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Posted Date: 11 May 2023

doi: 10.20944/preprints202305.0772.v1

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*Preprints* (www.preprints.org) | NOT PEER-REVIEWED | Posted: 11 May 2023 doi:10.20944/preprints202305.0772.v1

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*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 chickpea drinks have the desired properties, high content of fatty acids and phenolic compounds, that bring probiotic benefits.

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

## 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 cow's milk allergy. 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]. Legumes, 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, oodles, etc. [3].

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, mainly 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 [4].

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Functional drinks constitute one of the most developed segments in the market [5] 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 persons that do not drink dairy-based probiotic drinks due to ethical reasons [6], 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 [7].

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 probiotics, which enhance human intestinal health [8].

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. They were compared 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*, sweet 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, we used germinated and ungerminated seeds. The number of seeds used/experimental variant was 225 g/repetition (for each experimental variant, three repetitions were made), and all the grains were washed and soaked in water, in a ratio of 1:2, for 8 hours at 22 °C.

In addition to obtain lupine and chickpea beverages, we also used non-germinated oat seeds, a plant-based drink frequently found on the market [9]. These analyses were performed to report our results comparatively with a well-known product.

For germination, the water was drained after 8 hours of soaking, and the grains were placed in germinators for 48 hours at 22-25 °C. After 48 hours, the germinated seeds were removed from the germinators, rinsed, and used for the next steps. Lupine seeds have been dehulled to produce a beverage with low alkaloid content.

Plant-based beverages preparation: The ungerminated and germinated seeds were used to obtain plant-based beverages. Thus, the soaked or germinated seeds were placed in a blender, and 1275 ml of water was added. After both seeds were ground, the beverage was filtered and squeezed to obtain the desired product.

### *2.3. Fermentation of Probiotic Beverage*

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. For the experiment, $20x10^9$ colony forming unit (CFU) *Lactobacillus plantarum* 299v was used. *L. plantarum* 299v is a probiotic strain commonly found in fermented foods of plant origin, such as sauerkraut, brined olives, or pickled cucumbers [10]. The fermentation process was conducted for 20 h, and the plant-based beverage was transformed into a fermented/ probiotic beverage.

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*2.4. Analytical Methods*

2.4.1. Protein, fat, pH

We use Fulmatic Lactoscan Milk Analyzer Julie Z9, 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 seconds.

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 hours. The mixture was centrifuged for 5 minutes at 6700*g*. The fatty upper layer was separated, and the fatty acids were isolated using the method described in Copolovici et al. 2017 [11]. Briefly, the fatty acids were transmethylated 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). 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, respectively, an electrospray ionization (ESI) probe and quadrupole rod mass spectrometer. The separation was performed on a Luna C18 reversed-phase column (150 mm x 4.6 mm x 3 µm, 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/minute, resulting in a total analysis time of 8 minutes.

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/minute. 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 x 0.25 mm x 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

2.4.5.1. The Total Phenolic Content

The total phenolic content (TPC) was determined through the Folin–Ciocalteu method [12]. Briefly, 100 µL Folin–Ciocalteu reagent (0.2 N) was added to 10 µL of plant-based beverages and mixed with 80 µL sodium carbonate ($Na_2CO_3$) solution (1 M). After 20 minutes, 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

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were expressed as mg of gallic acid equivalent (GAE) / mL of plant-based beverages. The assays were run in triplicate.

2.4.5.2. 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 [13]. An exact volume of 25 µL of each sample was added to 100 µL distilled water and 10 µL of 5% sodium nitrate ($NaNO_2$) solution. After 5 minutes, 15 µL of 10% aluminum chloride ($AlCl_3$) was added. At 6 minutes, 50 µL of 1 M sodium hydroxide and 50 µL 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.

2.4.5.3. Individual Polyphenolic Compounds

The samples were analyzed whit 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 x 4.6 mm x 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/minute. 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

2.4.6.1. 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.[13] with slight modifications. Briefly, each sample's aliquot (40 µL) was mixed with 200 µL DPPH solution (0.02 mg/mL). Samples were kept for 15 minutes 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).

2.4.6.2. 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-etil-benzo-tiazolin-6-sulfonat). The ABTS radical scavenging activity assay was performed according to the method described by Re et al. [14]. 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 µL of each plant-based beverage sample for the assay. The absorbance was read after 6 minutes. The standard curve was linear between 0.04 and 0.4 mg Trolox. Results were expressed in mg Trolox equivalent/mL.

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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 hours, according to the protocol described by Criste et al., 2020 [15]. 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 0.5N in a sonicator for 30 minutes. The homogenate was centrifuged for 10 minutes at 5,000g and the pellet was resuspended in 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 [16]. The absorbance was measured at 435 nm. The results are expressed in % 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 see 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 and 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 | 1.99 | 39.96 | 8.28 |
| Lupine germinated | 2.93 | 1.27 | 27.03 | 5.97 |
| Lupine fermented | 2.54 | 1.17 | 23.11 | 4.59 |
| Lupine germinated fermented | 2.42 | 1.18 | 21.91 | 4.99 |
| **Chickpea** | 3.12 | 2.09 | 28.36 | 8.02 |
| Chickpea germinated | 2.52 | 1.38 | 22.78 | 7.29 |
| Chickpea fermented | 1.87 | 1.13 | 16.26 | 4.43 |
| Chickpea germinated fermented | 2.36 | 1.01 | 21.42 | 6.24 |
| **Oat** | 3.79 | 0.62 | 36.42 | 8.07 |
| Oat fermented | 2.82 | 0.71 | 26.37 | 5.26 |

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

We can observe 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.

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*3.2. Fatty Acids Content*

The FAC was analyzed by GC-MS (Shimadzu 2010 Plus). The obtained FAC is presented in Table 2. Lauric acid in the short-chain saturated fatty acids category shows close values between the plant-based beverages variants, the lowest values observed in the lupine germinated probiotic beverage 5.36±0.65 mg/100mL and 5.62±0.43 mg/100mL in fermented chickpea beverage. In contrast, the highest content was observed in chickpea-germinated fermented beverages 8.02±3.27 mg/100mL. 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, we determined the presence of myristic acid, palmitic acid, and stearic acid. Margaric acid was also observed in some lupine and chickpea beverages but not in oat beverages.

Myristic acid is detected in all samples, and the differences between samples are not significant ($p>0.05$). Lower values regarding the amount of myristic acid were in the chickpea probiotic beverage samples, namely 0.89±0.11 mg/100ml, it increases in chickpea germinated samples 1.47±0.32 mg/100mL (CG), 1.27±0.05 mg/100mL (CGF). Myristic acid is also present in the oat drink 0.97±0.21 mg/100mL, and the probiotic oat drink 0.82±0.02 mg/100mL.Palmitic acid is mainly present in lupine beverages 22.43±1.87 mg/100mL and increases insignificantly in beverages obtained from germinated lupine (25.44±4.72) mg/100mL but significantly by fermentation with Lactobacillus plantarum (30.94±3.29). Fermentation significantly increases the amount of palmitic acid in chickpea-germinated probiotic beverages from 14.75±7.99 (CG) to 27.49±2.09 mg/100mL (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 chickpea beverage 4.19±0.30 mg/100mL (CG), compared to 2.67±0.21 mg/100mL (C). Obtaining the probiotic drink by fermentation with L. plantarum leads to a significant decrease ($p<0.05$) in the amount of stearic acid observed in oat probiotic beverage to 0,23±0.03 mg/100mL, from 2.42±0.22 mg/100mL observed in oat beverage.

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

| | Lauric acid | Miristic 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 ($p < 0.05$).

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

Larger amounts are observed for oleic acid, especially in the case of lupine (21.73±1.35) and chickpea beverages (11.67±0.93), and an increase is observed through the germination process to 24.62±1.56 in lupine germinated beverage, and the increase is significant ($p<0.05$) in germinated chickpea beverage 26.30±7.73 mg/100mL. As with the stearic acid content, 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, we could detect the presence of linoleic acid, especially in the case of chickpea germinated beverage 37.26±2.24 and significantly increased ($p<0.05$) by fermentation to 33,50±2.09 (CGF). A significant increase ($p<0.05$) in linoleic acid by fermentation with L. plantarum is also observed in lupine seed beverage, from 12.97±1.18 to 22.75±2,32 mg/100mL.

If the content of short-chain saturated fatty acids values (Table 3) are close between the various plant-based drinks, consistently, higher amounts of long-chain saturated fatty acids can be observed in lupine beverages (between 0.21 and 0.36 mg/100 mL). Through germination and fermentation, we observed higher amounts in the probiotic beverage made from chickpeas 0.32 mg/100 mL.

**Table 3.** Fatty acids content of plant-based beverages (mg fatty acid/100 mL).

| | 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 |

SFA: saturated fatty acids (SCFA: short-chain fatty acids, LCFA: long-chain fatty acids). UFA: Unsaturated fatty acids (MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids).

Higher amounts of monounsaturated fatty acids (MUFA) are observed in lupine drinks, while polyunsaturated fatty acids are more present in chickpea drinks, making the MUFA/PUFA (polyunsaturated fatty acids) ratio between 0.24-0.27 mg fatty acid/100 mL in lupine drinks and

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between 0.37-0.75 mg fatty acid/100 mL in chickpea drinks. PUFA are in higher quantity in the oat drink 0.66 mg/100 mL compared to the probiotic oat drink (0.35 mg/100 mL), but the MUFA/PUFA ratio is 1.91 and 2.39 respectively.

### *3.3. Organic Acids Content*

The organic acids (Table 4) that we could detect in our samples are mainly represented by lactic acid resulting from the metabolism of carbohydrates and citric acid or fumaric acid.

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.

**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 ($p < 0.05$).

The fermentation with *L. plantarum* leads to an accumulation of lactic acid in the lupine and oat beverages. The amount of lactic acid increases from 1.16±0.18 mg/100 mL to 4.19±0.22 mg/100 mL in lupine probiotic beverage, and from 1.13±0.20 to 154.91±6.32 mg/100 mL in oat probiotic beverage.

Citric acid is detected especially after the fermentation process of the probiotic drinks obtained from ungerminated seeds, 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 (108,56±11.18 mg/100 mL) and the chickpea probiotic beverage (71.70±5.14 mg/100 mL).

Fumaric acid is detected only in the lupine fermented beverage (6,33±0.06 mg/100 ml) and in the oat beverage (0,46±0,05 mg/100 ml).

### *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. Other volatile compounds detected are thymine in chickpea-germinated drinks, Catechol chickpea-germinated fermented beverages, Ornithine chickpea fermented beverages, and 2-oxo-valeric acid chickpea germinated fermented beverages.

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**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 |
| **Chickpea** | astaxanthin | 426 | 0.4 | ±0.01 |
| Chickpea germinated | - | | | |
| Chickpea fermented | Thymine | 824 | 1.9 | ±0.02 |
| Chickpea germinated fermented | dl-mevalonic acid lactone | 821 | 3.2 | ±0.08 |
| **Lupine** | Ornithine | 826 | 60.7 | ±0.75 |
| Lupine germinated | Catechol | 881 | 6.5 | ±0.12 |
| | 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 Content

Total phenolic content was determined spectrophotometrically using the Folin–Ciocalteu assay and was expressed as mg of GAE/mL (gallic acid equivalents/mL). The results in Table 5 show the phenolic compounds content in different plant-based beverages. Still, they are significantly higher ($p < 0.05$), in lupine and chickpea probiotic beverages.

The TPC content is 8,60±0,33, respectively 8,92±0,08 mg GAE/mL in lupine, and germinated lupine beverages, and increase significantly ($p < 0.05$) in lupine probiotic beverages at 11,91±0,63 and 12,81±0,48 mg GAE/mL in lupine germinated probiotic drinks.

Chickpea beverages have a lower content of TPC, 2,00±0,56 mg GAE/mL respectively 3,02±0,29 mg GAE/mL chickpea germinated beverages, compared to lupine drinks. Still, the TPC content is significantly higher ($p < 0.05$) in probiotic beverages 6,22±0,22 mg GAE/mL chickpea fermented beverages and 10,22±0,53 mg GAE/mL chickpea germinated fermented beverages.

The oat drink has a lower TPC content of 2.26±0.05 mg GAE/mL and remains very close, 2.33±0.11 mg GAE/mL after fermentation with *L. plantarum*.

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**Table 5.** Total phenolic and flavonoids content.

| Sample | Total phenolic content mg GAE/mL | Total flavonoids mg QE/mL |
|---|---|---|
| **Lupine** | 8.60±0.33 $^{b}$ | 1.23±0.08 $^{c}$ |
| Lupine germinated | 8.92±0.08 $^{b}$ | 4.24±0.26 $^{b}$ |
| Lupine fermented | 11.91±0.63 $^{a}$ | 3.49±0.61 $^{b}$ |
| Lupine germinated fermented | 12.81±0.48 $^{a}$ | 6.55±0.10 $^{a}$ |
| **Chickpea** | 2.00±0.56 $^{c}$ | 0.26±0.07 $^{c}$ |
| Chickpea germinated | 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** | 2.26±0.05 $^{a}$ | 1.19±0.31 $^{a}$ |
| Oat fermented | 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 ($p < 0.05$).

3.5.2. Total Flavonoid Content

The TFC of plant-based beverages was determined using an aluminum chloride colorimetric method. The levels ranged from 0.26±0.07 mg QE/mL in the chickpea beverages and 1.23±0.08 mg Qe/mL in the lupine drinks. However, higher amounts were determined in lupine probiotic beverages (3.49±0.61 mg QE/mL) and germinated lupine beverages (6.55±0.10 mg QE/mL ), as well as chickpea probiotic beverages (2.62±0 .22 mg QE/mL) and germinated chickpea beverages (3.51±0.14 mg QE/mL).

The content of total flavonoids increases by fermentation with *L. plantarum* in the probiotic drink from ungerminated lupine (3.49±0.61 mg QE/mL) and in the one from germinated lupine (6.55±0.10 mg QE/mL). An increase in the flavonoid content following the fermentation process is also observed in the case of beverages obtained from chickpeas, which, although initially have a lower TFC (compared to those obtained from lupine), following fermentation reaches 2.62±0.22 mg QE/mL and 3.51±0.14 mg QE/mL in the case of the probiotic drink from sprouted chickpeas. In the case of beverages made from oat, TFC is lower, and the fermentation process does not change significantly ($p>0.05$).

3.5.3. 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. Sixteen phenolic compounds were detected using the Shimadzu Nexera I LC/MS - 8045 (Kyoto, Japan) UHPLC system.

**Table 6.** Individual Polyphenolic compounds detected in lupine, chickpea, and oat beverages.

| Sample mg/100mL | 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$^{a}$ |
| ***trans*-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-lupin fermented, LFG – 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 ($p < 0.05$).

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doi:10.20944/preprints202305.0772.v1

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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, chrysine, luteolin-7-O-glucoside, rutin; flavonol: myricetin; flavanones: naringenin; phenolic diterpenes - carnosol; secoiridoid glycoside- amarogentin. The phenolic compound detected in all types of plant-based beverages was the 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 (893.5±182.35 mg/100mL) increases significantly both by germination (1476.31±198.27) and by fermentation of the germinated product (2634.69±248.04 mg/100mL). Other compounds detected in lupine beverages such as ferulic acid (22.67±3.45 mg/100mL), caffeic acid (38.07±5.67 mg/100mL), *trans*-p-coumaric acid (4.19±1.14 mg/100mL), but are no longer detected after germination or fermentation. In contrast, compounds such as apigenin, rutin, vitexin, are only detected in probiotic drinks.In chickpea beverages, in addition to myricetin, naringenin can be detected in all samples, which can be significantly increased by fermentation with *L. plantarum* to 16.57±2.54 mg/100mL chickpea probiotic beverages and 14.22±0.51 mg/100mL chickpea germinated probiotic beverages. Other compounds that can be detected in chickpea-based probiotic drinks are apigenin at 2.04±0.36 mg/100mL (CF) and 0.61±0.04 mg/100mL (CGF) respectively, and salicylic acid at 3.57±1.12 mg/100mL (CF) and a significant increase of 10.03±1.24 mg/100mL in CGF.

Other phenolic compounds detected in lupine-based products are caffeic acid at 215.7±21 mg/100mL (CG), chlorogenic acid at 0.73±0.08 mg/100mL which increases significantly following the germination process to 10.92±1.02 mg/100mL, amarogentin which can also be detected in germinated chickpeas (1.02±0.05 mg/100mL) and the chickpea probiotic beverages (0.71±0.14 mg/100mL). Vanillin is a phenolic compound in chickpeas beverages (55.08±5.3 mg/100mL) and chickpea-germinated beverages (41.97±6.27 mg/100mL) but not in probiotic drinks.

### *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-etil-benzo-tiazolin-6-sulfonat). Values are represented as mean ± standard deviation. Data statistical analyses were achieved by using one-way ANOVA

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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 ($p < 0.05$).

3.6.1. Determination of DPPH Scavenging Activity

Plant-based beverages' scavenging activity (H/e transferring ability) against DPPH was evaluated spectrophotometrically and expressed as mg Trolox equivalent/mL (Table 8). Due to the low total polyphenol and flavonoid content, the antioxidant potential of plant-based beverages is expected to be reduced.The antioxidant potential was measured by DPPH assay and found to be between 1,37±0,17mg 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*.

Following the total content of phenolic compounds, higher antioxidant activity was observed in the drinks from sprouted chickpeas (1.59±0.02 mg Trolox equivalent/mL). The rest of the values regarding the antioxidant activity by the DPPH method are statistically insignificant ($p>0.05$).

The antioxidant activity by the DPPH method is significative lower (0,42±0,02 mg Trolox equivalent/mL) in oat probiotic beverage compared with oat-based beverage (0,62±0,01 mg Trolox equivalent/mL).

3.6.2. ABTS Radical Scavenging Activity

The antioxidant potential of the plant-based beverages was also measured using the ABTS assay (Table 8) and found to be 1.09±0.14 in lupine beverage and 0.54±0.11 mg Trolox equivalent/mL in chickpea beverage.

The germination process can lead to an increase in antioxidant activity as observed in chickpeas (0.92±0.13 mg Trolox equivalent/mL). 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 (1.07±0.22 mg Trolox equivalent/mL) and germinated lupine (1.29±0.04 mg Trolox equivalent/mL).

*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 with an alternative source of probiotics. Our results show that plant-based beverages obtained from germinated or ungerminated seeds may represent a suitable matrix as a source of probiotics.

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

| Sample | Fermented<br>log CFU/mL | Germinated fermented<br>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 ($p\geq0.05$).

The *L. plantarum* load observed at the end of the fermentation process was between 6.46±0.25 log CFU/mL in the lupine-based beverage and 7.69±0.36 log CFU/mL in the chickpea-based beverage. The germination of the seeds before preparation of the probiotic beverages does not significantly influence the microbial load in germinated or ungerminated chickpea beverages, the significant difference is 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.

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*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. [16] 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 |
| Dehulled lupine | 0.03±0.01 |
| Soaking water | 0.002±0.02 |

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. A loss of alkaloids is achieved through the soaking process, their presence being detected (0,002%) in the water used for soaking.

## 4. Discussion

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 [17].

Grains and legumes are important sources of macronutrients, micronutrients, and phytochemicals in the diet of various persons, and also a source of anti-nutritional compounds. These components represent a complex system found in food matrices, and interactions between these components result in insoluble complexes with reduced nutrient bioaccessibility. Nutrient interactions with anti-nutritional factors prevent their release, mainly referring to trypsin inhibitors and phytates inherent in grains and legumes that reduce protein digestibility and mineral release. Fermentation and germination are commonly used to release nutrients and phytochemicals, making them accessible to digestive enzymes [18].

Germination facilitates the enzymatic breakdown of carbohydrates into simple sugars by activating endogenous enzymes such as $\alpha$-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 simple sugars [2]. The duration of the process is an important factor. The maximum starch hydrolysis is between 48 and 72 hours when the amylase activity is at its maximum [19].

Fermentation is a desirable process of biochemical modification of the primary food matrix produced by microorganisms and their enzymes. Fermentation is used to increase the bioaccessibility and bioavailability of nutrients in various crops, improve organoleptic properties, and extend shelf life. The fermentation process can reduce the content of various antinutrients in lupine [20,21]. 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 [22]. Furthermore, LAB strains can increase the nutritional value of plant-based beverage analogs by influencing the content of vitamins such as B-complex [23].

*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 [24]. 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* [24]. Recent

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studies have shown an increase in glucose in the early stages of fermentation due to starch hydrolyzing, and the effect of activated maltase and $\alpha$-amylase [25]. 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 [26]. In humans, vegetable protein has poor digestibility compared to animal protein, and fermentation can increase the digestibility of vegetable proteins [28]. Unfortunately, microflora can also utilize amino acids and proteins during fermentation, leading to the loss of amino acids and proteins [27]. Therefore, the optimal fermentation conditions that could result in maximum protein digestibility with minimal protein loss remain unclear [27]. Probiotic drinks can be produced from various raw materials such as grains, millet, legumes, fruits, and vegetables [28]. In our study, we used germinated and ungerminated seeds of lupine and chickpea to obtain plant-based beverages. In addition, we also use non-germinated oat seeds, a plant-based beverage frequently found on the market and analyzed a fact for which we wanted to report our results compared to a well-known product.

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 [29]. Legume beverages present the most balanced composition, rich in proteins and minerals, with a low-glycemic index. Its protein content, ca. 3–4%, is similar to cow milk (3.3–3.5%), while other real and nut-based beverages typically display values between 0.1% and 1.0% [29]. In our study, the protein content is 5.63% for lupine and 3.12% in chickpea beverages but lower when using germinated seeds or probiotic beverages. The lupine beverages prepared during our 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 [30].

Mayuri Chavana [31], conducted 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 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. In our study, the pH is 4.59 in lupine and 4.43 in chickpea ungerminated probiotic beverages, respectively 4.99 and 6.24 in germinated probiotic beverages. Sharma P. et al.[7] 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 to 5 in the case of lupine and from 8 to 4 in the case of chickpea probiotic beverages.

The composition of fatty acids in plant-based beverages is very rarely analyzed in scientific studies, but we can compare our results with the types of fatty acids detected in the seeds of legumes. Thus, in the white lupine samples analyzed by Nouha Ferchichi et al. [32], seven long-chain fatty acids (palmitic acid, stearic acid, arachidic acid, behenic acid, lignoceric acid, myristic acid, margaric acid) were detected. In our study, in the lupine beverages, we managed to detect 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 our beverages are represented by palmitoleic acid, elaidic acid, oleic acid, and vaccenic acid, predominating oleic acid and vaccenic acid, similar to previous studies where the seeds of *L. albus* oleic and vaccenic acids represent cca. 35% [32], and *Cicer arietinum* L. oleic acid represents 32,22% [33] of total lipids.

Among the polyunsaturated fatty acids, we could detect the presence of linoleic acid, especially in the case of chickpea germinated beverage at 37.26±2,24 mg/100mL and significantly increased ($p<0.05$) by fermentation. A significant increase ($p<0.05$) in the amount of linoleic acid by fermentation with *L. plantarum* is also observed in lupine seed beverage, from 12.97±1.18 to 22.75±2,32 mg/100mL linoleic acid is the most important polyunsaturated fatty acid in lupine seeds representing on average 21.33% of total lipids [32], and between 51.2-61.62% în chickpea seeds [33].

The organic acids we could detect in our 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 is observed in all the analyzed samples. Also, the fermentation with *L. plantarum* leads to an accumulation of lactic acid in the lupine and oat beverages. Laaksonen et al., [34] observed

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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 our samples, 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.

*L. plantarum* 299v cannot produce acetic or propionic acids [24], also absent in our study, 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 freundi*, *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 [35,36].

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

Our results regarding the phenolic compounds content indicate differences between types of plant-based beverages. Still, they are significantly higher ($p < 0.05$), in lupine and chickpea probiotic beverages. Total polyphenol content (TPC), estimated by the Folin-Ciocalteu method for the lupine beverage, was 8,60±0,33 GAE/mL (L), a significant amount knowing that lupine seeds contain between 205.3 to 431.29 mg GAE/100 g TPC, [32], and 39.20 mg/kg in ungerminated and 75,60mg/kg in germinated chickpea seeds (methanolic extract) [33].

Flavonoids are one of the main phenolic compounds found in grain legumes [37]. In our study, flavonoid content was 1,23±0,08 mg QE/mL in lupine beverage, in the lupine seeds, it ranged from 87.23 to 125.27 mg QE/100g [32]. In the chickpea beverage, the flavonoid content was 2,00±0,56, while in chickpea seeds Kalefetoglu et al. [38], determined values from 114,3 to 118,1 mg QE/100g depending on chickpea varieties.

The ability of probiotic microorganisms to metabolize phenolic compounds depends on the species or strains [8]. However, differences in the total polyphenol content and antioxidant capacity have been shown between different plant-based beverages for the same probiotic strains [8], this is the case of our study using *L. plantarum*, TPC content is 11,91±0,63 (LF) and 12,81±0,48 mg GAE/mL (LGF) in lupine probiotic beverages and has a lower content of TPC 6,22±0,22 mg GAE/mL (CF) and 10,22±0,53 mg GAE/mL (CGF) in chickpea probiotic beverages.

Using UHPLC/triple quadrupole tandem mass spectrometry, Ferchichi et al. [32] detected 21 different phenolic compounds in lupine seeds but only 12 of them were detected in *L. albus* while using Shimadzu Nexera I LC/MS - 8045 (Kyoto, Japan) UHPLC system, while we detect 16 different phenolic compounds in our 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 the lupine, chickpea, and other species of legumes [39]. In our study as in various recent studies, myricetin is detected in an important amount in lupine (*L. albus*) [40], or chickpea [41].

Quercetin, caffeic acid, ferulic acid, and *trans*-p-cumaric acid were detected by Grela E. et al.[42] , as the main phenolic compounds in lupine and chickpea, but we didn't detect quercetin in any sample, and ferulic acid was detected only in the lupine samples. In the same study [42], DPPH radical scavenging activity was 5,04 for lupine and 2,97 for chickpea extracts, and we determined 1,37 to 1,71 mg Trolox equivalent/mL in lupine, and 0,56-1,59 mg Trolox equivalent/mL in chickpea beverages, the highest values being observed in beverages produced from germinated seeds. We obtained close values in terms of ABTS radical scavenging activity, that is 1.07 to 1.29 mg Trolox equivalent/mL in lupine, and 0,32-0,92 mg Trolox equivalent/mL in chickpea beverages, while in lupine seeds, Vollmannova et al.[40], determined ABTS radical scavenging activity 5.5 to 7.75 mg

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Trolox equivalent/g and DPPH radical scavenging activity 1.16 to 1.88 mg Trolox equivalent/g, in different cultivars.

The probiotic count was increased in all germinated samples: 8.16±0.35 log in lupine and 7.85±0.48 in chickpea-germinated beverages. Similar results were found in probiotic beverages having germinated or ungerminated barley, ragi, moth bean, soybean, almond, and coconut [31].

*L. plantarum* TMW 1,460 [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.[20], showed good fermentation performance, including a high number of viable cells, the formation of metabolic products, and substrate uptake. These studies highlight our results in the use of *L. plantarum* strains for obtaining probiotic beverages from lupine and chickpea. In oat probiotic beverage, we detect 7.32±0.45 log CFU/ml. In contrast, a study by Gupata S. et al. [43], 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.

*L. albus* seeds are low-alkaloid varieties and a valuable alternative source of proteins [44]. However, depending on the species, lupines contain varying contents of toxic alkaloids [45], 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 [46]. Soaking and dehulling the lupine seeds decreases the total alkaloid content and is recommended in lupine-based beverage production technology.

## 5. Conclusions

As consumers expect plant-based beverages to be equivalent to dairy-based products in terms of nutrition, appearance, taste, aroma, and shelf life, our study highlights these aspects of legume-based, non-dairy probiotic functional drinks. Both the lupin and the chickpea drinks analyzed in this study have the desired properties, as well as probiotic benefits, as those expected from dairy-based products, and also similar to oat-based beverages, which are well-established products on the market.

**Author Contributions:** Conceptualization, A.D.C.; methodology, A.D.C., A.C.U., C.O.C.; C.C., D.M.C. and N.K.O.; validation, A.D.C., L.C. and N.K.O.; writing—original draft preparation, A.D.C.; writing—review and editing, A.D.C. and C.O.C.; supervision, project administration, funding acquisition, A.D.C. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by USAMV Cluj-Napoca, Romania, grant number 23102/12.10.2021.

**Conflicts of Interest:** The authors declare no conflict of interest.

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