Development and Evaluation of Vegan Yogurts and Sour Milk Alternatives from White Lupin

András Misz  
New Champignons Ltd

Csaba Vágvölgyi  
University of Szeged

Csaba Csutorás (✉ csutoras.csaba@uni-esztehazy.hu )  
Eszterhazy Karoly Catholic University

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Abstract

A method to produce white lupin milk was devised, leading to the creation of fermented, dairy-like products. We developed vegan yogurt and sour milk alternatives using white lupin milk. Two mesophilic (CHN-11, CHN-22) and two thermophilic (YC-380, YC-X11) commercial yoghurt cultures were tested, with thermophilic ones yielding superior sensory outcomes for lupin-based yogurt alternatives. The sensory appeal of these products improved with inulin addition. Fourteen panelists assessed the products using a nine-point hedonic scale. Strawberry and peach-flavored white lupin yogurt alternatives achieved sensory scores comparable to cow milk yogurts, suggesting their potential as true substitutes. The sensory values of strawberry and peach-flavored white lupin-based yogurt alternatives closely matched those of cow milk yogurts, positioning them as viable alternatives. These lupin-based products could serve as functional foods for individuals with cow milk allergy or lactose intolerance. While current literature lacks reports on cross-reactivity between milk proteins and white lupin proteins, the potential allergenic proteins in white lupin do pose constraints on their broader application.

Introduction

Cow milk allergy (CMA) represents a clinical 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 [1, 2]. 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 composed of over 20 proteins (allergens) capable of inciting allergic reactions, with most research identifying casein and β-lactoglobulin as the primary allergens [2].

Numerous studies have delved into the potential of milk from varied animals, such as goats [3, 4, 5], camels [6], sheep [3], mares, donkeys [5, 7], and buffalos [3], as alternatives. The literature provides mixed outcomes. While some research points to the hypoallergenic properties of goat [8], mare, donkey [7], and camel milk [6], other studies indicate that milks from goats, sheep, and buffalo might elicit allergic reactions like cow milk. Intriguingly, even soy milk has been associated with allergic reactions in certain instances [9].

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 [10] or derived from peanut milk [11].

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 [12, 13].

Lupin seeds, boasting a 40–45% protein content, have emerged as a promising alternative. Comparable to soybeans in their protein-to-oil ratio, lupins have fewer anti-nutritional components. They offer not just proteins but a spectrum of nutrients: lipids, dietary fiber, minerals, vitamins [14], and phytochemicals like polyphenols [15]. The study here utilizes white lupin seeds to design yogurt alternatives devoid of cow milk, focusing on their development and key characteristics.

**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 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* subsp. *diacetylactis*), and CHN-22 (*Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuconostoc pseudomesenteroides*).

**Production of Lupin milk**

The production procedure mirrored the domestic method for soymilk. Specifically: 100 g of white lupin seeds were soaked in 500 mL of water overnight, with water changes twice. The soaked seeds were blended thoroughly with the water. The resulting puree was boiled for 30 minutes, followed by the addition of another 500 mL of water. Post boiling, the puree was allowed to cool down before filtering through a cheesecloth and a 0.5 mm sieve. The produced lupin milk can be refrigerated for up to 3 days. The milk's light bitter flavor can be minimized by increasing the number of rinses during soaking and maintaining a low boiling state for 30 minutes. Lupin milk's chemical composition was previously evaluated by Elsamani et al. [16].

**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 cultures: CHN-11, CHN-22, YC-380, and YC-X11. For comparison, cow milk yogurt (CMY) was produced using the YC-X11 culture.

Culture amounts varied from 4 mg to 20 mg. Mesophilic cultures incubated at 37°C, while thermophilic ones at 44°C. Incubation duration was shortened with increased culture amounts (around 3–4 hours). Post incubation, 20 g L⁻¹ of inulin was introduced for a sweeter taste. The yogurts were then flavored (strawberry and peach) and stored at 5°C for 24 hours.
Water holding capacity (WHC)

The WHC of the formulated yogurt-like products was assessed based on a modified method from Harte et al. [17]. The process consisted of centrifuging the stirred yogurt for 15 minutes at 8000 rpm, 4°C. WHC was calculated using the formula:

\[
\text{WHC} (%) = \left(1 - \frac{W_1}{W_2}\right) \times 100,
\]

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-hour cold storage at 5°C.

Susceptibility to syneresis

Syneresis susceptibility was assessed by draining 100 mL yogurt sample on filter paper for 6 hours. The whey volume collected helped gauge syneresis using:

\[
\text{STS} (%) = \left(\frac{V_1}{V_2}\right) \times 100,
\]

where \(V_1\) is the whey volume post-drainage and \(V_2\) is the initial yogurt sample volume. This evaluation was done post a 24-hour 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 from the sample extracts.

For the extraction process, both White Lupin Yogurt (WLY) and Cow Milk Yogurt (CMY) samples underwent oil extraction through an adaptation of the Rose-Gottlieb method [18]: 30 g of the milk or yogurt sample was precisely measured and placed into a Rose-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 minutes. Following the heating, the mixture was agitated for an additional 2 minutes, 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-second 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 minutes. The distinct etheric layer was carefully siphoned off and transferred to a distillation flask. Employing a vacuum, the solvent was thoroughly evacuated. A 1 mL aliquot of the residue was solubilized in isoctane. The ester derivative was then crafted using 2M KOH in methanol, which was later neutralized with sodium bisulfate (NaHSO4).

The resultant fatty acid methyl esters were then analyzed with a Shimadzu 2010 GC-MS, utilizing a Supelco-wax column. For accurate calibration and comparison, FAME-mix and Grain-mix standards from
Sigma-Aldrich were 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 color and texture), mouthfeel, flavor, and general acceptability.

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 [19]. For the evaluation, panelists were provided two distinct samples, each presented in cups labeled 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. The hedonic scale ratings were as follows:

9: Like extremely
8: Like very much
7: Like moderately
6: Like slightly
5: Neither like nor dislike
4: Dislike slightly
3: Dislike moderately
2: Dislike very much
1: Dislike extremely

Alongside their ratings, panelists were encouraged to provide additional comments or suggestions, particularly concerning the sensory texture, mouthfeel, and flavor profiles of the samples under evaluation.

**Results and Discussion**

**Water holding capacity**

The water holding capacity (WHC) of White Lupin yogurt-like products surpassed that of Cow Milk yogurt (CMY), registering at 42.35 g / 100 g (Table 1).
Table 1
WHC (water holding capacity) and STS (susceptibility to syneresis) values of White Lupin-based yogurt alternatives compared with cow milk yogurt (CMY)

<table>
<thead>
<tr>
<th>Examined parameter</th>
<th>*CHN-11</th>
<th>*CHN-22</th>
<th>*YC-380</th>
<th>*YC-X11</th>
<th>CMY</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHC (g/100 g)</td>
<td>46.61 ± 0.21</td>
<td>46.45 ± 0.27</td>
<td>47.03 ± 0.38</td>
<td>47.11 ± 0.35</td>
<td>42.35 ± 0.18</td>
</tr>
<tr>
<td>STS (mL/100 mL)</td>
<td>43.29 ± 0.19</td>
<td>43.45 ± 0.15</td>
<td>43.12 ± 0.22</td>
<td>43.07 ± 0.17</td>
<td>46.74 ± 0.26</td>
</tr>
</tbody>
</table>

*Two mesophilic (CHN−11, CHN−22) and two thermophilic (YC−380, YC−X11) cultures were applied to produce lupin-based sour milk and yogurt alternatives. CMY is cow milk yogurt.

Values are means ±SD based on 3 observations.

This variation in WHC 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 flavor [20]. Factors intrinsic to food proteins that affect WHC encompass amino acid composition, protein conformation, and attributes like surface polarity and hydrophobicity [21]. 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 hydrogen bonds [22]. 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 they enhance texture and hydration by interacting with proteins [23].

Susceptibility to Syneresis

White Lupin yogurt-like products exhibited lower Susceptibility to Syneresis (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 [24]. The fat globules in milk might operate as a copolymer alongside proteins, fortifying the gel network. Added inulin exerts a similar effect [22].

Fatty acid composition

Yogurt-like beverages derived from lupin boast a superior fatty acid profile when juxtaposed with cow milk yogurts (Table 2).

While the ratio of saturated to unsaturated fatty acids remains consistent across both yogurt types, the composition of unsaturated fatty acids in lupin-based yogurts is more beneficial nutritionally. The considerable quantities of n-3 and n-6 fatty acids present enrich their nutritional value. Lupin-based yogurt alternatives predominantly consist of saturated stearic acid (42.53%), palmitic acid (16.79%), and...
myristic acid (12.0%), but are also enhanced by essential unsaturated acids like linoleic acid (10.15%) and linolenic acid (8.43%).

Table 2
Fatty acid composition of cow milk yogurt (CMY) and white lupin-based yogurt alternative (WLY)

<table>
<thead>
<tr>
<th>Fatty acid methyl ester</th>
<th>*Tr (min)</th>
<th>WLY (%)</th>
<th>CMY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lauric acid methyl ester</td>
<td>7.7</td>
<td>-</td>
<td>2.38 ± 0.03</td>
</tr>
<tr>
<td>myristic acid methyl ester</td>
<td>12</td>
<td>0.26 ± 0.01</td>
<td>9.15 ± 0.09</td>
</tr>
<tr>
<td>palmitic acid methyl ester</td>
<td>16.3</td>
<td>16.79 ± 0.15</td>
<td>39.39 ± 0.24</td>
</tr>
<tr>
<td>palmitoleic acid methyl ester</td>
<td>17.1</td>
<td>0.38 ± 0.01</td>
<td>0.65 ± 0.01</td>
</tr>
<tr>
<td>cis-10-heptadecenoic acid methyl ester</td>
<td>20.7</td>
<td>2.06 ± 0.03</td>
<td>-</td>
</tr>
<tr>
<td>stearic acid methyl ester</td>
<td>24.2</td>
<td>42.53 ± 0.21</td>
<td>14.25 ± 0.14</td>
</tr>
<tr>
<td>oleic acid methyl ester</td>
<td>25.3</td>
<td>1.8 ± 0.02</td>
<td>29.52 ± 0.20</td>
</tr>
<tr>
<td>linoleic acid methyl ester</td>
<td>28</td>
<td>10.15 ± 0.10</td>
<td>4.34 ± 0.05</td>
</tr>
<tr>
<td>linolenic acid methyl ester</td>
<td>32.7</td>
<td>8.43 ± 0.08</td>
<td>-</td>
</tr>
<tr>
<td>arachidic acid methyl ester</td>
<td>39.5</td>
<td>6.9 ± 0.06</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>cis-11-eicosenoic acid methyl ester</td>
<td>41.2</td>
<td>3.44 ± 0.04</td>
<td>-</td>
</tr>
<tr>
<td>behenic acid methyl ester</td>
<td>52.4</td>
<td>6.6 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>erucic acid methyl ester</td>
<td>53.7</td>
<td>0.66 ± 0.01</td>
<td>-</td>
</tr>
</tbody>
</table>

*Tr: retention time of different methyl esters in GC.

Values are means ± SD based on 3 observations.

Sensory evaluation

The sensory evaluation outcomes of yogurts and yogurt-like beverages, categorized by appearance, texture, and flavor, are consolidated in Figs. 1–3. All ratings for the assessed sensory attributes remained within the commercially accepted range, which is between 4 and 9 scores. Unflavored yogurt-like products scored lower in aspects like mouthfeel, flavor, and overall appeal when pitted against CMY. This difference can be attributed to the distinct lupin flavor. While heat-treating the lupin milk mitigates this pronounced flavor, its complete elimination remains challenging. Some test participants expressed aversion to this specific taste. However, flavor enhancements using fruit concentrates from strawberries and peaches led to improved flavor 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 YC-380 and YC-X11 cultures.

Conclusions

White lupin emerges as a promising alternative protein source for human nutrition. We developed a straightforward procedure to produce white lupin milk. From this milk, we formulated fermented dairy-like products. Our research indicates that various starter cultures can be employed, and the sensory attributes of lupin-based yogurt and sour milk alternatives can be enhanced using inulin. All the products we crafted exhibited satisfactory physico-chemical and sensory properties. The most favorable sensory outcomes were achieved with the YC-X11 yogurt culture. Notably, the sensory values of strawberry and peach-flavored white lupin-based yogurt alternatives closely matched those of cow milk yogurts, positioning them as viable alternatives. White lupin-based yogurt alternatives hold potential as functional foods, particularly for individuals with cow milk allergies or lactose intolerance. While current literature lacks reports on cross-reactivity between milk proteins and white lupin proteins, the potential allergenic proteins in white lupin do pose constraints on their broader application.

Declarations

Author contributions

András Misz: investigation, methodology, writing original draft. Csaba Vágvölgyi: conceptualization, methodology, writing-review, project administration. Csaba Csutorás: investigation, conceptualization, methodology, writing-review, project administration.

Consent to Participate

All participants agreed to participate in the research and to publish the results in Plant Foods for Human Nutrition.

Research funding

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Competing interests
The authors declare that they have no competing interests.

**Ethics approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

**References**


Figures
Figure 1

Sensory evaluation of unflavored lupin-based yogurt alternatives.

CHN-11, CHN-22, YC-380, YC-X11 are different starter cultures for the production of lupin-based yogurt alternatives, CMY is cow milk yogurt. Bars are means ±SD based on 3 observations.
Figure 2

Sensory evaluation of strawberry flavored lupin-based yogurt alternatives.

CHN-11, CHN-22, YC-380, YC-X11 are different starter cultures for the production of lupin-based yogurt alternatives, CMY is cow milk yogurt. Bars are means ±SD based on 3 observations.
Figure 3

Sensory evaluation of peach flavored lupin-based yogurt alternatives.

CHN-11, CHN-22, YC-380, YC-X11 are different starter cultures for the production of lupin-based yogurt alternatives, CMY is cow milk yogurt. Bars are means ±SD based on 3 observations.