Volume 67(2):195-201, 2023
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs
DOI:10.14232/abs.2023.2.195-201

ARTICLE

Development and Evaluation of Vegan Yogurts and Sour Milk Alternatives from White Lupin (*Lupinus albus* L.)

András Misz[1,2], Sándor Kocsubé[2], Máté Vágvölgyi[3], Judit Bajzát[1], Csaba Vágvölgyi[2], Csaba Csutorás[4,*]

[1]New Champignons Ltd., H-1224 Budapest, Hungary
[2]Department of Microbiology, Faculty of Science and Informatics, University of Szeged, H-6726 Szeged, Hungary
[3]Institute of Pharmacognosy, Faculty of Pharmacy, University of Szeged, Zrínyi u. 9., H-6720 Szeged, Hungary
[4]Institute of Chemistry and Physics, Eszterházy Károly Catholic University, H-3300 Eger, Hungary

ABSTRACT Cow milk allergy (CMA) triggers a clinically abnormal immunological response to cow milk proteins. To address this issue, extensive studies have explored milk alternatives from various animal and plant sources. This study introduces a method for producing white lupin milk, resulting in fermented dairy-like products (vegan yogurt and sour milk alternatives). Four commercial yogurt cultures, including two mesophilic (CHN-11, CHN-22) and two thermophilic (YC-380, YC-X11) mixed bacterial cultures, were tested, with thermophilic ones yielding superior sensory outcomes for lupin-based yogurt alternatives. Incorporating inulin (2%) enhanced sensory appeal, particularly evident in strawberry and peach-flavoured variants, which achieved sensory scores comparable to cow milk yogurts. Furthermore, white lupin-based yogurts demonstrated superior water-holding capacity (up to 47.11 g/100 g in comparison with 42.35 g/100 g measured for cow milk yogurt), influencing texture and mouthfeel. They also exhibited favourable fatty acid profiles, notably rich in beneficial unsaturated fatty acids such as linoleic and linolenic acid (up to 10.15% and 8.43%, respectively), indicating potential health benefits. Sensory evaluation underscored the impact of starter cultures on product attributes, with certain cultures yielding more favourable results. In conclusion, white lupin emerges as a promising alternative protein source with the potential to produce high-quality dairy-like products. While white lupin-based products hold promise as functional foods for individuals with specific dietary needs, further research is necessary to address potential allergenic concerns associated with white lupin proteins.
Acta Biol Szeged 67(2):195-201 (2023)

KEY WORDS
cow milk allergy
functional food
sensory evaluation
vegan foods
white lupin
yogurt alternatives

ARTICLE INFORMATION
Submitted
24 April 2024
Accepted
30 April 2024
***Corresponding author**
E-mail: csutoras.csaba@uni-eszterhazy.hu

Introduction

Cow milk allergy (CMA) represents a clinically abnormal immunological response to cow milk proteins, stemming from interactions between certain milk proteins and various immune mechanisms, which can cause immediate IgE-mediated reactions (Zepeda-Ortega et al. 2021; Vandenplas et al. 2021). Differently, reactions that do not involve the immune system are classified as cow milk protein intolerance. CMA is particularly prominent in early childhood, affecting 2-3% of infants in developed countries. However, it is notable that approximately 85-90% of affected children outgrow this sensitivity by the age of three. Cow milk is comprising of over 20 proteins (allergens) capable of inciting allergic reactions, with most research identifying casein and β-lactoglobulin as the primary allergens (Vandenplas et al. 2021).

Several investigations have extensively explored the potential of milk from a variety of animals, such as goats (Restani et al. 2002; Alvarez and Lombardero 2002; Clark and García 2017), camels (El-Agamy et al. 2009), sheep (Restani et al. 2002), mares, donkeys (Clark and García 2017; Sarti et al. 2019), and buffalos (Restani et al. 2002), as alternatives. However, the literature presents diverse and sometimes conflicting findings regarding their suitability as substitutes. While some research points to the hypoallergenic properties of goat (Wang et al. 2018), mare, donkey (Sarti et al. 2019), and camel milk (El-Agamy et al. 2009), other studies indicate that milks from goats, sheep, and buffalo might elicit allergic reactions like cow milk. Intriguingly, even soymilk has been associated with allergic reactions in certain instances (Katz et al. 2014).

Beyond animal-derived alternatives, plant proteins are emerging as potential substitutes for CMA patients. Commercial milk alternatives like rice, soy, oat, coconut, and almond milk are available, yet are not always suitable for infants. Conversely, specialized infant formulas based on soy, rice, almonds, or carob seeds are accessible, along with plant protein-based products, such as soy yogurts and those with inulin (Rinaldoni et al. 2012) or derived from peanut milk (Isanga and Zhang 2009).

Aside from CMA, a significant population segment grapples with lactose intolerance, a digestive issue arising from the body's inability to process lactose found predominantly in dairy. Plant-based dairy substitutes offer potential remedies (Mäkinen et al. 2016; Silva et al. 2020).

The white lupin (*Lupinus albus* L.), commonly referred to as field lupin, is a perennial plant naturally thriving in abundance throughout the Mediterranean region. It exhibits robust growth in sandy and acidic soils, a trait that has contributed to its cultivation for centuries. Even today, it continues to be cultivated in various regions, especially in the Mediterranean and the Middle East, with Egypt being one of the areas where white lupin cultivation remains prominent (Chiofalo et al. 2012).

The utilization of white lupin seeds has recently gained significant attention, particularly in the field of animal nutrition and feed formulation (Abraham et al. 2019). These seeds are nutritionally rich, with a high protein content typically ranging from 32.9% to 36.0%. Their oil content, between 9% and 13%, is notably high in beneficial polyunsaturated fatty acids (PUFA). They also contain numerous biologically beneficial compounds, such as dietary fibres, minerals, vitamins (Martínez-Villaluenga et al. 2006), phenolic acids, flavonoids, and isoflavonoids (Oomah et al. 2006), which possess, e.g., antioxidant properties and are beneficial for health. White lupin can affect lipid and glucose metabolism and may have a functional effect on inflammatory processes and the gut microbiome, influencing a wide range of physiological parameters, including metabolism, nutrient absorption, and immune function. Therefore, it is not surprising that there is intense research focused on using white lupin as feed (Gresta et al. 2023).

The promising results of its use as feed inspire research into the utilization of white lupin as human food (Sedláková et al. 2016). Our primary aim was to explore the feasibility of white lupin-derived milk as a viable substitute for cow's milk in the production of fermented dairy products. By employing methods like those used in traditional dairy processing, we aimed to create lupin-based products with sensory attributes comparable to conventional dairy products. Through the utilization of starter cultures and formulation strategies, our study sought to establish white lupin-based alternatives as functional and enjoyable options for individuals with specific dietary requirements. However, it's important to note that the aim of this study was not to perform a detailed, potentially standard-based analysis of lupin-based experimental yogurts. Rather, our goal was to lay the groundwork for such developments in yogurt production, demonstrating that with an economical and scalable technology, yogurt of adequate sensory quality can be produced from this ingredient.

Materials and methods

Raw materials

White lupin seeds (*Lupinus albus* cv. Nelly) were sourced from The Center for Agricultural and Applied Economic Sciences at the University of Debrecen (Nyíregyháza, Hungary). Four different freeze-dried DVS (Direct Vat Set) commercial yogurt starter cultures were tested: YC-380 (thermophilic; *Lactobacillus delbrueckii* sp. *bulgaricus, Streptococcus thermophilus*), YC-X11 (thermophilic; *Lactobacillus delbrueckii* sp. *bulgaricus, Streptococcus thermophilus*), CHN-11 (mesophilic; *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactococcus lactis* subsp. *diacetylactis*), and CHN-22 (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Leuconostoc pseudomesenteroides*). All starter cultures were purchased from Hungarian Dairy Farming Experimental Institute Ltd. (Mosonmagyaróvár, Hungary). Cow milk was purchased from Egertej Ltd. (Eger, Hungary).

Production of lupin milk

The production procedure mirrored the domestic method for soymilk. Specifically, 100 g of white lupin seeds were soaked overnight in 500 mL of deionized (DI) water changes twice. The soaked seeds were blended thoroughly with DI water. The resulting puree was boiled at 100 °C for 30 min and added with 500 mL of DI water. After boiling, the puree was allowed to cool down to room temperature before filtering through a cheesecloth and a 0.5 mm sieve. The produced lupin milk can be refrigerated at 4-6 °C for up to 3 days. The milk's light bitter flavour can be minimized by increasing the number of rinses during soaking and maintaining a low boiling state for 30 min. Lupin milk's chemical composition was previously evaluated by Elsamani et al. (2014).

Production of white lupin yogurt alternatives (WLY)

Vegan yogurt-like beverages were crafted using white lupin milk. The process involved treating 0.5 L of white lupin milk with various mixed bacterial cultures: CHN-11, CHN-22, YC-380, and YC-X11. For comparison, cow milk yogurt (CMY) was produced using the YC-X11 culture.

Ten mg cultures were applied, mesophilic cultures were incubated at 37 °C, while thermophilic ones were at 44 °C. Incubation duration was 4 h. Post incubation, 20 gL^{-1} of inulin (Sigma Aldrich, St. Louis, MO, USA) was introduced for a sweeter taste. The yogurts were then flavoured with strawberry and peach (Sigma Aldrich, St. Louis, MO, USA) and stored at 5 °C for 24 h.

Water holding capacity (WHC)

The WHC of the formulated yogurt-like products was assessed based on a modified method from Harte et al. (2003). The process consisted of centrifuging the stirred yogurt for 15 min at 8000 rpm, 4 °C. WHC was calculated using the following formula:

$$WHC\ (\%) = (1-W_1/W_2) \times 100,$$

where W_1 is the whey weight post-centrifugation, and W_2 is the yogurt weight. These measurements were conducted thrice, and WHC was determined after a 24-h cold storage at 5 °C.

Susceptibility to syneresis (STS)

Syneresis susceptibility was assessed by draining 100 mL yogurt sample on filter paper (Whatman® quantitative filter paper, ashless, Grade 41, circles, diam. 150 mm) for 6 h. The whey volume collected helped gauge syneresis using the following formula:

$$STS\ (\%) = (V_1/V_2) \times 100,$$

where V_1 is the whey volume post-drainage, and V_2 is the initial yogurt sample volume. This evaluation was done after a 24 h incubation at 5 °C storage.

Fatty acid composition

The fatty acid composition of the samples was ascertained using Gas Chromatography-Mass Spectrometry (GC-MS). Preparation began with the formation of fatty acid methyl esters (FAME) from the sample extracts.

Fat extraction

For the extraction process, white lupin yogurt (WLY) and cow milk yogurt (CMY) samples underwent oil extraction by adopting the Röse-Gottlieb method (Manirakiza et al. 2001). Briefly, 30 g of the milk or yogurt sample was precisely measured and placed into a Röse-Gottlieb extraction flask. 3.75 mL of ammonia solution was introduced, after which the flask was securely closed and shaken vigorously. This mixture was subsequently heated in a 60 °C water bath for 5 min. Following the heating, the mixture was agitated for an additional 2 min, post which 30 mL of 95% ethanol was added. After a series of shakes, the concoction was cooled to ambient temperature using cold water. 75 mL of diethyl ether was then poured into the mixture, followed by a 30-s shake. An equivalent volume (75 mL) of petroleum ether was subsequently added. Following a final series of shakes, the mixture was left undisturbed, allowing for the complete separation of the etheric layer, a process taking approximately 30 min. The distinct etheric layer was carefully removed and transferred to a distillation flask. Employing a vacuum, the solvent was thoroughly evacuated. A 1 mL aliquot of the residue was solubilized in isooctane.

FAME preparation

The ester derivative was then crafted using 2 M KOH in methanol, which was later neutralized with sodium bisulphate ($NaHSO_4$).

Gas chromatography (GC) analysis

The resultant fatty acid methyl esters were then analysed with a quadrupole GC/MS system (GCMS-QP2010 Plus, Shimadzu, Kyoto, Japan) equipped with a Supelcowax 10 Capillary GC Column (30 m × 0.25 mm × 0.25 µm; Supelco, Bellefonte, PA, USA). Temperature program: 140 °C (5 min) to 240 °C at 4 °C /min. Carrier gas: helium, 20 mL/s; Injection: 1 µL, split 100:1; Injection temperature: 260 °C.

Identification and calculation of fatty acids

For accurate calibration and comparison, FAME-mix (Supelco 37-Component FAME Mix (47885-U)) from Sigma-Aldrich (Bellefonte, PA, USA) was employed. The entire analytical procedure was reiterated three times across different yogurt samples. The reported results represent the mean values of these three separate runs.

Sensory evaluation

After being stored overnight at 5 °C, both yogurt-like beverages and traditional yogurt samples were subjected to a sensory evaluation, focusing on attributes such as appearance (encompassing colour and texture), mouthfeel, flavour, and general acceptability. The panelists were chosen voluntarily, and their participation was in accordance with the ethical standards of the institutional research committee (EKCU) and with the 1964 Helsinki Declaration and its later amendments.

A group of fourteen volunteer panelists, proficient in food science and familiar with sensory evaluation techniques for yogurt, assessed the samples. Their evaluations used the nine-point hedonic scale, as defined by Stone and Sidel (1993). For the evaluation, panelists were provided two distinct samples, each presented in cups labelled with a unique three-digit number containing roughly 25 mL of the product. The samples comprised a mix of white lupin yogurt-like products and traditional cow milk yogurts were rated on a 9-point hedonic scale

Table 1. Water holding capacity and susceptibility to syneresis values of white lupin-based yogurt alternatives compared with cow milk yogurt.

	White lupin yogurt				Cow milk yogurt
	Mesophilic starter		Thermophilic starter		
Examined parameter	CHN-11	CHN-22	YC-380	YC-X11	YC-X11
Water-holding capacity (g/100 g)	46.61 ± 0.21[b]	46.45 ± 0.27[b]	47.03 ± 0.38[b]	47.11 ± 0.35[b]	42.35 ± 0.18[a]
Susceptibility to syneresis (mL/100 mL)	43.29 ± 0.19[b]	43.45 ± 0.15[b]	43.12 ± 0.22[b]	43.07 ± 0.17[b]	46.74 ± 0.26[a]

Values are means ±SD based on three observations. Means with different letters indicate significant differences between an experimental yogurt and CMY. P-values lower than 0.05 were considered as significant.

ranging from 1 (dislike extremely) to 9 (like extremely) across various sensory attributes, including appearance, aroma, flavour, fruity character, and overall impression. The scale also included descriptors such as "Like very much," "Like moderately," "Like slightly," "Neither like nor dislike," "Dislike slightly," "Dislike moderately," and "Dislike very much." Alongside their ratings, panelists were encouraged to provide additional comments or suggestions, particularly concerning the sensory texture, mouthfeel, and flavour profiles of the samples under evaluation.

Statistical analysis

Statistical analysis was performed with Microsoft Excel version 2402 (Microsoft Corp., Redmond, WA, USA). Comparison of the samples was carried out using Two-sample t-test with unequal variance. P-values lower than 0.05 were considered as significant.

Results and discussion

Water holding capacity

The water-holding capacity of white lupin-based yogurt products surpassed that of CMY, registering at 42.35 g / 100 g (Table 1).

This variation in water holding capacity between the yogurts can be credited to the differing properties of proteins and carbohydrates within them. The bond between proteins and water plays a pivotal role in fermented products, influencing their viscosity, mouthfeel, texture, and flavour (Yu et al. 2007). Factors intrinsic to food proteins that affect WHC encompass amino acid composition, protein conformation, and attributes like surface polarity and hydrophobicity (Barbut 1999). Another influencing element for the elevated WHC in white lupin products could be the presence of inulin, known for its remarkable water retention ability. It also acts as a thickener, forming complexes with proteins through

Table 2. Fatty acid composition of white lupin yogurt (WLY) and cow milk yogurt (CMY) prepared with YC-X11 cultures analysed by gas chromatography.

Fatty acid methyl ester	Time Retention (tR)	White Lupin Yogurt	Cow Milk Yogurt
	min	%	%
lauric acid methyl ester	7.7	-	2.38 ± 0.03
myristic acid methyl ester	12	0.26 ± 0.01b	9.15 ± 0.09a
palmitic acid methyl ester	16.3	16.79 ± 0.15b	39.39 ± 0.24a
palmitoleic acid methyl ester	17.1	0.38 ± 0.01b	0.65 ± 0.01a
cis-10-heptadecenoic acid methyl ester	20.7	2.06 ± 0.03	-
stearic acid methyl ester	24.2	1.8 ± 0.02b	14.25 ± 0.14a
oleic acid methyl ester	25.3	42.53 ± 0.21b	29.52 ± 0.20a
linoleic acid methyl ester	28	10.15 ± 0.10b	4.34 ± 0.05a
linolenic acid methyl ester	32.7	8.43 ± 0.08	-
arachidic acid methyl ester	39.5	6.9 ± 0.06b	0.32 ± 0.01a
cis-11-eicosenoic acid methyl ester	41.2	3.44 ± 0.04	-
behenic acid methyl ester	52.4	6.6 ± 0.06	-
erucic acid methyl ester	53.7	0.66 ± 0.01	-

Values are means ± SD based on three observations.
Means with different letter indicate significant differences between the experimental yogurt and CMY. P-values lower than 0.05 were considered as significant.

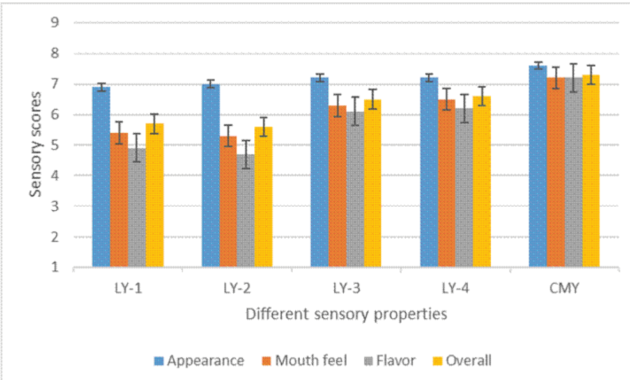

Fig. 1: Sensory evaluation of unflavoured lupin-based yogurt alternatives. LY-1, LY-2, LY-3, and LY-4 are lupin-based yogurts produced with CHN-11, CHN-22, YC-380, and YC-X11 starter cultures, respectively. CMY is cow milk yogurt. Bars are means ±SD taken from three observations. Groups sharing the same letter indicate no significant difference between an experimental yogurt (LY) and CMY in terms of collectively evaluating four attributes in each case. *P*-values lower than 0.05 were considered as significant.

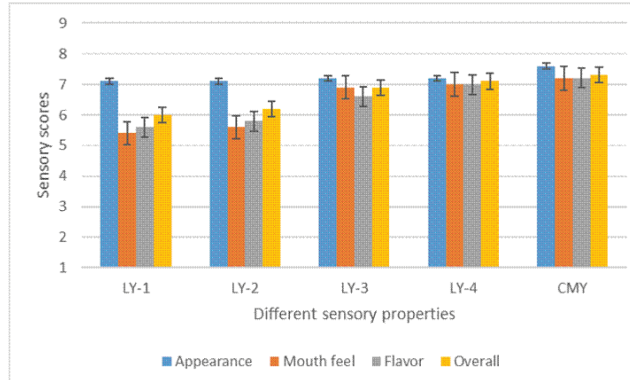

Fig. 2: Sensory evaluation of strawberry-flavoured lupin-based yogurt alternatives. LY-1, LY-2, LY-3, and LY-4 are lupin-based yogurts produced with CHN-11, CHN-22, YC-380, and YC-X11 starter cultures, respectively. CMY is cow milk yogurt. Bars are means ±SD taken from three observations. Groups sharing the same letter indicate no significant difference between an experimental yogurt (LY) and CMY in terms of collectively evaluating four attributes in each case. *P*-values lower than 0.05 were considered as significant.

hydrogen bonds (Aryana and McGrew 2007). The incorporation of stabilizers can further enhance WHC values. These stabilizers serve dual purposes: they mitigate water movement in the yogurt matrix due to their water-binding ability and enhance texture and hydration by interacting with proteins (Thaiudom and Goff 2003).

Susceptibility to syneresis

White lupin yogurt-like products exhibited lower STS compared to CMY, with a value of 46.74 mL/100 mL (Table 1). This reduced STS can be attributed to the richer fat content in white lupin seeds (9-10%) in contrast to cow milk (3-4%). Typically, low-fat yogurts demonstrate a higher degree of syneresis compared to their high-fat counterparts (Staff 1998). The fat globules in milk might operate as a copolymer alongside proteins, fortifying the gel network. Added inulin exerts a similar effect (Aryana and McGrew 2007).

Fatty acid composition

Yogurt-like beverages derived from lupin boast a superior fatty acid profile when compared to cow's milk yogurts (Table 2).

Although the ratio of saturated to unsaturated fatty acids is similar in both types of yogurts, lupin-based yogurts exhibit a more nutritionally beneficial composition of unsaturated fatty acids. Notably, they are rich in n-3 and n-6 fatty acids, enhancing their nutritional value. The primary constituents of lupin-based yogurt alternatives include unsaturated oleic acid (42.53%), linoleic acid (10.15%), and linolenic acid (8.43%), complemented by a significant presence of palmitic acid (16.79%) among the saturated fatty acids.

The fatty acid composition of lupin-based yogurts aligns with previous literature. Al-Amrousi et al. (2022), observed that the thermal treatment of lupin seeds does not significantly alter their fatty acid composition, which remains consistent with our findings. Furthermore, another study by Rybiński et al. (2018), that analysed a collection of various lupin seeds and reported similar fatty acid profiles, predominantly characterized by high levels of unsaturated fatty acids and low concentrations of stearic and myristic acids.

Sensory evaluation

The sensory evaluation outcomes of yogurts and yogurt-like beverages, categorized by appearance, texture, flavour, and overall attributes are consolidated in Fig. 1-3. All ratings for the assessed sensory attributes remained within the commercially accepted range, which is between 4 and 9 scores. Unflavoured yogurt-like products scored lower in aspects like mouthfeel, flavour, and overall appeal when pitted against CMY. This difference can be attributed to the distinct lupin flavour. While heat-treating the lupin milk mitigates this pronounced flavour, its complete elimination remains challenging. Some panelists expressed aversion to this specific taste. However, flavour enhancements using fruit concentrates from strawberries and peaches led to improved flavour scores for lupin-based yogurt alternatives. White lupin sour milk products formulated with CHN-11 and CHN-22 mesophilic cultures generally garnered lower scores for mouthfeel, suggesting these cultures might be less optimal for lupin yogurt production compared to the

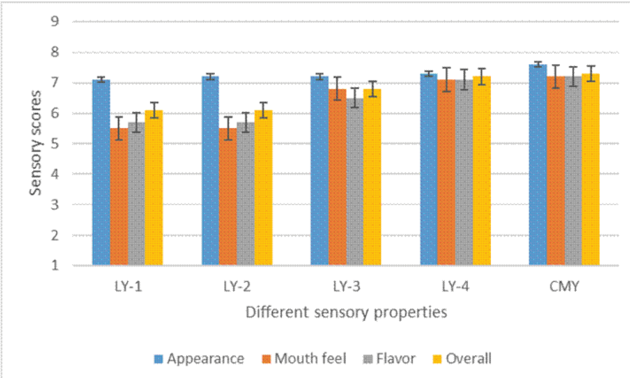

Fig. 3: Sensory evaluation of peach-flavoured lupin-based yogurt alternatives.LY-1, LY-2, LY-3, and LY-4 are lupin-based yogurts produced with CHN-11, CHN-22, YC-380, and YC-X11 starter cultures, respectively. CMY is cow milk yogurt. Bars are means ±SD taken from three observations. Groups sharing the same letter indicate no significant difference between an experimental yogurt (LY) and CMY in terms of collectively evaluating four attributes in each case. *P*-values lower than 0.05 were considered as significant.

YC-380 and YC-X11 cultures.

Canon et al. (2022), developed several yogurt alternatives incorporating lupin; however, they predominantly utilized cow milk in their formulations, with lupin serving as a supplementary ingredient. According to their findings, substituting milk proteins with lupin proteins at a 67:33 ratio was more favourable than the 50:50 ratio. They proposed further exploration into strains capable of enhancing aroma profiles and producing additional textural agents, such as exopolysaccharides, to mask the potentially undesirable aromas associated with lupin. Vieira et al. (2022) suggested that the sensory attributes of yogurt alternatives containing lupin flour could render them suitable for children's nutrition.

Conclusions

The requirement to explore alternative protein sources is gaining traction due to the substantial environmental footprint associated with conventional livestock farming. Cultivating economically viable and environmentally sustainable crops offers a compelling solution to mitigate the ecological burden induced by animal agriculture. White lupin (*Lupinus albus* L.) emerges as a promising candidate in this regard, benefiting from its rich cultivation history and agricultural practices.

Our study highlights the potential of white lupin as a valuable alternative protein source for human nutrition, marking a significant milestone in dietary diversification. We present a simple method for producing white lupin milk, serving as a foundational ingredient for a range of fermented dairy-like products. Through our investigations, we demonstrate the versatility of employing various starter cultures and the efficacy of enhancing the sensory attributes of lupin-based yogurt and sour milk alternatives through the incorporation of inulin.

Importantly, all products developed in our study exhibit satisfactory physico-chemical and sensory properties, with the YC-X11 yogurt culture yielding the most favourable outcomes. The remarkable similarity in sensory values between strawberry and peach-flavoured white lupin-based yogurt alternatives and traditional cow milk yogurts underscores their viability as credible substitutes. These findings highlight the potential of white lupin-based yogurt alternatives as functional foods, particularly beneficial for individuals with cow milk allergies or lactose intolerance.

Further research directions may involve product development, which will inevitably entail examining the parameters of lupin-based yogurts. Additionally, it will be essential to clarify whether lupin-based products can trigger any allergic reactions.

Acknowledgements

Support for this research came from the Doctoral Student Scholarship Program of the Co-operative Doctoral Program of the Ministry of Innovation and Technology, funded by the National Research, Development and Innovation Fund (A. Misz: KDP-2021-C1764158; J. Bajzát: KDP-2023-C2298833).

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