

Article

Plant-Based Beverages from Germinated and Ungerminated Seeds, as a Source of Probiotics, and Bioactive Compounds with Health Benefits—Part 1: Legumes

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Abstract: Consumption of plant-based milk replacers has increased in recent years due to health benefits, benefits attributed mainly to the content of phenolic compounds, fatty acids, or bioactive compounds with antioxidant activity. In this context, we proposed to obtain two types of less studied plant-based beverages, namely lupine and chickpea beverages, as well as the possibility of getting these beverages using germinated seeds and even obtaining probiotic drinks through fermentation with *Lactobacillus plantarum* 299v. To evaluate the quality of the obtained products, we determined their content of proteins, fatty acids, organic acids, volatile compounds, and phenolic compounds. We evaluated the antioxidant activity of the obtained herbal drinks, and a load of probiotic microorganisms present after the fermentation process. Both lupine and chickpeas are legumes with high protein content and a range of health benefits. Fermentation with *L. plantarum* introduces probiotic properties and enhances the nutritional profile of these beverages. Plant-based beverages inoculated with *L. plantarum* can offer a convenient way to incorporate probiotics into plant-based diets, providing consumers with the benefits of both plant-based nutrition and probiotic supplementation.

Keywords: plant-based beverages; lupine; chickpea; *Lactobacillus plantarum*; probiotic drinks; bioactive compounds



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1. Introduction

The consumption of plant-based beverage substitutes has increased significantly in recent years due to the numerous health benefits and the growing number of people suffering from lactose intolerance or allergy to milk proteins. These beverages are preferred by consumers who follow a plant-based diet for various reasons, ranging from a desire for a healthy lifestyle and awareness of environmental pollution to aspects such as aversion to animal cruelty [1]. Members of the pea family (Fabaceae), have a higher protein content than cereal grains [2]. Examples of legumes are soybeans, chickpeas, lentils, common beans, mung beans, and lima beans. Sweet lupin and chickpea have been utilized in numerous products ranging from traditional fermented foods (Tempe, Miso, etc.) to dairy substitutes, pasta, noodles, etc. [3].

Only a few legumes and oilseeds have been widely used for the preparation of healthy, affordable, and nutritious plant-based beverages alternatives, as the consumer demands that these plant-based beverages are a comparable alternative to cow's milk in terms of nutritional value, appearance, taste, aroma, and stability [4].

Legumes are an essential source of macronutrients, micronutrients, and anti-nutritional compounds in several diets, including vegan and vegetarian choices. Nutrient interactions with anti-nutritional factors prevent their release, referring to trypsin inhibitors and phytates that reduce protein digestibility and mineral release.

Fermentation and germination are commonly used methods for releasing nutrients and phytonutrients, making them more accessible to digestive enzymes. Sprouted grains are more nutritious than raw grains and are rich in digestible energy, bioavailable vitamins, minerals, amino acids, proteins, and phytochemicals [5].

Germination facilitates the enzymatic breakdown of carbohydrates into simple sugars by activating endogenous enzymes such as α -amylase and thus digestibility due to starch degradation to provide energy for seed development [2]. The effect of germination on carbohydrates depends largely on the activation of hydrolytic and amylolytic enzymes that lead to decreased starch and increase of simple sugars [2]. The duration of the process is an important factor. The maximum starch hydrolysis is between 48 and 72 h when the amylase activity is at its maximum [6].

Fermentation is used to increase the bioaccessibility and bioavailability of nutrients in various crops, improve organoleptic properties, and extend shelf life so fermentation is a desirable process of biochemical modification of the primary food matrix produced by microorganisms and their enzymes. The fermentation process can reduce the content of various antinutrients in lupine [7,8]. Lactic acid bacteria (LAB) can affect the flavor of fermented foods in several ways, depending on the composition of the raw material. LAB fermentation can also be used to improve the nutritional quality of vegetables or the functionality of their proteins [9]. Furthermore, LAB strains can increase the nutritional value of plant-based beverage analogs by influencing the content of vitamins such as B-complex [10].

L. plantarum 299v is a probiotic strain of LAB naturally appearing in the human gut, which can modulate the immune system. Its immunomodulating properties are observed to decrease anti-inflammatory cytokines [11]. This strain has a high tolerance to low pH from the stomach and high pH from the duodenum. The ability of *L. plantarum* 299v to adhere to the intestinal wall also classifies the strain as a good probiotic since it is able to reside in human mucosal cells in vivo [11]. Recent studies have shown an increase in glucose levels in the early stages of fermentation due to starch hydrolyzing, and the effect of activated maltase and α -amylase [12]. Glucose released during fermentation is a preferred substrate for food-fermenting microorganisms and could partially explain the decrease in total carbohydrates after 24 h of fermentation [13]. In humans, vegetable protein has poor digestibility compared to animal protein, and fermentation can increase the digestibility of vegetable proteins. Unfortunately, microflora can also utilize amino acids and proteins during fermentation, leading to the loss of amino acids and proteins. Therefore, the optimal fermentation conditions that could result in maximum protein digestibility with minimal protein loss remain unclear [14]. Probiotic drinks can be produced from various raw materials such as grains, millet, legumes, fruits, and vegetables [15]. *L. albus* seeds are low-alkaloid varieties and a valuable alternative source of proteins [16]. However, depending on the species, lupines contain varying contents of toxic alkaloids [17], which partly limits their utilization. The major alkaloids of *L. albus* are lupanine (55–75% of total alkaloids), albine (6–15%), multiflorin (3–14%), 13-hydroxylupanine (4 to 12%), 13-angeloyloxylupanine (1–3%). Minor alkaloids of the seeds are ammodendrine, angustifolin, 5,6-dehydrolupanine, isoangustifoline, α -isolupanine, 17-oxolupanine, 11,12-seco-12,13-didehydro-multiflorin (previously N-methyl-albin), sparteine, tetrahydrocytisine, tetrahydrorhombifoline, various esters of 13-hydroxylupanine and 13-hydroxymultiflorin, 5,6-dehydromultiflorin, lupanine N-oxide and 13- α -hydroxy-5-dehydromultiflorin [18]. Soaking and dehulling the lupine seeds decreases the total alkaloid content and is recommended in lupine-based beverage production technology [19].

Functional drinks constitute one of the most developed segments in the market [20] and are highly valued for their nutritional characteristics [3]. As non-dairy probiotic

beverages that can be consumed by people with lactose intolerance, with an allergy to cow's milk proteins, and people that do not drink dairy-based probiotic drinks due to ethical reasons [21], they are a choice that brings a supply of probiotic bacteria, increase the digestibility, reduce the flatulence, fight against the unwanted pathogens, while also improving taste and texture [22].

Legumes are carriers of prebiotics as they contain non-digestible oligosaccharides that microorganisms can metabolize. The non-dairy probiotic beverages based on legumes are rich in bioactive compounds, prebiotics, and can be fortified with probiotics, which enhance human intestinal health [23].

In this context, this work aimed to obtain and evaluate plant-based beverages using germinated and ungerminated seeds of lupine and chickpea in order to obtain probiotic drinks by fermentation with *Lactobacillus plantarum* 299v and compare them with the much more available and consumed oat beverages.

2. Materials and Methods

2.1. Materials and Chemicals

We used two types of seeds, both from the legume family *Fabaceae*, white lupin (*Lupinus albus* L.) and chickpea (*Cicer arietinum* L.), as well as oat (*Avena sativa* L.) seeds, all obtained from local suppliers. Man Rogosa Sharp Agar and Peptone water for microbial growth were purchased from Merck (Darmstadt, Germany). Standards of sugars and phenolic compounds were purchased from Sigma-Aldrich Co. and Fluka (Saint Louis, MO, USA), and the rest of the reagents were bought from Merck (Darmstadt, Germany). Before analysis, the samples were filtered through a 0.45 µm MF-Millipore™ Membrane Filter from Merck (Darmstadt, Germany).

2.2. Germination and Plant-Based Beverages Preparation

To obtain the plant-based beverages, were used germinated and ungerminated lupine and chickpea seeds. 225 g of each germinated and ungerminated seeds were used for the experiment. All the grains were washed and soaked in water, in a ratio of seeds: water 1:2, for 8 h at 22 °C. For each experimental variant, three repetitions were made.

To obtain the germinated seeds, the water was drained after 8 h of soaking, and the grains were placed in germinators for 48 h at 22–25 °C, according to the literature data [24,25]. After 48 h, the germinated seeds were removed from the germinators, rinsed, and used for the next steps. Lupine seeds were dehulled to produce a beverage with low alkaloid content.

Plant-based beverages preparation: The seeds thus prepared, were placed in a blender, and 1275 mL of water was added. After both seeds were ground, the beverage were filtered and squeezed to obtain the plant-based beverages.

Oat-based beverages, prepared in the same way, were used to compare the obtained results with the results obtained for a well-known plant-base beverage [26].

2.3. Fermentation of Plant-Based Beverages

L. plantarum 299v is a probiotic strain commonly found in fermented foods of plant origin, such as sauerkraut, brined olives, or pickled cucumbers [27]. *L. plantarum* 299v was purchased in lyophilized form and was grown in MRS (Man Rogosa Sharpe) broth at 37 °C for 20 h and transferred twice prior to inoculating the plant-based beverages. After that, the obtained biomass was centrifugated (Eppendorf R 5804 centrifuge, Hamburg, Germany) at 2000× *g*, 10 min, at 4 °C and washed three times with sterile water. The optical density of the thus obtained suspension was measured at a wavelength of 600 nm, after which it was brought to the concentration of 20 × 10⁹ CFU/mL to be used for the inoculation of plant-base beverages. After the grinding and straining process, 750 mL of each plant-based beverage category was dispensed into sterile containers and seeded with the probiotic culture. The fermentation process was conducted for 20 h, at 37 °C and the plant-based beverage was transformed into a fermented/probiotic beverage.

2.4. Analytical Methods

2.4.1. Protein, Fat, pH

We use Fulmatic Lactoscan Milk Analyzer Julie Z9, Scope Electric Instruments, Razgrad, Bulgaria, an automatic Test Milk Analyzer that uses a small 5–10 mL sample for protein, fat content, and pH determination. The device analyzes animal, plant, and other specialty milk beverages in 60 s.

2.4.2. Fatty Acids Content (FAC)

A 10 mL sample of plant-based beverage was extracted with 25 mL diethyl ether at room temperature for 2 h. The mixture was centrifuged for 5 min at $6700\times g$. The fatty upper layer was separated, and the fatty acids were isolated using the method described in Copolovici et al., 2017 [28]. Briefly, the fatty acids were transmethyalted into corresponding fatty acid methyl esters using a methanol/toluene/sulphuric acid mixture (88/10/2 *v/v/v*). The resulting methyl esters were extracted twice with *n*-heptane and analyzed by GC-MS (Shimadzu 2010 Plus), Shimadzu Europa GmbH, Duisburg, Germany. The constituents have been identified based on fatty acid standards and National Institute of Standards and Technology 14 and Wiley 09 mass spectra libraries. The results were expressed as mg fatty acid/100 mL plant-based beverages.

2.4.3. Organic Acids Content

The liquid chromatography–mass spectrometry (LC/MS) method was performed on a Shimadzu Nexera I LC/MS–8045 (Kyoto, Japan) ultra-high-performance liquid chromatography (UHPLC) system equipped with a quaternary pump and autosampler, an electrospray ionization (ESI) probe and quadrupole rod mass spectrometer. The separation was performed on a Luna C18 reversed-phase column (150 mm $\times$ 4.6 mm $\times$ 3 μ m, 100 Å), from Phenomenex (Torrance, CA, USA). The column was maintained at 35 °C during the analysis.

The mobile phase was a gradient made from acetonitrile (Merck, Darmstadt, Germany) and ultra-purified water prepared by Simplicity Ultra Pure Water Purification System (Merck Millipore, Billerica, MA, USA). The organic modifier was formic acid (Merck, Darmstadt, Germany). Both the acetonitrile and the formic acid were of LC/MS grade. The flow rate was 0.5 mL/min, resulting in a total analysis time of 8 min.

The detection was performed on a quadrupole rod mass spectrometer operated with ESI, in positive multiple reaction monitoring ion modes. The interface temperature was set at 30 °C. For vaporization and drying gas, nitrogen was used at 35 psi and 10 L/min. The capillary potential was set at +3000 V. The results were expressed as mg/100 mL plant-based beverages.

2.4.4. Volatile Compounds

The volatile compounds content was evaluated by GC-MS. A Dani Master GC-MS system was used, along with an SH-Rxi-5ms column with dimensions of 30 cm $\times$ 0.25 mm $\times$ 0.25 mm and nitrogen as the carrier gas, with a 10 mL/min flow rate and gradient temperature. The ESI mass spectrometry detector identified the compounds with molecular weights from 50 to 600 Daltons, and the ion source was operated at 20 °C. The results were expressed as a percentage (%) of the volatile fraction.

2.4.5. Total and Individual Content of Phenolic Compounds

The Total Phenolic Content

The total phenolic content (TPC) was determined through the Folin–Ciocalteu method [29]. Briefly, 100 μ L Folin–Ciocalteu reagent (0.2 N) was added to 10 μ L of plant-based beverages and mixed with 80 μ L sodium carbonate (Na_2CO_3) solution (1 M). After 20 min, the absorbance of the resulting, blue-colored solution was measured at 765 nm. For quantification, a calibration curve of gallic acid was prepared with solutions in the range of

0.025–0.15 mg/mL ($R^2 = 0.9992$). The results were expressed as mg of gallic acid equivalent (GAE)/mL of plant-based beverages. The assays were run in triplicate.

Total Flavonoid Content

Total flavonoid content (TFC) was measured by the aluminum chloride colorimetric assay on a 96-well microplate reader (Synergy™ HT BioTek Instruments, Winooski, VT, USA), using quercetin as a reference standard [30]. An exact volume of 25 μ L of each sample was added to 100 μ L distilled water and 10 μ L of 5% sodium nitrate (NaNO_2) solution. After 5 min, 15 μ L of 10% aluminum chloride (AlCl_3) was added. At 6 min, 50 μ L of 1 M sodium hydroxide and 50 μ L of distilled water were added to the mixture. The absorbance of the mixture was determined at 510 nm. For quantification, a calibration curve of quercetin was prepared with solutions in the range of 0.025–0.2 mg/mL ($R^2 = 0.9987$). The results were expressed as mg of quercetin equivalent (QE)/mL of plant-based beverages. The assays were run in triplicate.

Individual Polyphenolic Compounds

The samples were analyzed with a Shimadzu Nexera I LC/MS–8045 (Kyoto, Japan) UHPLC system equipped with a quaternary pump and autosampler, respectively, an ESI probe and quadrupole rod mass spectrometer. The separation was performed on a Luna C18 reversed-phase column (150 mm $\times$ 4.6 mm $\times$ 3 mm, 100 Å), from Phenomenex (Torrance, CA, USA). The column was maintained at 40 °C during the analysis. The mobile phase was a gradient made from methanol (Merck, Darmstadt, Germany) and ultra-purified water prepared by Simplicity Ultra Pure Water Purification System (Merck Millipore, Billerica, MA, USA). The organic modifier was formic acid (Merck, Darmstadt, Germany). The initial gradient was 5:90:5, methanol:water:formic acid water. The methanol and the formic acid were of LC/MS grade. The flow rate used was 0.5 mL/min. The detection was performed on a quadrupole rod mass spectrometer operated with ESI in negative and positive multiple reaction monitoring ion mode. The interface temperature was set at 30 °C. Gas nitrogen was used at 35 psi and 10 L/min for vaporization and drying. The identification was performed by comparison of MS spectra and their transitions between the separated compounds and standards. The identification and quantification were made basely on the main transition from the MS spectra of the substance. Quantification was performed using calibration curves.

2.4.6. Antioxidant Activity

Determination of 2,2-diphenyl-1-picrylhydrazyl Radical Scavenging Activity

The scavenging activity of the tested plant-based beverages against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) was evaluated spectrophotometrically by the method of Criste et al. [30] with slight modifications. Briefly, each sample's aliquot (40 μ L) was mixed with 200 μ L DPPH solution (0.02 mg/mL). Samples were kept for 15 min at room temperature, and then the absorbance was measured at 517 nm. The radical scavenging activity is expressed in milligram equivalent Trolox per gram of sample (mg Trolox equivalent/mL).

ABTS Radical Scavenging Activity

The method is based on the ability of the tested antioxidant to capture the cationic radical of ABTS (2,2-azino-bis(3-ethylbenzotiazolin-6-sulfonat). The ABTS radical scavenging activity assay was performed according to the method described by Re et al. [31]. The ABTS^+ + cation radical was produced by the reaction between 7 mM ABTS solution and 2.45 mM potassium persulfate solution, stored in the dark at room temperature for 12 h. Before usage, the ABTS^+ + solution was diluted to an absorbance of 0.700 ± 0.025 at 734 nm with ethanol. The resulting solution was mixed with 17 μ L of each plant-based beverage sample for the assay. The absorbance was read after 6 min. The standard curve was linear between 0.04 and 0.4 mg Trolox. Results were expressed in mg Trolox equivalent/mL.

2.4.7. Determination of the Number of Lactic Bacteria in Fermented Products

Enumeration of viable lactic acid bacteria (LAB) was performed by estimation of CFU number on Man Rogosa Sharp Agar plates after incubation at 37 °C for 48 h, according to the protocol described by Criste et al., 2020 [32]. Triplicate platings of each sample were made, and the average value was represented as log CFU/mL.

2.4.8. Total Alkaloids Content

Lupine seeds (5 g) were homogenized in 25 mL of HCl 0.5 N in a sonicator for 30 min. The homogenate was centrifuged for 10 min at 5000× g and the pellet was resuspended in 5 mL of 0.5 N HCl. 2 mL of Dragendorff's reagent was added to 5 mL of the extract solution, and the precipitate formed was centrifuged. The precipitate was further washed with ethanol according to the method described by Sreevidya & Mehrotra, 2003 [33]. The absorbance was measured at 435 nm. The results are expressed in percentages and represent the mean of three independent determinations.

2.5. Statistical Analysis

All measurements were performed in triplicate, and the results were represented as mean ± the standard deviation of the mean. Statistical analyses were performed with the GraphPad Prism 9.3.0 statistics program. Data statistical analyses were achieved using one-way ANOVA followed by the post hoc Tukey test to observe significant differences ($p < 0.05$) between all samples from each plant-based beverage type.

3. Results

3.1. Protein, Fat, Density, and pH

The results regarding the protein, fat content, density, and pH of plant-based beverages are presented in Table 1.

Table 1. Automatic characterization of plant-based beverages (protein, fat, density, and pH).

Sample	Protein	Fat	Density	pH
	%	%		
Lupine	5.63 ± 0.25 ^a	1.99 ± 0.10 ^a	39.96 ± 0.76 ^a	8.28 ± 0.15 ^a
Lupine germinated	2.93 ± 0.12 ^b	1.27 ± 0.13 ^a	27.03 ± 0.53 ^b	5.97 ± 0.13 ^b
Lupine fermented	2.54 ± 0.10 ^b	1.17 ± 0.17 ^a	23.11 ± 0.48 ^c	4.59 ± 0.10 ^c
Lupine germinated fermented	2.42 ± 0.22 ^b	1.18 ± 0.13 ^a	21.91 ± 0.21 ^d	4.99 ± 0.11 ^c
Chickpea	3.12 ± 0.25 ^a	2.09 ± 0.22 ^a	28.36 ± 0.31 ^a	8.02 ± 0.16 ^a
Chickpea germinated	2.52 ± 0.11 ^b	1.38 ± 0.12 ^b	22.78 ± 0.36 ^b	7.29 ± 0.13 ^a
Chickpea fermented	1.87 ± 0.12 ^c	1.13 ± 0.17 ^b	16.26 ± 0.28 ^c	4.43 ± 0.12 ^b
Chickpea germinated fermented	2.36 ± 0.23 ^b	1.01 ± 0.10 ^b	21.42 ± 0.32 ^b	6.24 ± 0.11 ^c
Oat	3.79 ± 0.24 ^a	0.62 ± 0.12 ^a	36.42 ± 0.25 ^a	8.07 ± 0.11 ^a
Oat fermented	2.82 ± 0.11 ^b	0.71 ± 0.13 ^a	26.37 ± 0.33 ^b	5.26 ± 0.17 ^b

Values are represented as mean ± standard deviation. Within the same column, different letters indicate significant differences ($p < 0.05$).

It can be observed that the germination process and the fermentation with *L. plantarum* lead to decreased protein and fat content. Still, the differences are statistically insignificant ($p > 0.05$). As expected, pH drops significantly when *L. plantarum* ferments plant-based beverages.

3.2. Fatty Acids Content (FAC)

The FAC was analyzed by GC-MS (Shimadzu 2010 Plus). The obtained FAC is presented in Table 2. From the category of short-chain saturated fatty acids, lauric acid shows close values between plant-based drink variants, the lowest values observed being in the probiotic drink sprouted from lupine of 5.36 ± 0.65 mg/100 mL and in the drink from

fermented chickpea, namely, 5.62 ± 0.43 mg/100 mL. In contrast, the highest content was observed in chickpea-germinated fermented beverages. No significant differences ($p > 0.05$) were observed in the amount of lauric acid between lupine, chickpea, or oat beverages regardless of whether the seeds are germinated, or the obtained beverage is subjected to the fermentation process, except for the probiotic drink obtained from sprouted chickpeas ($p < 0.05$).

Among the long-chain saturated fatty acids, the presence of myristic, palmitic, and stearic acid was detected. Margaric acid was also observed in some lupine and chickpea beverages but not in oat beverages. Myristic acid was detected in all samples including the oat drinks, but the differences between samples are not significant ($p > 0.05$). Lower values regarding the amount of myristic acid were detected in the chickpea probiotic beverage samples, compared to chickpea germinated samples. Palmitic acid is mainly present in lupine beverages and increases insignificantly in beverages obtained from germinated lupine, but significantly after the fermentation with *L. plantarum*. Fermentation significantly increases the amount of palmitic acid in chickpea-germinated probiotic beverages from 14.75 ± 7.99 mg/100 mL (CG) to 27.49 ± 2.09 mg/100 mL (CGF).

In the case of stearic acid, an increase in its quantity is observed by germination in all types of seeds. However, the differences are significant only in the case of germinated chickpea beverage compared to chickpea beverage. Obtaining the probiotic drink by fermentation with *L. plantarum* leads to a significant decrease ($p < 0.05$) in the amount of stearic acid from 2.42 ± 0.22 mg/100 mL observed in oat beverage to 0.23 ± 0.03 mg/100 mL observed in oat probiotic beverage.

The detected monounsaturated fatty acids are represented by palmitoleic acid, elaidic acid, oleic acid, and vaccenic acid.

Higher amounts of oleic acid were observed, especially in the case of drinks with lupine and chickpeas. In addition, an increase in the value of oleic acid was observed after the germination process for both lupine and chickpea. However, the oleic acid content decreases significantly following the fermentation process with *L. plantarum* in the case of oats beverages. Palmitoleic acid has similar values between all types of plant beverages, with higher amounts observed following the fermentation process.

Among the polyunsaturated fatty acids, linoleic acid was detected, especially in the case of chickpea germinated beverage which significantly decreased ($p < 0.05$) by fermentation. Instead a significant increase ($p < 0.05$) in linoleic acid by fermentation with *L. plantarum* is observed in lupine seed beverage.

Table 3 shows the summary of the fatty acids presented in Table 2 by category: short-chain fatty acids (SCFA), long-chain fatty acids (LCFA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), unsaturated fatty acids (UFA), as well as the ratios between SFA/UFA and PUFA/MUFA.

If the values of SCFA (Table 3) of the different plant-based beverages are very close, consistently higher amounts of long-chain saturated fatty acids can be observed in lupine beverages compared to chickpea beverages.

Higher amounts of monounsaturated fatty acids (MUFA) are observed in lupine drinks, while polyunsaturated fatty acids (PUFA) are more present in chickpea drinks, making the PUFA/MUFA ratio between 0.24–0.27 mg fatty acid/100 mL in lupine drinks and between 0.37–0.75 mg fatty acid/100 mL in chickpea drinks. PUFA are in higher quantity in the oat drink compared to the probiotic oat drink, but the PUFA/MUFA ratio is 1.91 and 2.39, respectively.

Table 2. Fatty acids content of plant-based beverages (mg fatty acid/100 mL plant-based beverages).

	Lauric Acid	Myristic Acid	Palmitoleic Acid	Palmitic Acid	Margaric Acid	Linoleic Acid	Elaidic Acid	Oleic Acid	Vaccenic Acid	Stearic Acid
Lupine (L)	6.50 ± 0.77 ^a	1.07 ± 0.08 ^a	0.23 ± 0.04 ^a	22.43 ± 1.87 ^{cd}	nd	12.97 ± 1.18 ^{bc}	28.69 ± 3.08 ^a	21.73 ± 1.35 ^b	2.65 ± 0.29 ^{bc}	1.51 ± 1.88 ^a
Lupine germinated (LG)	5.71 ± 0.86 ^a	1.02 ± 0.22 ^a	0.32 ± 0.08 ^a	25.44 ± 2.72 ^{bc}	nd	17.20 ± 1.89 ^b	34.68 ± 7.41 ^a	24.62 ± 1.56 ^b	3.28 ± 0.55 ^{bc}	3.25 ± 0.35 ^a
Lupine fermented (LF)	5.63 ± 1.01 ^a	1.33 ± 0.41 ^a	0.40 ± 0.09 ^a	30.94 ± 3.29 ^{ab}	0.27 ± 0.01 ^a	22.75 ± 2.32 ^a	27.66 ± 0.44 ^a	52.38 ± 8.11 ^a	4.77 ± 0.57 ^a	3.93 ± 0.45 ^a
Lupine germinated fermented (LGF)	5.36 ± 0.65 ^a	1.19 ± 0.46 ^a	0.40 ± 0.09 ^a	17.29 ± 1.40 ^d	0.04 ± 0.02 ^b	10.35 ± 0.87 ^c	14.94 ± 0.01 ^b	21.06 ± 2.80 ^b	1.90 ± 0.21 ^c	2.53 ± 0.21 ^a
Chickpea (C)	5.95 ± 0.56 ^b	1.11 ± 0.29 ^a	0.36 ± 0.02 ^a	19.91 ± 1.33 ^{ab}	nd	9.41 ± 0.68 ^c	12.42 ± 2.63 ^{bc}	11.67 ± 0.93 ^{ab}	0.88 ± 0.09 ^c	2.67 ± 0.21 ^c
Chickpea germinated (CG)	6.23 ± 0.30 ^b	1.47 ± 0.32 ^a	0.37 ± 0.09 ^a	14.75 ± 7.99 ^b	0.59 ± 0.09 ^a	37.26 ± 2.24 ^a	32.09 ± 4.32 ^a	26.30 ± 7.73 ^a	2.60 ± 0.02 ^a	4.19 ± 0.30 ^a
Chickpea fermented (CF)	5.62 ± 0.43 ^b	0.89 ± 0.11 ^a	0.39 ± 0.03 ^a	9.42 ± 0.01 ^{bc}	0.20 ± 0.08 ^b	3.10 ± 0.30 ^d	3.83 ± 0.25 ^{cd}	2.29 ± 0.03 ^{bc}	0.20 ± 0.07 ^d	1.73 ± 0.08 ^d
Chickpea germinated fermented (CGF)	8.02 ± 0.32 ^a	1.27 ± 0.05 ^a	0.44 ± 0.04 ^a	27.49 ± 2.09 ^a	0.29 ± 0.20 ^{ab}	33.50 ± 2.09 ^{ab}	20.81 ± 6.11 ^b	21.16 ± 8.70 ^a	1.96 ± 0.16 ^b	3.36 ± 0.26 ^b
Oat (O)	5.21 ± 0.46 ^a	0.97 ± 0.21 ^a	0.17 ± 0.24 ^a	33.59 ± 2.89 ^a	nd	35.40 ± 2.93 ^b	16.84 ± 8.62 ^a	16.47 ± 5.93 ^a	1.14 ± 0.06 ^a	2.42 ± 0.22 ^a
Oat fermented (OF)	4.58 ± 0.64 ^a	0.82 ± 0.02 ^a	0.33 ± 0.05 ^a	19.95 ± 1.83 ^a	nd	66.02 ± 5.36 ^a	13.80 ± 0.82 ^a	0.55 ± 0.01 ^b	0.13 ± 0.05 ^b	0.23 ± 0.03 ^b

nd—not detected; Values are represented as mean ± standard deviation. Data statistical analyses were achieved by using one-way ANOVA followed by post hoc Tukey test to see significant differences between all samples from each plant-based beverage type, different letters show the significant differences within each group ($p < 0.05$).

Table 3. Fatty acids content of plant-based beverages (mg fatty acid/100 mL). SCFA: short-chain fatty acids; LCFA: long-chain fatty acids; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: Unsaturated fatty acids.

	SCFA	LCFA	SFA	MUFA	PUFA	UFA	SFA/UFA	PUFA/MUFA
Lupine	0.06	0.25	0.32	0.53	0.13	0.66	0.48	0.24
Lupine germinated	0.06	0.30	0.35	0.63	0.17	0.80	0.44	0.27
Lupine fermented	0.06	0.36	0.42	0.85	0.23	1.08	0.39	0.27
Lupine germinated fermented	0.05	0.21	0.26	0.38	0.10	0.49	0.54	0.27
Chickpea	0.06	0.24	0.30	0.25	0.09	0.35	0.85	0.37
Chickpea germinated	0.06	0.21	0.27	0.61	0.37	0.99	0.28	0.61
Chickpea fermented	0.06	0.12	0.18	0.07	0.03	0.10	1.82	0.46
Chickpea germinated fermented	0.08	0.32	0.40	0.44	0.33	0.78	0.52	0.75
Oat	0.05	0.37	0.42	0.35	0.66	1.01	0.42	1.91
Oat fermented	0.05	0.40	0.44	0.15	0.35	0.50	0.88	2.39

3.3. Organic Acids Content

The organic acids (Table 4) that were detected in the analyzed samples are mainly represented by lactic acid resulting from the metabolism of carbohydrates and citric acid or fumaric acid.

Table 4. Organic acids content in plant-based beverages.

Sample	Lactic Acid mg/100 mL	Citric Acid mg/100 mL	Fumaric Acid mg/100 mL
Lupine	1.16 ± 0.18 ^c	nd	nd
Lupine germinated	396.38 ± 15.08 ^a	nd	nd
Lupine fermented	4.19 ± 0.22 ^c	405.43 ± 26.18 ^a	6.33 ± 1.27
Lupine germinated fermented	117.94 ± 6.27 ^b	0.07 ± 0.01 ^b	nd
Chickpea	136.42 ± 6.48 ^b	nd	nd
Chickpea germinated	153.80 ± 5.43 ^a	nd	nd
Chickpea fermented	29.13 ± 1.28 ^c	71.70 ± 5.14	nd
Chickpea germinated fermented	29.56 ± 2.02 ^c	nd	nd
Oat	1.13 ± 0.20 ^b	7.79 ± 2.12 ^b	0.46 ± 0.05
Oat fermented	154.91 ± 6.32 ^a	108.56 ± 11.18 ^a	nd

nd—not-detected; Values are represented as mean ± standard deviation. Data statistical analyses were achieved by using one-way ANOVA followed by post hoc Tukey test to see significant differences between all samples from each plant-based beverage type, different letters show the significant differences within each group ($p < 0.05$).

Through the germination process, a significant increase in the amount of lactic acid is observed in all analyzed samples. The highest values are observed in the case of beverages obtained from germinated seeds when the amount of lactic acid increases to 396.38 ± 15.08 mg/100 mL in the lupine germinated beverage and 153.80 ± 5.43 mg/100 mL in chickpea germinated beverage.

The fermentation with *L. plantarum* leads to an accumulation of lactic acid in the lupine and oat beverages.

Citric acid is detected especially after the fermentation process of the probiotic drinks obtained from ungerminated seeds, with the highest amounts being observed in the lupine probiotic product, 405.43 ± 26.18 mg/100 mL. Higher amounts of citric acid are also found in the fermented oat beverage and the chickpea probiotic beverage.

Fumaric acid was detected only in the lupine fermented beverage and in the oat beverage.

3.4. Volatile Compounds

The most frequently detected compound, dl-mevalonic acid lactone, is detected in lupine and chickpea probiotics beverages, while astaxanthin is seen only in lupine germinated samples (Table 5). Other volatile compounds detected are thymine in chickpea-germinated drinks, catechol in chickpea-germinated fermented beverages, ornithine in chickpea fermented beverages, and 2-oxo-valeric acid in chickpea germinated fermented beverages.

Table 5. Volatile compounds detected in plant-based beverages.

Sample	Identified Compounds	Match Factor	Concentration in Volatile Fraction, by Normalization, %	sd
Lupine				
Lupine germinated	astaxanthin	415	3.2	±0.08
Lupine fermented	dl-mevalonic acid lactone	836	0.3	±0.01
Lupine germinated fermented	dl-mevalonic acid lactone	807	3.7	±0.10
	astaxanthin	426	0.4	±0.01
Chickpea				
Chickpea germinated	Thymine	824	1.9	±0.02
Chickpea fermented	dl-mevalonic acid lactone	821	3.2	±0.08
	Ornithine	826	60.7	±0.75
	Catechol	881	6.5	±0.12
Chickpea germinated fermented	dl-mevalonic acid lactone	785	1.0	±0.02
	2-oxo-valeric acid	786	16.2	±0.24

sd = standard deviation, Values are represented as mean ± standard deviation.

3.5. Total and Individual Content of Phenolic Compounds

3.5.1. The Total Phenolic and Flavonoid Content

Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophotometrically using the Folin–Ciocalteu assay, respectively aluminum chloride colorimetric method. The results are presented in Table 6 and show the TPC and TFC from the analyzed plant-based beverages.

Table 6. Total phenolic and flavonoids content.

Sample	Total Phenolic Content mg GAE/mL	Total Flavonoids mg QE/mL
Lupine		
Lupine germinated	8.60 ± 0.33 ^b	1.23 ± 0.08 ^c
Lupine fermented	8.92 ± 0.08 ^b	4.24 ± 0.26 ^b
Lupine germinated fermented	11.91 ± 0.63 ^a	3.49 ± 0.61 ^b
	12.81 ± 0.48 ^a	6.55 ± 0.10 ^a
Chickpea		
Chickpea germinated	2.00 ± 0.56 ^c	0.26 ± 0.07 ^c
Chickpea fermented	3.02 ± 0.29 ^c	2.83 ± 0.35 ^b
Chickpea fermented	6.22 ± 0.22 ^b	2.62 ± 0.22 ^b
Chickpea germinated fermented	10.22 ± 0.53 ^a	3.51 ± 0.14 ^a
Oat		
Oat fermented	2.26 ± 0.05 ^a	1.19 ± 0.31 ^a
	2.33 ± 0.11 ^a	1.22 ± 0.18 ^a

GAE—gallic acid equivalents, QE—quercetin equivalents. Values are represented as mean ± standard deviation. Data statistical analyses were achieved by using one-way ANOVA followed by the post hoc Tukey test to see significant differences between all samples from each plant-based beverage type. Different letters show significant differences within each group ($p < 0.05$).

Of all the samples analyzed, the highest content of TPC was determined in samples derived from lupine, while the lowest content was observed in oat drink samples. In both sprouted lupine drink samples and sprouted chickpea drink samples, germination

seems to have increased the amount of total polyphenols. Regarding the drinks obtained by fermentation with *L. plantarum*, all showed a significantly ($p < 0.05$) higher value of polyphenols.

The highest content of flavonoids was measured in the lupine beverage samples where TFC had values between 8.60 ± 0.33 mg QE/mL for lupine beverage and 12.81 ± 0.48 mg QE/mL lupine germinated fermented beverages.

A significant ($p > 0.05$) increase in the flavonoid content following the fermentation process of the plant-based beverages with *L. plantarum* was observed in the case of bouth beverages obtained from lupine and chickpeas. In the case of beverages made from oat, TFC is lower, and the fermentation process does not change significantly ($p > 0.05$) their content.

3.5.2. Individual Polyphenolic Compounds

Although the determination of the total content of polyphenols and flavonoids allows us to appreciate the functional character of plant-based beverages, the determination of individual polyphenolic compounds allows us to appreciate their health benefits (Table 7). Sixteen phenolic compounds were detected using the Shimadzu Nexera I LC/MS-8045 (Kyoto, Japan) UHPLC system.

Among these, phenolic acids or hydroxycinnamic acid derivatives: caffeic acid, chlorogenic acid, *trans*-p-coumaric acid, ferulic acid; or hydroxybenzoic acid derivatives: salicylic acid, salicin, vanillin; flavonoids: apigenin, chrysin, luteolin-7-O-glucoside, rutin; flavonol: myricetin; flavanones: naringenin; phenolic diterpenes—carnosol; secoiridoid glycoside—amarogentin. The phenolic compound detected in all types of analyzed plant-based beverages was flavonol myricetin. In contrast, the rest of the phenolic compounds were detected only in certain kinds of plant-based drinks.

In lupine beverages, the amount of myricetin increases significantly both by germination and by fermentation of the germinated product. Phenolic acids were detected in lupine beverages such as ferulic acid, caffeic acid, *trans*-p-coumaric acid, but are no longer detected after germination or fermentation. In contrast, compounds such as apigenin, rutin, vitexin, were only detected in probiotic drinks. In chickpea beverages, besides myricetin, naringenin can be detected in all samples, which can be significantly increased due to the fermentation with *L. plantarum*. In chickpea-based probiotic drinks apigenin and salicylic acid were also detected.

Other phenolic compounds detected in chickpea-based products are caffeic acid, chlorogenic acid, amarogentin which increases significantly following the germination process, Vanillin is a phenolic compound in chickpeas beverages and chickpea-germinated beverages, but was not present in probiotic drinks.

Table 7. Individual Polyphenolic compounds detected in lupine, chickpea, and oat beverages.

Sample mg/100 mL	L	LG	LF	LGF	C	CG	CF	CGF	O	OF
Caffeic acid	38.07 ± 5.67 ^a	nd	nd	nd	nd	215.7 ± 21 ^a	nd	nd	nd	nd
Chlorogenic acid	0.95 ± 0.18 ^b	1.62 ± 0.41 ^a	nd	0.94 ± 0.05 ^b	0.73 ± 0.08 ^b	10.92 ± 1.02 ^a	nd	nd	nd	3.21 ± 0.44 ^a
<i>trans</i> -p-cumaric acid	4.19 ± 1.14 ^a	nd	nd	nd	3.45 ± 0.54 ^a	nd	nd	nd	136.57 ± 8.21 ^a	28.35 ± 4.55 ^b
Ferulic acid	22.67 ± 3.45 ^a	nd	nd	nd	nd	nd	nd	nd	125.51 ± 8.21 ^a	52.29 ± 6.22 ^b
Salicylic acid	nd	4.87 ± 1.2 ^b	4.48 ± 1.11 ^b	9.35 ± 2.08 ^a	10.61 ± 2.24 ^b	17.42 ± 2.12 ^a	3.57 ± 1.12 ^c	10.03 ± 1.24 ^b	33.21 ± 5.54 ^b	65.5 ± 8.44 ^a
Amarogentin	nd	nd	nd	nd	nd	1.02 ± 0.05 ^a	0.71 ± 0.14 ^a	nd	nd	nd
Apigenin	nd	nd	nd	1.49 ± 0.26 ^a	nd	0.63 ± 0.03 ^b	2.04 ± 0.36 ^a	0.61 ± 0.04 ^b	3.45 ± 0.54 ^a	0.67 ± 0.12 ^b
Carnosol	0.14 ± 0.02 ^a	0.12 ± 0.02 ^a	nd	nd	0.24 ± 0.04 ^a	nd	0.16 ± 0.03 ^a	nd	nd	0.15 ± 0.03 ^a
Chrysine	nd	5.81 ± 0.84 ^a	nd	nd	3.32 ± 1.05 ^b	3.32 ± 0.4 ^b	16.14 ± 2.05 ^a	nd	nd	5.26 ± 0.22 ^a
Salicin	nd	nd	nd	nd	nd	702.92 ± 23.21 ^a	567.79 ± 42.02 ^b	nd	nd	nd
Luteolin-7-O-glucosid	nd	nd	nd	nd	nd	nd	0.2 ± 0.02 ^a	nd	nd	nd
Myricetin	893.5 ± 182.35 ^c	1476.31 ± 198.27 ^b	568.69 ± 0.87 ^c	2634.69 ± 248.04 ^a	3704.52 ± 142.2 ^a	1606.85 ± 105.32 ^c	1352.27 ± 25.01 ^d	1994.24 ± 35.21 ^b	1897.68 ± 24.15 ^a	947.7 ± 22.66 ^b
Naringenin	nd	0.38 ± 0.08 ^a	nd	nd	0.59 ± 0.21 ^b	0.47 ± 0.15 ^b	16.57 ± 2.54 ^a	14.22 ± 0.51 ^a	nd	0.50 ± 0.03 ^a
Rutin	nd	nd	1.08 ± 0.57 ^a	nd	0.95 ± 0.45 ^a	nd	nd	nd	0.83 ± 0.05 ^b	1.93 ± 0.22 ^a
Vitexin	nd	nd	2.08 ± 0.33 ^a	1.04 ± 0.14 ^a	nd	0.37 ± 0.05 ^a	nd	nd	nd	nd
Vanillin	nd	nd	nd	nd	55.08 ± 5.3 ^a	41.97 ± 6.27 ^b	nd	nd	157.38 ± 6.88 ^a	nd

nd = not detected. Values are represented as mean ± standard deviation. L-lupine, LG-lupine germinated, LF-lupine fermented, LGF—lupine germinated fermented; C—chickpea, CG—chickpea germinated, CF—chickpea fermented, CFG—chickpea germinated fermented; O—oat, OF—oat fermented; Data statistical analyses were achieved by using one-way ANOVA followed by the post hoc Tukey test to see significant differences between all samples from each plant-based beverage type, different letters show the significant differences within each group ($p < 0.05$).

3.6. Antioxidant Activity

The total polyphenolic and flavonoid content provides general information about the expected antioxidant activity and allows a comparison of the antioxidant potential between samples. Two different methods, DPPH radical scavenging and ABTS radical cation quenching, were used to estimate the total antioxidant activity of the samples (Table 8).

Table 8. Determination of potential for antioxidant activity of plant-based beverages.

Sample	DPPH Radical Scavenging Activity mg Trolox Equivalent/mL	ABTS Radical Scavenging Activity mg Trolox Equivalent/mL
Lupine (L)	1.37 ± 0.17 ^a	1.09 ± 0.14 ^a
Lupine germinated (LG)	1.71 ± 0.26 ^a	1.03 ± 0.17 ^a
Lupine fermented (LF)	1.65 ± 0.32 ^a	1.07 ± 0.22 ^a
Lupine germinated fermented (LGF)	1.63 ± 0.04 ^a	1.29 ± 0.04 ^a
Chickpea (C)	0.56 ± 0.01 ^c	0.54 ± 0.11 ^b
Chickpea germinated (CG)	1.59 ± 0.02 ^a	0.92 ± 0.13 ^a
Chickpea fermented (CF)	0.66 ± 0.05 ^{b,c}	0.32 ± 0.02 ^b
Chickpea germinated fermented (CGF)	0.69 ± 0.08 ^b	0.32 ± 0.09 ^b
Oat (O)	0.62 ± 0.01 ^a	0.28 ± 0.01 ^a
Oat fermented (OF)	0.42 ± 0.02 ^b	0.29 ± 0.07 ^a

DPPH—2,2-diphenyl-1-picrylhydrazyl, ABTS—2,2-azino-bis(3-ethyl-benzo-thiazolin-6-sulfonate). Values are represented as mean ± standard deviation. Data statistical analyses were achieved by using one-way ANOVA followed by the post hoc Tukey test to see significant differences between all samples from each plant-based beverage type, different letters show the significant differences within each group ($p < 0.05$).

Due to the low total polyphenol and flavonoid content, the antioxidant potential of plant-based beverages is expected to be reduced. The antioxidant potential measured by DPPH assay and found to be between 1.37 ± 0.17 mg Trolox equivalent/g and 1.71 ± 0.26 mg Trolox equivalent/mL in lupin-based beverages. The differences between the samples were insignificant regardless of the process of germination or fermentation with *L. plantarum*.

Higher antioxidant activity was observed in the drinks from sprouted chickpeas. The differences between the rest of the values regarding the antioxidant activity obtained by the DPPH method are statistically insignificant ($p > 0.05$). Regarding the antioxidant activity tested by the DPPH method of the oat-based drink samples, it was significantly lower than that of the lupine and chickpea-based drinks.

The results obtained by the ABTS method were similar to those from the DPPH method, and were 1.09 ± 0.14 mg Trolox equivalent/g for lupine beverage and 0.54 ± 0.11 mg Trolox equivalent/mL for chickpea beverage.

The germination process can lead to an increase in antioxidant activity as observed in chickpeas. Still, the fermentation process is not always associated with an increase in antioxidant activity, however higher values of antioxidant activity, but not statistically significant ($p > 0.05$), can be observed in the lupine probiotic beverage and germinated lupine.

3.7. Determination of the Number of Lactic Bacteria in Fermented Products

Obtaining non-dairy probiotic drinks is a challenge, but also a necessity to provide people with lactose intolerance or vegans an alternative source of probiotics. The results of this study show that plant-based beverages obtained from germinated or ungerminated seeds may represent a suitable matrix as a source of probiotics. The *L. plantarum* load observed at the end of the fermentation process were presented in Table 9.

Table 9. Number of lactic bacteria in fermented plant-based beverages.

Sample	Fermented log CFU/mL	Germinated Fermented log CFU/mL
Lupine	6.46 ± 0.25 ^a	8.16 ± 0.35 ^b
Chickpea	7.69 ± 0.36 ^a	7.85 ± 0.48 ^a
Oat	7.32 ± 0.45	-

Values are represented as mean ± standard deviation. Different superscript letters within the same row indicate significant differences ($p \geq 0.05$).

The germination of the seeds before preparation of the probiotic beverages did not significantly influence the microbial load in germinated or ungerminated chickpea beverages, instead a significant difference was observed in the lupine probiotic beverage with an increase from 6.46 ± 0.25 log CFU/mL to 8.16 ± 0.35 log CFU/mL in the germinated lupine probiotic beverage.

3.8. Determination of Total Alkaloids in Lupine Seeds

To obtain a lupine-based beverage with low alkaloid content, lupine seeds were peeled. To evaluate the influence of lupine seed dehulling, we use the method described by Sreevidya, N., & Mehrotra, S. [33] to determine the total alkaloid content in soaked seeds and after dehulling them. Since the soaking process also reduces the alkaloids' content, we determined their presence in the water used for soaking (Table 10).

Table 10. Total alkaloids content.

Sample	% of Alkaloid Content
Soaked lupine	0.06 ± 0.02 ^a
Dehulled lupine	0.03 ± 0.01 ^a
Soaking water	0.002 ± 0.02 ^a

Values are represented as mean ± standard deviation. Different superscript letters within the same column indicate significant differences ($p \geq 0.05$).

Soaking and dehulling the lupine seeds leads to a decrease in the total alkaloid content. Thus, if in the soaked seeds, the alkaloid content is 0.06%, it drops to 0.03% in the dehulled seeds.

4. Discussion

Plant-based beverages have gained significant popularity in recent years as an alternative to traditional dairy products [34]. In this study, it was used germinated and ungerminated seeds of lupine and chickpea to obtain probiotic plant-based beverages. Oat-based beverages, were used to compare the obtained results with those for a well-known plant-based beverage.

The results regarding the protein, fat content, density, and pH of plant-based beverages are in accordance with results regarding the seeds of lupine and chickpea obtained by Lopes et al. [35]. Legume beverages present the most balanced composition, rich in proteins and minerals, with a low-glycemic index. The protein content, ca. 3–4%, is similar to cow milk (3.3–3.5%), while other plant and nut-based beverages typically display values between 0.1% and 1.0% [35]. In this study, the protein content was 5.63% for lupine beverage and 3.12% in chickpea beverage, but the content decreases in probiotic beverages. The lupine beverages prepared during this study were lower in fat (1.99%) than the fat content of 5.00 g/100 g observed in the lupine drink characterized by Kavas [36], this difference was probably due to the use of different variety of lupine.

The values obtained in this study for the pH value are in line to those reported by Mayuri Chavana [37], in a study regarding the development of non-dairy fermented probiotic drinks based on germinated and ungerminated barley, ragi, moth bean, soybean, almond, and coconut, and who reported that in all cases, the pH was between 5.84 to 5.33,

in germinated probiotic drinks, and from 6.48 to 4.56, in ungerminated probiotic drinks. Sharma P. et al. [22] demonstrated that the pH change of legume probiotic drinks is due to the hydrolysis of starch into sugars during germination, which is readily used by organisms and converted to lactic acid. Thus, in the case of probiotic drinks made from germinated or ungerminated seeds, the pH drops from 8.28 ± 0.15 to 4.99 ± 0.11 in the case of lupine and from 8.02 ± 0.16 to 4.43 ± 0.12 in the case of chickpea probiotic beverages.

The composition of fatty acids in plant-based beverages is very rarely analyzed in scientific studies, reason for which the obtained results were compared with the types of fatty acids detected in the seeds of legumes. Thus, in the white lupine samples analyzed by Nouha Ferchichi et al. [38], seven long-chain fatty acids (palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, myristic acid, margaric acid) were detected. In this study, the lupine beverages, were detected 4 of them (palmitic acid, stearic acid, myristic acid, and margaric acid), but margaric acid could only be detected in the probiotic beverages.

The monounsaturated fatty acids detected in the obtained beverages are represented by palmitoleic acid, elaidic acid, oleic acid, and vaccenic acid, predominating oleic acid and vaccenic acid, this results are similar with those presented in previous studies where the seeds of *L. albus* was reported to have oleic and vaccenic acids representing ca. 35% [38], and *Cicer arietinum* L. was reported to have oleic acid representing 32.22% [39] of total lipid content.

Among the polyunsaturated fatty acids, has been detected the presence of linoleic acid, especially in the case of chickpea germinated beverage and it had a value of 37.26 ± 2.24 mg/100 mL and significantly increased ($p < 0.05$) by fermentation. A significant increase ($p < 0.05$) in the amount of linoleic acid after the fermentation with *L. plantarum* is also observed in lupine seed beverage, from 12.97 ± 1.18 to 22.75 ± 2.32 mg/100 mL. In other studies, linoleic acid was reported to be the most important polyunsaturated fatty acid in lupine seeds representing on average 21.33% of total lipids [38], and between 51.2–61.62% in chickpea seeds [39].

In the process of lactic fermentation of plant-based beverages, the fatty acid content can exhibit both increments and decrements, aspect that could be observed in this study (Table 2), but also in others carried out by other authors [40,41]. The rise in fatty acid content can be ascribed to lipolysis, de novo synthesis by specific lactic acid bacteria strains, and carbohydrate metabolism. Conversely, a reduction in fatty acid content can occur due to bacterial utilization, conversion into alternative metabolites, and interactions with other microorganisms. The specific changes depend on factors such as the beverage's composition, lactic acid bacteria strain, fermentation conditions, and duration.

The organic acids detected in the analyzed samples were mainly represented by lactic acid, but also citric acid or fumaric acid were detected. Through the germination process, an increase in the amount of lactic acid was observed in all the analyzed samples. A possible explanation according to Emkani et al. [42] for the presence of lactic acid in lupine germinated seeds is as result of lactate fermentation, a natural metabolic process that occurs during the germination stage. This process helps the plant cells generate energy when the oxygen supply is limited. Also, the fermentation with *L. plantarum* leads to an accumulation of lactic acid in the lupine and oat beverages. Laaksonen et al. [34] observed a significant increase in lactic acid content and a reduction of sucrose in all the analyzed samples using different LAB starters or starter mixtures. Other acids were also detected (citric, malic, maleic, succinic, fumaric, and quinic).

In the obtained beverages, citric acid was detected, especially after fermentation in the probiotic drinks obtained from ungerminated seeds, the highest amounts were observed in the lupine probiotic product, and the fumaric acid was also detected only in the fermented lupine beverage. The presence of citric acid in fermented lupine and chickpea beverages can be attributed to a couple of possible explanations, it is possible that *L. plantarum* utilizes certain sugars present in the lupine seeds as substrates and produces metabolic intermediates that eventually lead to the formation of citric acid [43]. Another possibility is that the presence of citric acid in the fermented lupine seeds is not directly produced by *L.*

plantarum but is a result of co-culturing or contamination by other microorganisms. During fermentation, the fermentation environment can be complex, with different microorganisms present [44]. Further research and analysis are necessary to elucidate the exact mechanisms and microorganisms involved in the production of citric acid during lupine fermentation.

L. plantarum 299v cannot produce acetic or propionic acids [11], compounds also absent in the analyzed samples, but can affect the metabolic activity of other bacteria in the colon and also inhibit the growth of potentially pathogenic bacteria such as *Listeria monocytogenes*, *Bacillus cereus*, *Yersinia enterocolitica*, *Citrobacter freundii*, *Enterobacter cloacae*, and *Enterococcus faecalis* [25]. The ability of *L. plantarum* 299v to adhere to the intestinal wall, based on the mechanism of mannose-binding, also classifies the strain as a good probiotic since it is able to reside in human mucosal cells in vivo, which is essential for immunomodulating properties of this strain [45,46].

The most frequently volatile compound detected in plant-based beverages was dl-mevalonic acid lactone, detected in lupine and chickpea probiotics beverages, while as-taxanthin is seen only in germinated lupine samples. Other volatile compounds detected are thymine in chickpea-germinated beverages, catechol in chickpea-germinated probiotic drinks, ornithine in chickpea fermented beverages, and 2-oxo-valeric acid in chickpea-germinated probiotic beverages.

Regarding TPC, the results indicate differences between different types of plant-based beverages. However, total polyphenolic compounds are significantly higher ($p < 0.05$) in lupine probiotic drinks compared to chickpeas [38].

In this study, a TPC amount of 8.60 ± 0.33 GAE/mL was determined for the lupine beverage. This value is small considering the fact that other authors [32,39] reported values between 205.3 and 431.29 mg GAE/100 g [32] for lupine seeds. In the case of chickpeas, the values obtained in this study were also lower than those reported by other authors [39] for TPC determined from methanolic extracts of ungerminated chickpea seeds, namely 39.20 mg/kg and from chickpea sprouts, namely 75.60 mg/kg. These differences between the values may be due to the different variety of lupine analyzed, different extraction solvents, but also to the different ways of reporting the results.

Flavonoids are one of the main phenolic compounds found in grain legumes [47]. In this study, flavonoid content was 1.23 ± 0.08 mg QE/mL in lupine beverage while other authors reported ranged from 87.23 to 125.27 mg QE/100 g in the lupine seeds [38]. In the chickpea beverage, the flavonoid content was 2.00 ± 0.56 mg QE/mL while in chickpea seeds Kalefetoglu et al. [48], determined values from 114.3 to 118.1 mg QE/100 g depending on chickpea varieties. In the case of the oat drink, it seems that the amount of polyphenols and flavonoids did not show significant differences after fermentation, this may also be due to enzymatic activity during germination can contribute to the breakdown or modification of polyphenols, potentially reducing their content [49].

The ability of probiotic microorganisms to metabolize phenolic compounds depends on the species or strains [23]. However, differences in the total polyphenol content and antioxidant capacity have been shown between different plant-based beverages for the same probiotic strains [23], these differences were also observed in this study, where *L. plantarum* was used for the probiotic fermentation of the beverages.

Using UHPLC/triple quadrupole tandem mass spectrometry, Ferchichi et al. [38] detected 21 different phenolic compounds different varieties of lupine seed, but only 12 of them were detected in *L. albus* while using Shimadzu Nexera I LC/MS-8045 (Kyoto, Japan) UHPLC system, furthermore in the present study were detected 16 different phenolic compounds in lupine beverages. This shows that the phenolic composition varies, quantitatively and qualitatively, not only between the species of lupine, but also between the ecotypes, an aspect previously documented for lupine, chickpea, and other species of legumes [50]. In the samples analyzed, as in various recent studies, myricetin is detected in an important amount in lupine (*L. albus*) [51], or chickpea [52].

Quercetin, caffeic acid, ferulic acid, and *trans*-p-cumaric acid were detected by Grela E. et al. [53], as the main phenolic compounds in lupine and chickpea, but in this study

it was not detected quercetin in any sample. Ferulic acid was detected only in the lupine samples. Regarding, the increase in the total content of polyphenols during probiotic fermentation, coupled with the decrease in specific compounds, can be attributed to the metabolic activities of probiotic bacteria, including degradation, transformation, conversion to microbial metabolites, enhanced extraction or release, and synergistic effects with other components present in the fermentation matrix [54].

In the same study [53], DPPH radical scavenging activity was 5.04 mg Trolox equivalent/mL for lupine and 2.97 mg Trolox equivalent/mL for chickpea extracts, while in the present study the values obtained were lower, the highest values being observed in beverages produced from germinated seeds [51].

Similar values were obtained by Vollmannova et al. [51], who reported values between 5.5 and 7.75 mg Trolox equivalent/g for ABTS radical scavenging activity and values between 1.16 and 1.88 mg Trolox equivalent/g for DPPH radical scavenging activity, in different lupine types. The rise of plant-based diets has led to an increased demand for plant-based alternatives to traditional animal-based products. Plant-based beverages inoculated with *L. plantarum* can offer a convenient way to incorporate probiotics into plant-based diets, providing consumers with the benefits of both plant-based nutrition and probiotic supplementation.

The probiotic value were increased in all germinated samples the values obtained being of 8.16 ± 0.35 log CFU/mL in lupine and 7.85 ± 0.48 log CFU/mL in chickpea-germinated beverages, while in oat probiotic beverage, was detected a quantity of 7.32 ± 0.45 log CFU/mL. This concentration is within the typical range observed for probiotic products [55]. Similar results were found in probiotic beverages having germinated or ungerminated barley, ragi, moth bean, soybean, almond, and coconut [37]. The tested plant-based beverages generally provide favorable nutritive conditions for the cultivation of *L. plantarum*. These beverages contain carbohydrates, which serve as essential energy and carbon sources for the bacterium's growth and fermentation. Additionally, this plant-based beverages can offer proteins that provide amino acids necessary for *L. plantarum* metabolism and protein synthesis. Moreover, the pH of lupine germinated beverages is within the range that supports the growth of *L. plantarum*, typically slightly acidic, or it can be corrected very easily.

L. plantarum TMW 1460 [32], showed measurable growth in nutrient broth with oligosaccharides (stronger for raffinose), but also other LAB are suitable microorganisms for lupine fermentation. Most of the *L. plantarum* strains tested by Fritsch et al. [7], showed good fermentation performance, including a high number of viable cells, the formation of metabolic products, and substrate uptake. These studies support the use of *L. plantarum* strains to obtain probiotic beverages from lupine and chickpea. In contrast, a study made by Gupata S. et al. [56], regarding the process optimization for the development of a functional beverage based on lactic acid fermentation of oats, found a viable cell count at the end of the 8 h fermentation period of 10.4 log CFU/mL. Adding to the fact that the stability of *L. plantarum* during storage was reduced with 0.9 log CFU/mL at the end of the 21 days storage period means that it has viability for a long period in these matrices.

Fermenting lupine and chickpea beverages with *L. plantarum* can offer unique and nutritious plant-based probiotic beverages. Both lupine and chickpeas are legumes with high protein content and a range of health benefits. Fermentation with *L. plantarum* introduces probiotic properties and enhances the nutritional profile of these beverages. *L. plantarum* utilizes the carbohydrates present in the beverages to produce lactic acid, resulting in a tangy flavor and a decrease in pH. The fermentation process may also contribute to improved digestibility and bioavailability of proteins, making the nutrients more accessible to the body, but future studies are required. Additionally, *L. plantarum* can promote the growth of beneficial gut bacteria, potentially enhancing gut health and supporting the immune system. Incorporating *L. plantarum* into lupine and chickpea beverages offers a promising way to create probiotic-rich plant-based options with added health benefits. However, in the future formulation and fermentation conditions should be

optimized to ensure optimal growth and activity of *L. plantarum* and to achieve desirable sensory attributes in the final products [19].

5. Conclusions

The potential of lupine and chickpea as valuable sources for the production of nutrient-rich beverages was evaluated. The fermentation process with *L. plantarum* 299v resulted in the enhancement of bioactive compounds, including polyphenols, fatty acids, and organic acids, which contribute to the overall nutritional value and potential health benefits of the beverages. Additionally, the plant-based beverages exhibited significant antioxidant activity, indicating their potential as functional beverages with free-radical scavenging properties. These findings highlight the viability of using lupine and chickpea as alternative ingredients for the development of probiotic drinks, providing consumers with nutritious options that promote gut health and offer potential antioxidant benefits. Further investigations into the health benefits, optimization of fermentation conditions, and consumer acceptance are warranted to fully explore the potential of lupine and chickpea-based probiotic beverages in the market. Overall, this study provides valuable insights into expanding the range of plant-based beverages and offers potential options for consumers seeking diverse and nutritious alternatives.

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