanetti et al., 2011). A similar mechanism should be considered for the reduction or generation of other aroma active compounds (e.g. hexanal), too. Interestingly, D. Kaneko et al. (2014) found that the addition of L-valine in soymilk fermentation leads to changes in gene expression (repression of *ilvC* and stimulation of *als* and *aldB*) of *S. thermophilus*, which resulted in a reduction of the diacetyl production in favor of acetoin. They also suggested that a proteolytic treatment of the soymilk might be applicable to release L-valine from soy protein and therefore, to reduce diacetyl production during fermentation. In a different study, it was shown that the final

TABLE 6 Modification of the flavor by variation of the fermentation parameters

Strain	Substrate	Variation	Observed changes	Source
L. lactis TIL46	Potassium phosphate buffer enriched with alkohols and (activated) fatty esters	Reduction of water activity by freezing	The synthesis of esters (ethyl butanoate, ethyl acetate) was approx. 100-fold higher at low water acitivites (frozen) compared with high water activities (liquid).	(Nardi et al., 2002)
Lactobacillus sanfranciscensis LSCE1	Hydrolyzed wheat flour medium	pH shift from 5.6 to 3.6	A shift from the carbohydrate to the amino acid metabolism resulted in a up to 7-fold production of 2-/3-methylbutanoic acid from leucine.	(Serrazanetti et al., 2011)
Streptococcus thermophilus NBRC 13957	Soymilk	Addition of L-valine	The production of diacetyl was shifted significantly toward acetoin.	(D. Kaneko et al., 2014)
Streptococcus thermophilus (34 different strains)	LM17 medium	Addition of L-threonine	Production of acetaldehyde was increased in all strains.	(Chaves et al., 2002)
Lactococcus lactis subsp. *cremoris* AM2 *Lactococcus lactis* subsp. *cremoris* HP	Cheddar cheese	Prolonged fermentation time	Due to cell lysis, the lipolytic activity increased up to the 7-fold of the original activity. Therefore, free fatty acids were released into the food matrix. Increasing amounts of up to 59% of the oxidizable fatty acid linoleic acid were observed.	(Collins et al., 2003)
Lactococcus lactis IL1403	Defined basal medium with addition of glucose and branched chain amino acids	Depletion of carbohydrates by prolonged fermentation time (21 days) until the onset of the non-culturable state	After carbohydrate depletion, a shift toward the amino acid metabolism was observed, resulting in branched chain fatty acids (2-methylbutanoic acid) produced from branched chain amino acids (leucine).	(Ganesan et al., 2006)
Pediococcus pentosaceus P133 *Pediococcus acidilactici* P75 *Lactobacillus plantarum* L1047 *Lactobacillus perolens* L532 *Lactobacillus paracasei* L1150 *Lactobacillus amyllolyticus* TL5	Lupine protein extract Pea protein extract	Addition of glucose	For all strains and both substrates, the sensory evaluation resulted in an elevated impression of sour. This correlated with an increased pH drop upon fermentation.	(Klotzbücher, 2009)
Lactobacillus brevis 25a	Sourdough	Addition of fructose	With an increasing fructose addition (0–10 g/100 g flour), the concentration of lactic acid dropped up to 16% while the concentration of acetic acid increased sharply up to 400%. This led to a decline of the fermentation quotient from approx. 4 to 1.	(Wolfgang Röcken, Martina Rick, & Marita Reinkemeier, 1992)

(Continues)

TABLE 6 (Continued)

Strain	Substrate	Variation	Observed changes	Source
Mixed culture of *Bifidobacterium lactis* Bb 12 and *Lactobacillus acidophilus* (strain further specified)	Milk	Addition of RFO	By adding 2% (w/v) RFO to the milk, the resulting fermentation quotient was increased depending on the ratio of bifidobacteria to LAB in the inoculum to 104% (ration 10:1; FQ 0.76 to 1.55), 24% (ratio 1:1, FQ 3.15 to 3.49), and 11% (ratio 1:10, FQ 3.40 to 4.23).	(C. Martínez-Villaluenga et al., 2005)
Lactobacillus plantarum Al 30 *Lactobacillus paralimentarius* Al 28 *Lactobacillus helveticus* Al 26	Amaranth based sourdough	Temperature increase from 30°C to 35°C	The production of lactic acid and acetic acid increases especially in the first 48 h. At this point changes of approx. 25% (lactic acid) and approx. 20% (acetic acid) were reported.	(Mario Jekle, Andreas Houben, Martin Mitzscherling, & Thomas Becker, 2010)
Bifidobacterium adolescentis NCIMB 702204 *Bifidobacterium infantis* NCIMB 702205 *Bifidobacterium breve* NCIMB 702257 *Bifidobacterium longum* NCIMB 702259	Malt-based beverage	Prolonged fermentation time	For the different strains, prolonging the fermentation time from 12 to 24 h led to an increase of lactic acid (99–231%) and of acetic acid (48–103%).	(Rozada-Sánchez et al., 2008)
Bifidobacterium infantis CCRC 14633 *Bifidobacterium longum* B6	Soymilk	Prolonged fermentation time	Prolonging the fermentation time from 12 to 48 h increased the concentrations of lactic and acetic acid for 50–108% and 20–57%, respectively. The fermentation quotient increased slightly from 0.52–0.54 to 0.68–0.69.	(Hou et al., 2000)

concentration of acetaldehyde in yogurt produced with *S. thermophilus* could be increased by adding L-threonine (Chaves et al., 2002). Gobbetti et al. (2005) concluded that the addition of arginine increased the 2-acetyl-pyrroline production in sourdough. The release of these amino acids could be achieved by using technical enzymes as well as native enzymatic activity elevated by germination as part of the substrate modulation.

The resistance of bacteria against stress factors is affected by the physiological condition of the cells, the genetic differences among the different species, but also by the environment of the microorganism (pH, water activity, etc.) (Serrazanetti et al., 2009). Hence, knowing the food matrix is important for predicting the stress answer. By changing the fermentation parameters, stress can be induced, which may lead to changes in the metabolic pathways and consequently to a different aroma profile.

The overall aroma is also influenced by the length of the LAB fermentation time. As summarized by Smid and Kleerebezem (2014), it leads to a different aroma as intracellular enzymes are set free into the food matrix by cell lysis and start to interact with substances in the food matrix. Nevertheless, cell lysis might also release unpleasant compounds into the beverage like lipids and oxidizable fatty acids (Collins et al., 2003). Ganesan et al. (2006) showed that *L. lactis* changes to branched chain amino acid catabolism after carbohydrate depletion, which leads to the production of 2-methyl butanoic acid from leucine. As legume-based substrates are very rich in proteins, it should be considered that aroma compounds are generated via possible amino acid pathways during growth or after the growth phase of the LAB.

To summarize, lactic acid fermentation was successfully used in many cases to improve the aroma of legume-based products. Depending on the used legume and its pretreatment, the LAB need to be selected with care, as they highly influence the resulting aroma profile. Therefore, in the development of legume-based beverages, a strain selection should always be performed for the pretreated substrate instead of simply relying on a strain known from a different process. Moreover, the fermentation parameters seem to have a high potential in directing the metabolic activities and therefore influencing the production of certain aroma compounds. Further scientific effort is required to fill the knowledge gaps in the understanding of the involved mechanisms. The use of isotope-labeled compounds like hexanal-d_{12} might reveal the metabolic pathways used by LAB in the degradation of hexanal. Moreover, this approach could not only identify the resulting metabolic products, but might also prove how changes in the fermentation parameters affect the involved metabolic pathways.

4.2 | Enhancing taste acceptability

Lactic acid fermentation improves the flavor by producing acids and hence introducing a refreshing character to the beverage. Labba et al. (2021) concluded that acids trigger a salivary reflex and therefore lead to mouth wetting, which is associated with the perception of refreshing. Moreover, they stated a positive correlation between the sourness of a beverage and its refreshing character. Nevertheless, the acidification of the food matrix varies strongly with the used strain and the available sugars. In soymilk, the different strains of *S. thermophilus* resulted in a lower pH (4.5–5.2) than *L. plantarum* (5.3–5.7) (Harlé, 2020). Klotzbücher (2009) evaluated the impact of different LAB on the taste of lupine protein extract and compared results of fermentations with and without additional glucose. In all the cases, the taste resulted from the different LAB (*L. paracasei*, *L. perolens*, *L. plantarum*, *P. acidilactici*, *P. pentosaceus*) was described as more sour when glucose was added (Klotzbücher, 2009).

No information is available in the literature about the impact of RFOs on the degree of acidification. A low acetic/lactic acid ratio is desirable (Rozada-Sánchez et al., 2008), as high contents of acetic acid compared with lactic acid were described as pungent, sour, and acid (Nsogning Dongmo et al., 2016; Peralta et al., 2016). This ratio is also a quality criterion in the sourdough production known as "Fermentation Quotient" (FQ) (= molar ratio of lactic acid/acetic acid). Recommendations for the FQ are given from 2 to 2.7 for rye sourdough (Hammes & Gänzle, 1998) and approximately 4.0 for wheat sourdough (Barber et al., 1991). So far, no values are reported as recommended for beverages. As the food matrix of sourdough differs highly from a beverages' one, the values cannot simply be transmitted. Thus, desirable FQ-values should be identified by varying the lactic acid/acidic acid ratio and evaluating the resulting beverage in sensory panel.

In sourdough fermented monoseptically with *L. brevis*, the acetic acid production increased almost linearly with the concentration of fructose added to the flour while the lactic acid production was inhibited (W. Röcken et al., 1992). Fructose acts as electron acceptor for the regeneration of NADH in the production of acetic acid (Gobbetti et al., 2005). C. Martínez-Villaluenga et al. (2005) showed that the addition of RFOs to the fermentation of milk elevates the lactic/acetic acid ratio. In amaranth sourdough inoculated with *Lactobacillus plantarum* Al30, *L. paralimentarius* Al28, and *L. helveticus* Al26 (one strain per experiment series), it was shown that lower fermentation temperatures (30°C vs. 35°C) generally increase the production of acetic and lactic acid, while the fermentation quotient varies with the strain (Jekle et al., 2010). The

temperature dependency of the acidification is further backed by Hadaji et al. (2006), who fermented goat milk with LAB and observed that the lactic acid production increases with higher temperatures.

Nsogning Dongmo et al. (2016) concluded that a customized lactic/acetic acid ratio would improve customer acceptance. With bifidobacteria, a prolonged fermentation shifted the lactic/acidic acid ratio toward lactic acid in malt-based beverages (Rozada-Sánchez et al., 2008) and soy milk (Hou et al., 2000). Hence, a longer fermentation might improve the taste by reducing the pungent acetic acid impression of the resulting beverage. However, no information is given about changes in the overall aroma profile in the aforementioned studies. As longer fermentation times lead to an increased cell lysis, which might affect the overall aroma impression negatively. Such an increase in off-flavors would negate the gain in the taste profile. Nevertheless, the sugar spectrum in the substrate, the choice of strain, and the fermentation pH, temperature, and time should be considered as factors for changing the taste and the refreshing character of the beverage.

Lactic fermentation shows a high potential for reducing bitterness by degrading the bitter peptides and masking the bitter impression. First, the enzymatic degradation of bitter peptides into smaller peptides and amino acids reduces the bitter impression (Meinlschmidt et al., 2016). This effect can be applied by using the high proteolytic activity of LAB (Bouchier et al., 1999; Bouchier et al., 2001; Shimamura et al., 2009).

Rodriguez-Serrano et al. (2018) reviewed the proteolytic system of LAB and stated that due to the auxotrophy for 4–14 amino acids, LAB possess a variety of proteolytic enzymes to obtain amino acids from peptides and complex proteins (endopeptidase, aminopeptidase, oligopeptidase, metallopeptidase, etc.). The conclusion of different reviews concerning the proteolytic system was that the degradation of high-molecular proteins happens extracellularly, whereas the resulting peptides are transported into the cells and decomposed further (Kieliszek et al., 2021; Rodriguez-Serrano et al., 2018; Savijoki et al., 2006). Due to cell lysis, intracellular peptidases are set free which could further hydrolyze bitter peptides (Baankreis et al., 1995). Moreover, the resulting amino acids might be transformed into further aroma active compounds (Peralta et al., 2013). In addition, LAB fermentation can mask bitterness by increasing the umami taste (Zhao et al., 2016). A study on human bitter taste receptors proved that five umami peptides acted as noncompetitive inhibitors on those receptors and therefore reduced the bitter taste response in the presence of umami peptides (M. J. Kim et al., 2015). LAB possess a cytoplasmic glutaminase and therefore convert glutamine to glutamate during growth. The resulting glutamate is partially converted into α-ketoglutarate, further metabolized and partially excreted into the food matrix (Teixeira et al., 2014; Vermeulen et al., 2007). Furthermore, due to the formed lactic and acetic acid and the binary bitter–sour taste interaction, the bitter impression might be suppressed (Keast & Breslin, 2002). Depending on the choice of strain, the bitter impression of the resulting beverage can be highly reduced.

To conclude, LAB show a high potential to improve the taste of a legume-based beverage by introducing a refreshing character, by degrading bitter peptides, and by suppressing bitterness via bitter–sour taste interactions and via inhibiting bitter taste receptors. Nevertheless, increasing proteolysis by prolonged fermentation times might also lead to new off-flavors from cell lysis. This needs to be regarded when designing the fermentation as a process step in the production of beverages.

5 | CONCLUSION

Lupines and faba beans are suitable as substrates for lactic acid fermentation in order to produce beverages and food for human consumption. High protein content, in particular, is an asset in both legumes.

While both are good sources of most essential amino acids, the scarcity in sulfuric amino acids needs to be balanced in order to increase protein bioavailability and provide full benefit from the high protein content. This can be achieved by increasing the consumer awareness to combine legume-based beverages with food rich in sulfuric amino acids, like methionine. More feasible but probably less acceptable would be the addition of methionine and cysteine directly into the beverage. Besides that, high protein bioavailability also depends on the decomposition of the high amounts of phytic acid in lupines and faba beans.

The high amounts of RFOs in both legumes are a major obstacle. Even as RFOs might contribute to a probiotic aspect, such flatulence-causing substances are generally not acceptable in beverages. As quinolizine alkaloids, condensed tannins, and vicine/covicine were highly reduced by breeding, those substances no longer limit the use of lupines and faba beans in food or beverages. Nevertheless, their concentration should be monitored to avoid an accumulation to unacceptable amounts while processing the legumes.

In food products, polyunsaturated fatty acids are mostly regarded as positive. But they are precursors of aroma active compounds like linoleic acid for hexanal. As deoiling can reduce but not entirely remove those precursors, the impact of low-threshold aroma compounds from the oxidation of fatty acids on the overall aroma needs to be mitigated during the production of beverages and food.

Multiple studies showed that the aroma of legume-based products can be improved by lactic acid fermentation, whereas the resulting aroma depends highly on the applied strain and the fermentation parameters.

Regarding the aroma, hexanal seems to be an important aroma compound in the interactive effect of the overall aroma of legumes and its degradation might reduce the typical beany aroma impression. Nevertheless, a prerequisite of the degradation of hexanal is that the resulting reaction products either show an acceptable aroma impression or result in concentrations beneath their aroma threshold. As different metabolic products are named in the literature and the metabolic pathways of the hexanal reduction in LAB are not entirely known, further research should identify the possible products. Moreover, it should be investigated whether the metabolic pathways can be manipulated by changing the fermentation parameters and/or the substrate composition. Once this knowledge is available, LAB can be utilized to reduce the beany aroma impression, generate organic acids and therefore add a refreshing character, and to improve consumer acceptance of legume-based beverages. This will release the high potential of lupines and faba beans as raw materials for new protein-rich, sustainable, vegan, and gluten-free beverages.

Only a fraction of LAB is known to grow on RFOs. In order to focus the strain selection exclusively on the aroma improvement, a proper preprocessing or substrate modulation is required. This substrate modulation should be applied as an interacting process rather than a number of independent process steps. Therefore, phytic acid and RFOs could be reduced by germinating lupines and faba beans. Drying would result in a storable malt while adding positive aroma compounds. Subsequent enzymatic treatment would hydrolyze proteins and increase their solubility. The substrate modulation would not only decrease the amount of antinutrients and increase the protein content in the beverage, but also provide sufficient phosphate, amino nitrogen, and fermentable sugars for lactic acid fermentation.

The presented approach of a combined process of a substrate modulation to enhance the nutritional value and a subsequent LAB fermentation to improve the aroma and taste profile could raise the consumer acceptance of legume-based products and additives. This facilitates the use of lupines and faba beans in novel products like refreshing beverages, but also in meat analogs, milk substitutes, or bakery goods. However, further research is required to fully understand the aroma profile of faba beans and to identify its key aroma compounds. Another knowledge gap lies within the complex phenomenon of the beany aroma impression. Most likely, it is due to an interactive effect with hexanal being one of its main contributors. Further studies should substantiate this assumption and might prove that the reduction of hexanal removes or diminishes the beany aroma impression. It was shown that LAB can degrade hexanal whereas the exact metabolic pathways and metabolic products are not entirely known. Therefore, scientific effort should be placed on the identification of the LAB metabolism of hexanal and whether variations of the fermentation parameters could trigger metabolic reroutings toward more or less desired compounds in food products.

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AUTHOR CONTRIBUTIONS

Stefan W. Ritter: conceptualization, investigation, visualization, writing-original draft. Martina I. Gastl: conceptualization, investigation, project administration, supervision, writing-original draft, writing-Review & editing. Thomas M. Becker: conceptualization, investigation, resources, supervision, writing-Review & editing.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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