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Research Article

Fermented Beverage Based on Lupines (*Lupinus luteus*) Using Water Kefir

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Abstract: This research aimed to elaborate and characterize a fermented lupine vegan drink from water kefir grains. Firstly, the standard protocols were carried out to extract and identify alkaloids in lupine. The second stage consisted of elaborating the water-soluble lupine vegetable extract (WLVE), and the effect of sucrose, inulin, and xanthan gum concentration on the extract was evaluated. The formulations were characterized for cell growth of kefir grains, beverage yield, soluble solids, and lactic acid. In the third step, the optimized drink (fermented and non-fermented) was characterized for moisture content, ash, total lipids, crude protein, and determination of total carbohydrates. The elaboration and characterization of a fermented vegan drink from water kefir grains using lupine was conducted. The optimized fermented drink was the formulation given by the central point. The lupine seed characterization revealed a high protein content, and the optimized beverage characterization showed a considerable carbohydrate content. The optimized drink has enzymatic activity with an emphasis on lipases. The drink's development as a new product using water-soluble lupine plant extract brought an exciting application for this legume and an additional food option for vegan consumers or consumers with dietary restrictions related to dairy products.

***Keywords*:** vegan market, experimental design, product development, dietary restrictions

1. Introduction

In recent years, the dietary profile of consumers has shown a trend towards healthier, more natural, accessible, low-cost, safe, and convenient products [1-2]. People are increasingly concerned about protecting natural resources and ethical issues in their lifestyles, and, together with this, the population consuming vegan food has grown. The option for a vegan diet can come from several reasons, such as nutrition and health, allergies and/or intolerance to dairy products, and concern for animal welfare [3].

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Furthermore, the increased consumption of vegan foods can also be associated with physiological factors such as allergies to milk proteins and/or lactose intolerance [3]. Thus, the most effective treatment for individuals with milk protein allergy and/or lactose intolerance is excluding milk and derived dairy products from their diet. Therefore, the food sector needs to invest in research focusing on developing new products that meet the demand of the vegan public and/or those who have some physiological restrictions on milk consumption [2].

According to Ballco and Gracia [4], the food industry has been improving in research and technologies that include developing new products, ingredients, and production methods to serve this type of consumers with special needs. In this way, analysis of vegetable-based raw materials in traditional products already consolidated in the market has been made on fermented drinks based on coconut milk [5], creamy cocoa-flavored desserts [6], and vegan dulce de leche [7], among others. Thus, water-soluble vegetable extracts are an attractive non-dairy alternative for developing vegan products [5].

The fermented drink is a food that is part of the Brazilian diet for its practicality and healthy appeal. However, it is excluded from the menu of vegan people and/or people with milk-induced allergies. After the 2019 pandemic, many people became interested in the benefits of fermented products, such as kombucha and different types of kefir, milk, and water. Kombucha is a fermented, lightly effervescent, sweetened black tea drink, while kefir is a fermented milk drink similar to a thin yogurt. Mainly, water kefir has aroused great interest from people interested in consuming plant-based and vegan diets or people allergic to milk proteins or lactose intolerant [3]. The food industry has invested in research and technology to enable the use of other raw materials to create diversified products that meet the demand of vegan consumers allergic to milk and soy, such as alternative water-soluble plant extracts [8].

Kefir is a type of fermented drink that can be produced based on plant extracts, which contain various microorganisms considered probiotics, which, through metabolic and symbiosis reactions, contribute positively to the sensory and nutritional characteristics of the product [3]. After fermentation of such plant extracts, this beverage presents a certain degree of turbidity, with a fruity, acidic, sour aroma, low alcohol content, high organic acids, and live probiotic microorganisms [3]. Kefir combines bacteria and yeasts in a matrix of proteins, lipids, and sugars. Due to its technological characteristics, kefir can be used in the fermentation of water-based beverages such as water-soluble vegetable extracts, fruit-based drinks, nuts, and almonds, among others [9].

One possible use of kefir is in the fermentation of lupine water-soluble plant extract. The nutritional characteristics of lupine seeds may contribute to reducing the risk of obesity, diabetes, and cardiovascular disease in humans. In contrast, some varieties may contain a significant amount of quinolizidine alkaloids, which cause bitterness and toxicity when presented in high concentrations [10]. Lupine are classified within the order Fabales, Leguminosae family Fabaceae, tribe Genisteae, and genus Lupinus and are traditionally used in human food due to their composition [11].

B vitamins and antioxidant components make this legume an excellent food and can be used as a raw material in developing food products [11]. Utilizing lupine water-soluble vegetable extract as a raw material in creating food products can be an exciting and innovative alternative, especially considering the growing interest in plant-based and sustainable food options. Some potential benefits are: Protein Content-Lupine extract is known for its high protein content. This can be advantageous for creating protein-rich food products, appealing to consumers looking for plant-based protein sources. Nutrient Profile-Lupine is rich in essential nutrients such as fiber, vitamins, and minerals, providing additional nutritional value to the products. Allergen-Friendly-Lupine is often considered a hypoallergenic alternative to soy and other common allergens, making it suitable for individuals with various dietary restrictions. Sustainability-Lupine is known for its ability to fix nitrogen in the soil, which can contribute to soil health. As a legume, it may require fewer synthetic fertilizers, making it a potentially sustainable crop. Versatility-Lupine extract can be used in various food applications, including baked goods, beverages, plant-based meat alternatives, and more, offering versatility in product development.

Thus, using lupine water-soluble vegetable extract as a raw material in creating food products for the public above can be an exciting alternative. Therefore, this research aimed to evaluate the use of the lupine water-soluble vegetable extract in developing a vegan fermented drink from water kefir grains.

2. Material and methods

This work was divided into three stages (Figure 1). In the first stage, the treatment was performed to remove the alkaloids in the lupine grains. The second step aimed to verify the effect of sucrose, inulin, and xanthan gum concentration through experimental planning of the central composite design (CCD) 2^3 type, containing three central points, in elaborating the fermented drink with water kefir. The third step was characterizing the optimized fermented drink.

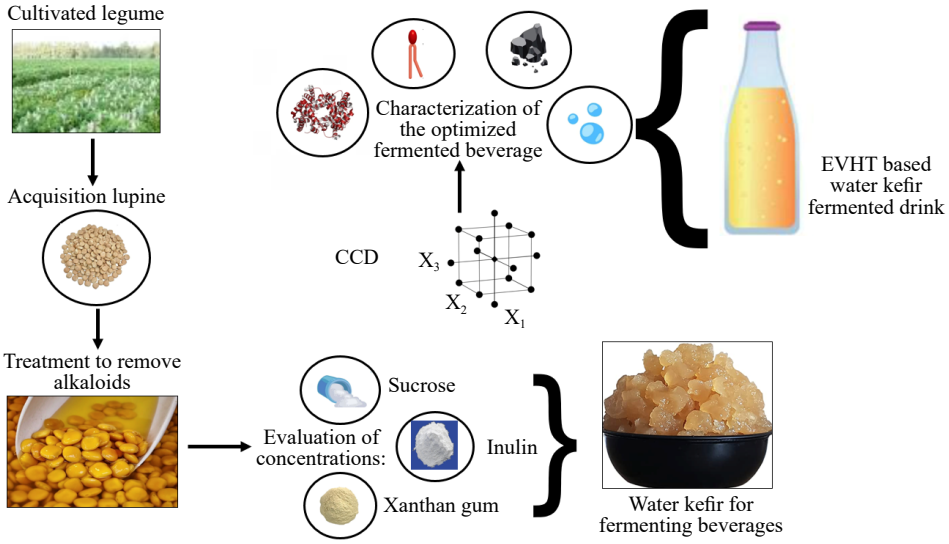

Figure 1. Stages of elaborating the fermented drink with water kefir

2.1 *Alkaloid removal treatment from lupine grains*

Removing alkaloids from lupine grains followed the methodology described by Hashimoto et al. [12], followed by certain modifications. Initially, 40 g of grains were soaked in water in a ratio of 1:5 (grains: water) at 4 ℃ overnight. Then, the water was discarded, and the grains were heated at 60 ℃, also in a 1:5 ratio, for 6 hours in a 0.5% tartaric acid solution, with this solution changed every 30 minutes. After this process, the grains were heated in the same proportion and temperature in a 0.5% sodium carbonate solution for 15 minutes, with two exchanges of this solution, and subsequently subjected to boiling for 30 minutes in the same solution, with two solution changes. Then, the grains were immersed in water at a ratio of 1:5 (grains: water) at 4 ℃ and allowed to stand overnight. Afterward, the water was discarded, and the grains were again immersed in water and boiled twice for 30 minutes. After treatments, the grains were dried in an oven at 30 ℃ for 24 hours and stored in glass bottles at room temperature until use.

2.2 *Extraction of alkaloids from treated lupine grains*

To verify the presence or absence of alkaloids in lupine grains and extracts, the methodology proposed by Simões et al. [13], with little modifications. Initially, 2 g of lupine kernels, crushed with 20 mL of 1% sulfuric acid (H_2SO_4), were boiled for 2 minutes on a hot plate. Then, this solution was filtered using a cotton funnel, cooled, and alkalized with ammonium hydroxide solution (NH_4OH) until it reached basic pH. Subsequently, 20 mL of this alkaline solution was added into a separatory funnel along with 20 mL of chloroform ($CHCl_3$), where they were carefully extracted through phase separation for 10 minutes. The chloroform layer was added to two porcelain capsules, and the solvent was evaporated in a water bath until dry. Next, 5 mL of 1% H_2SO_4 was added to the prepared capsules to dissolve the residue in the aliquot and distributed in test tubes for later analysis of alkaloids in the sample.

2.3 *Identification of the presence of alkaloids*

To prove the presence of alkaloids, the methodology described by Simões et al. [13], with adaptations. The samples distributed in tubes in the previous step were added with two drops of Dragendorff (D), Bouchardat (Wagner) (B), and Meyer (M) reagents. In parallel, a control sample (blank) was prepared, with only reagents without a sample. Tubes containing samples were compared with the blank. It was considered positive (has an alkaloid present) for forming insoluble complexes of reddish orange color for Bouchardat's reagent, brick red for Dragendorff, and white for Mayer. The test was performed on the lupine grain sample in nature (untreated) to compare.

2.4 *Experimental planning*

The elaboration of the water-soluble lupine vegetable extract (WLVE) followed the methodology described by Cruz-Chamorro et al. [14] with adaptations. About 100 g of lupine grains previously treated for alkaloid extraction were homogenized with the aid of 500 mL of water in a specific jar for the preparation of plant extracts (Vegan Milk Machine) with crushing, stirring (3,000 rpm) and heating (80 ℃) for 26 minutes. Subsequently, the WLVE was subjected to a filtering process twice, and then pasteurized at 95 ℃ ± 2 °C for 3 minutes, and packaged in a sanitized and sterilized glass bottle and stored in a refrigerator. After this step, the base extract proceeded to the central composite design step.

The variable levels (Table 1) of the central composite design (CCD) 2^3, containing three central points, followed the methodology described by Alves et al. [5] with modifications. The independent variables analyzed were the concentration of sucrose (X_1), inulin concentration (X_2), and xanthan gum concentration (X_3). The dependent variables (responses analyzed) by the proposed model were: (Y_1) cell growth of grains (Δx), (Y_2) beverage yield (R), (Y_3) soluble solids (SS), and (Y_4) lactic acid (LA). Coded and absolute levels and respective responses are shown in Table 1.

Initially, for seven days, the kefir grains were placed in an aqueous solution (filtered water) of sucrose (brown sugar) with concentrations of soluble solids of approximately 5 ºbrix and a temperature of 25 ± 1 °C in an air circulation oven, with an exchange of nutrients (sucrose solution) every 24 hours.

After seven days, 5% m/m of the kefir culture was inoculated into the WLVE, previously added with the specified concentrations of sucrose, inulin, and xanthan gum (Table 1) and incubated at 25 ± 1°C for 22 hours, then be analyzed.

Table 1. CCD defined sucrose, inulin, and xanthan gum concentrations for the fermented beverage formulations in the WLVE

Essay	Independent Variables (coded and actual values)			Response Variables			
	Sucrose (%w/v) X_1	Inulin (%w/v) X_2	Xanthan gum (%w/v) X_3	Grain cell growth (g/100g) Y_1	Yield (g/100g) Y_2	Soluble solids (ºbrix) Y_3	Determination Lactic Acid (g/100 mL) Y_4
F1	-1 (2.0)	-1 (2.5)	-1 (0.00)	4.66	97.35	4.6	1.35
F2	1 (10.0)	-1 (2.5)	-1 (0.00)	13.38	97.69	11.3	1.39
F3	-1 (2.0)	1 (3.5)	-1 (0.00)	25.60	97.62	5.6	1.48
F4	1 (10.0)	1 (3.5)	-1 (0.00)	6.06	97.99	12.4	1.51
F5	-1 (2.0)	-1 (2.5)	1 (0.16)	25.88	94.60	5.1	1.28
F6	1 (10.0)	-1 (2.5)	1 (0.16)	46.03	94.89	11.6	1.55
F7	-1 (2.0)	1 (3.5)	1 (0.16)	20.52	94.91	6.2	1.47
F8	1 (10.0)	1 (3.5)	1 (0.16)	59.11	93.98	11.8	1.42
F9	0 (6.0)	0 (3.0)	0 (0.08)	30.47	95.90	8.8	1.46
F10	0 (6.0)	0 (3.0)	0 (0.08)	25.40	96.43	8.3	1.28
F11	0 (6.0)	0 (3.0)	0 (0.08)	26.50	95.90	8.5	1.33

2.5 *Analysis of fermented WLVE in experimental planning*

2.5.1 *Cellular growth of kefir grains*

The cell growth of kefir grains (Δm) fermented in WLVE was through Equation 1, in which the kefir grain masses (g) were weighed at the beginning (m_{ko}) and after fermentation (m_{kf}).

$$\Delta m\left(\frac{g}{100g}\right)=\frac{\left(m_{kf}-m_{ko}\right)}{m_{ko}}\times 100 \quad (1)$$

2.5.2 *Fermented beverage yield*

Yield (R) was determined through Equation 2, where m_{so} corresponds to the initial mass and m_{sf} is the final mass of the fermented beverage.

$$R\left(\frac{g}{100g}\right)=\frac{m_{sf}}{m_{so}}\times 100 \quad (2)$$

2.5.3 *Determination of soluble solids*

The soluble solids (SS) contents were verified in a digital refractometer, with two to three drops of the WLVE filtrate, and the results were expressed in ºBrix [15].

2.5.4 *Determination of lactic acid*

To determine the production of lactic acid in the WLVE fermented beverage, the titratable acidity method by the titrimetric way of the Association of Official Analytical Chemists was used [16].

2.5.5 *Physicochemical analysis of optimized fermented WLVE*

The optimized formulation (unfermented and fermented) in the experimental design and the lupine grains (untreated and treated) were evaluated in triplicate for centesimal analysis.

The characterization followed the official methods of the Association of Official Analytical Chemists for estimating the parameter's moisture, ash, lipids, and protein. The difference method determines total carbohydrates through Equation 3 [16].

$$\text{g/100 g carbohydrates} = 100 - (\text{moisture} + \text{lipids} + \text{crude protein} + \text{ash}) \quad (3)$$

2.5.6 *Quantification of enzyme activity*

Amylase, cellulase, laccase, lipase, peroxidase, and protease activities were quantified from fermented WLVE. For amylase, the extract reacted from the dilution of starch (1%) in a sodium acetate solution (0.1 mol/L, pH 5.0), in the proportion of 1:25 (m/v), at 38 ± 1 °C for 10 minutes, and the resulting quantification was determined from the total reducing sugars (TRS) by spectrophotometry (UV-M51, Bel Photonics, Monza, Italy), at 540 nm by the dinitrosalicylic acid (DNS) method [17]. The enzymatic activity of amylase was calculated based on the glucose standard curve and the results expressed by U/mL.

For the activity of peroxidases, a reaction medium was prepared, adding phosphate solution (5.10-3 mol/L and pH 5.0), guaiacol (1%), hydrogen peroxide (0.08%), and distilled water at 35 ± 1 °C, for 10 minutes, according to the methodology of Khan and Robinson [18]. The oxidized compounds were measured at 470 nm, and the enzymatic activity was determined by the oxidative reaction of the substrate by tetraguaiacol and expressed in U/mL.

2.5.7 *Analysis of organic compounds*

The quantification of organic compounds (acetic acid, formic acid, arabinose, cellobiose, ethanol, fructose, and

glucose) produced during WLVE fermentation was obtained by High-Performance Liquid Chromatography (HPLC) (HPLC; LCMS-2020, Shimadzu). Assays were conducted in an HPLC instrument equipped with a RID10-A refractive index detector and an AMINEX® BIORAD HPX87H column. The previously filtered fermented beverage was diluted in distilled water (1:5 v/v), with subsequent injection of a volume of 20.0 µL of the sample to the chromatograph, coupled to a PDA 10-A detector, operated with a C18 column, eluted with acetonitrile 1:8 mL/minute of water and 1% acetic acid, thermostated at 30 ± 1 °C and mobile phase flow rate of 0.8 mL/minute. The organic compounds were determined from the calibration of each combination›s curves, according to Bazoti et al.'s methodology [19].

2.6 *Statistical analysis*

The Protimiza software (https://experimental-design.protimiza.com.br/) was used for data processing, with a 95% confidence level ($p < 0.05$).

3. Results and discussion

3.1 *Alkaloid extraction*

Treatment of lupin grains with tartaric acid was efficient in removing alkaloids (Figure 2), as it showed negative results (no alkaloids were present) with the three reagents tested (Dragendorff, Bouchardat, and Meye) (Figure 2b). The test with the control seed (without treatment) showed a positive result (alkaloid is present), evidenced by residue in the three tubes (Figure 2a).

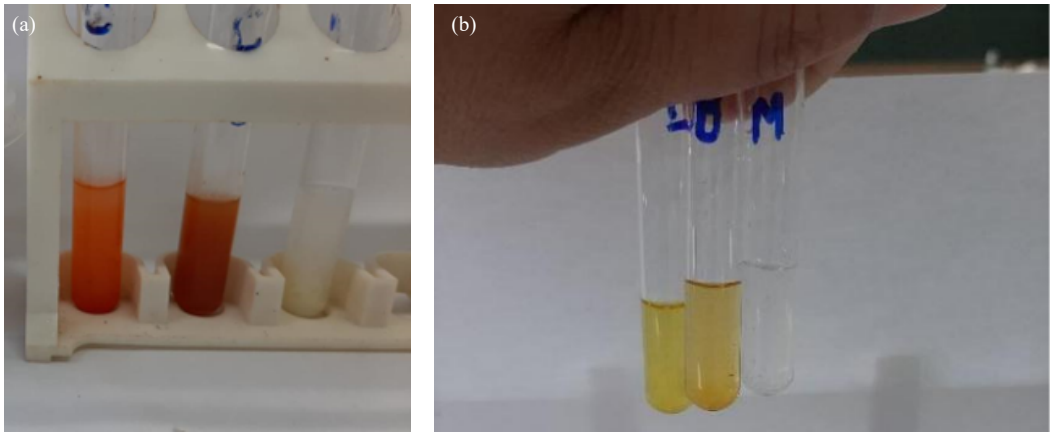

Figure 2. Results of tests for alkaloids in (a) control sample* (b) sample treated with tartaric acid
*It was considered positive (has an alkaloid present) that presented the formation of insoluble complexes of reddish orange color for Bouchardat's reagent, brick red for Dragendorff, and white for Mayer

The anti-nutritional substances found in lupine seeds are several alkaloids of the quinolizidine group, which promote bitterness and unpleasant taste [20-22]. As a result, several methods exist to reduce such factors and make their use feasible. Alkaloids are mostly water soluble, so a certain amount in the lupine grain can be eliminated and/or reduced. In addition to water, combining chemical and thermal treatment can be an alternative [23-25]. Despite the reports in the literature, in the present work, only the treatments with tartaric acid solution and heating for more than 4 hours were efficient. It is worth mentioning that the tests carried out were only qualitative, that is, whether or not they had the presence of alkaloids, and did not quantify them.

Despite the reports in the literature, in the present work, only the treatments with tartaric acid solution and heating for more than 4 hours were efficient. The tests carried out were only qualitative indicators for the presence of alkaloids and did not quantify them. Despite efficiently removing the alkaloids in the grains in the present work, using tartaric

acid resulted in an acidic taste. This can be seen from the initial time WLVE was prepared after this treatment, which had a pH of 4.06. Thus, the treatment was made with calcium carbonate and water to remove this acidic taste. After this additional treatment, the WLVE had a pH of 6.82. Calcium carbonate is an oenological alternative to reduce wine acidity through chemical deacidification, which neutralizes excess acidity and precipitates the salt formed (calcium tartrate) [26].

3.2 *Effect of fermentation parameters on response variables*

The significant variables for the model were the growth of kefir grains, yield, and total soluble solids. The results obtained in the formulations performed through the Central Composite Design (CCD) were subjected to regression analysis to evaluate the significant interactions ($p \leq 0.05$) in a confidence interval of 95% ($\alpha = 5\%$).

The regression coefficients for analyzing the effects of the kefir grains (i) enabled the writing of the mathematical model (Equation 3). Once the mathematical model was obtained, for statistical validity, it was necessary to perform Analysis of Variance (ANOVA), in which a p-value lower than 0.05 was obtained. Therefore, the mathematical model (Equation 4) was validated with a coefficient of determination (R^2) of 86.12%.

$$Y_1 = 25.78 + 5.99X_1 + 12.73X_3 - 1.23X_1X_2 + 8.70X_1X_3 - 0.74X_2X_3 \quad (4)$$

It is possible to notice that to obtain maximized kefir grain growth, it is necessary to work with maximum concentrations of sucrose (X_1) and xanthan gum (X_3) and minimum concentrations of inulin (X_2).

The yield of kefir grain (Y_2) had the effects analysis performed, and the mathematical coefficients made it possible to write the mathematical model (Equation 5). It was necessary to perform Analysis of Variance (ANOVA), which obtained a p-value less than 0.05. Therefore, the mathematical model was validated with a coefficient of determination (R^2) of 98.04%.

$$Y_2 = 96.11 + 0.01X_1 + 0X_2 - 1.53X_3 - 0.15X_1X_2 - 0.17X_1X_3 - 0.15X_2X_3 \quad (5)$$

The content of soluble solids (Y_3) had the same analysis done, and the regression coefficients for this parameter are presented in Table 2. These coefficients enabled the writing of the mathematical model (Equation 6). It was necessary to perform Analysis of Variance (ANOVA), which obtained a p-value less than 0.05. Therefore, the mathematical model was validated with a coefficient of determination (R^2) of 99.70%.

$$Y_3 = 8.56 + 3.20X_1 + 0.43X_2 + 0.10X_3 - 0.10X_1X_2 - 0.18X_1X_3 - 0.10X_2X_3 \quad (6)$$

Table 2. Regression coefficients for total soluble solids content (Y_3)

Name	Coefficient	Standard Error	t-calculated	p-value
Mean	8.56	0.08	112.39	0.0000
Sucrose (X_1)	3.2	0.09	35.82	0.0000
Inulin (X_2)	0.43	0.09	4.76	0.0089
Xanthan gum (X_3)	0.1	0.09	1.12	0.3257
(X_1 X_2)	-0.1	0.09	-1.12	0.3257
(X_1 X_3)	-0.18	0.09	-1.96	0.1217
(X_2 X_3)	-0.1	0.09	-1.12	0.3257

Thus, to obtain a maximized total soluble solids (TSS) value, it is necessary to work with maximum concentrations of sucrose (X_1) and high concentrations of inulin (X_2) combined with low concentrations of xanthan gum (X_3).

Observing the results obtained and statistically validated, for all responses (growth of kefir grains, yield, and total soluble solids), two variables had the same tendency, the third being antagonistic. Phenomenologically, the higher the

concentration of sucrose (X_1) and inulin (X_2), the higher the value of total soluble solids. This observation is consistent and expected since more solids are added to the vegetable drink formulation.

As for the growth of maximized kefir grains, sucrose and xanthan gum are used in higher concentrations, and this result is consistent with that described by Alves et al. [5]. For this response and yield of kefir grains, xanthan gum showed the opposite behavior. The analysis of the CCD data verified that while there was an increase in the growth of kefir grains, there was a reduction in the yield of the fermented beverage. A possible explanation for this is that due to the higher cellular concentration of kefir grains, there is a greater substrate consumption, thus reducing the yield of kefir-fermented beverages. A possible explanation for this is that, due to the higher cellular concentration of kefir grains, there is a greater substrate consumption, thus reducing the yield of kefir-fermented beverages [27].

Inulin (X_2), when available for metabolization in fermentation, provides a synergistic effect with kefir due to its prebiotic properties that act positively. Inulin use in fermentation is primarily due to the low availability of simple sugars in plant extracts, as with lupine [28].

Thus, to obtain maximized performance, it is necessary to work with maximum concentrations of sucrose (X_1) combined with low concentrations of inulin (X_2) or work with the inverse situation: low concentrations of sucrose (X_1) combined with high concentrations of inulin (X_2). For maximum yield, xanthan gum (X_3) must be in minimal concentrations.

The lactic acid content (Y_4) analysis indicated this parameter as insignificant, considering the levels evaluated in this study.

3.3 *Bromatological characterization of the optimized drink*

The interpretation of the response surface graphs, obtained through the application of the experimental design, reveals that the optimized drink is the formulation corresponding to the central point of the plan (Formulation 9-11).

Continuing the work, the centesimal characterization of the pure lupine extract (ETP), the non-fermented lupine extract (ETNF), the fermented lupine extract (ETF), the lupine seed in nature (T), and the lupine seed treated lupine (TT). These results are shown in Table 3.

As shown in Table 3, the fresh lupine seed contains 30.15% (m/m) of protein in its composition, a result close to that determined by Ruiz-Lopez et al. [29], who chose the protein content for white lupine seeds (*Lupins albums*) to be 36.30% (m/m). We have also obtained a value close (11.5%) but higher than that described in Table 3 for lipids (9.13%). The protein and lipid results determined in this work and by Ruiz-Lopez et al. [29] are consistent with those determined by Monteiro et al. [30].

There are studies on lupine proteins that predominantly identified globulins, responsible for 90% of the total content, and the remainder is represented by albumins [31-33]. The results on composition aligned with what was expected. The lupine used to prepare the optimized fermented drink is high in protein and carbohydrates [34].

Table 3. Bromatological characterization of the optimized drink

	Moisture % (m/m)	Ashes % (m/m)	Proteins % (m/m)	Lipids % (m/m)	Carbohydrates % (m/m)	Total titratable acidity (mEq.kg^{-1})
ETP	98.1 ± 0.06	< 0.02	0.86 ± 0.02	0.36 ± 0.01	0.68 ± 0.00	1.7 ± 0.3
ETNF	90.1 ± 0.02	< 0.02	0.78 ± 0.01	0.39 ± 0.06	8.73 ± 0.00	2.0 ± 0.0
ETF	90.3 ± 0.03	< 0.02	0.70 ± 0.03	0.36 ± 0.04	8.64 ± 0.00	31.5 ± 0.3
T	10.9 ± 0.21	2.65 ± 0.04	30.15 ± 0.69	9.13 ± 0.14	47.17 ± 0.26	202.5 ± 3.7
TT	78.2 ± 0.60	0.71 ± 0.02	9.28 ± 0.20	2.44 ± 0.10	9.37 ± 0.20	16.0 ± 0.3

Note: ETP: pure lupine extract; ETNF: unfermented lupine extract; ETF: Fermented lupine extract; T: Lupine seed in nature; TT: Treated lupine seed

3.4 *Quantification of enzyme activity*

The activities of six enzymes in the optimized vegetable drink were quantified: amylase, cellulose, peroxidase,

lipase, protease, and laccase (in decreasing order of activity). The enzymatic activity was determined in U/mL; the results are presented in Table 4. Activity at a higher intensity of peroxidase and amylase was reported by Alves et al. [5] in a fermented drink with coconut water kefir added with inulin. The activity of amylase can be explained by the supply of substrate for fermentation, mainly sucrose and inulin, preferentially metabolizing sugars by bacteria through enzymatic hydrolysis [35].

Table 4. Determination of enzymatic activity

Enzymes	Enzyme Activity (U/mL)
Amylases	264.63
Celluloses	56.93
Laccases	0.01
Lipases	5.06
Peroxidases	11.83
Proteases	0.56

The determined value of amylase activity, 264.63 U/mL, is much higher than that reported by Alves et al. [5], around 9 U/mL, and compared to that reported by Konki and Kim [35]. The latter worked with fermented yogurts and determined enzymatic activity for amylase of 5.41 U/mL.

Amylase acts on the texture and promotes an increase in food volume [36]; it can be applied in the production of sweeteners and can also act as a moisture regulator, maximizing the shelf life of foods [37]. On the other hand, peroxidase has redox characteristics to form free radicals and has been applied to encapsulate bioactive compounds [38].

In determining the activity of the amylase enzyme, 9.53 g of total reducing sugars per L of vegan drink was observed, with the cellulase enzyme (20.51 g/L) more than double this value.

The high-performance liquid chromatography (HPLC) technique was used to identify components present in Fermented Beverages [39]. The results are shown in Table 5.

Table 5. Characterization of the fermented beverage

Compound	Concentration (g/L)
Glucose	16.62
Cellobiose	1.49
Acetic acid	0.44
Fructose	28.84
Ethanol	1.98
Glycerol	0.02
Citric acid	0.01

The presence of arabinose, xylitol, and formic acid was not identified. Due to the great diversity of compounds in kefir grains, other authors also seek to identify and quantify them using the HPLC technique [39]. Paredes and his collaborators [40] quantified the content of organic acids such as lactic, acetic, citric, malic, and succinic by the same method in probiotic beverages fermented by kefir grains with mixed substrates of fruits and vegetables. Due to the great diversity of possible compounds in kefir cultures, it is unlikely to find similar results. Generally, it can be said that kefir grains significantly affect the organic acid content.

4. Conclusions

The elaboration and characterization of a fermented vegan drink from water kefir grains using lupine was conducted. The optimized fermented drink was the formulation given by the central point. The lupine seed characterization revealed a high protein content, and the optimized beverage characterization showed a considerable carbohydrate content. The optimized drink has enzymatic activity with an emphasis on lipases. The drink's development as a new product using water-soluble lupine plant extract brought an exciting application for this legume and an additional food option for vegan consumers or consumers with dietary restrictions related to dairy products.

Authors' contributions

Helen Treichel and Larissa Canhadas Bertan conceived and designed the study.

Claudia Moreira Santa Catharina Weis analyzed the data and drafted the manuscript.

Luan Gabriel Techi Diniz, Gessica Suiany Andrade, Luciane Mendes Monteiro, Jane Manfron, Aline Frumi Camargo, Simone Kubeneck, Gabriel Henrique Klein, Larissa Capeletti Romani, Vitoria Dassoler Longo, Julia Pieper Nerling, Luciano Tormen, Catia Tavares Dos Passos Francisco carried out the experimental procedures and helped with writing and carefully revised the manuscript.

Helen Treichel and Larissa Canhadas Bertan critically reviewed and supervised the development of the paper.

All authors reviewed and approved the final manuscript.

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Consent for publication

All authors agreed with this publication.

Availability of data and materials

The datasets generated for this study are available to the corresponding author on request.

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Conflict of interest

The authors declare no competing financial interest.

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