applied sciences

MDPI

Communication

Bacterial Profile and Changes in the Protein–Peptide Fraction in Spontaneously Fermented *Lens culinaris* Medik.

Katarzyna Skrzypczak [1,*], Katarzyna Michalak [2], Jakub Wyrostek [3], Ewa Jabłońska-Ryś [1], Aneta Sławińska [1], Wojciech Radzki [1] and Waldemar Gustaw [1]

[1] Department of Fruits Vegetables and Mushrooms Technology, Faculty of Food Science and Biotechnology, University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland
[2] Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland
[3] Department of Analysis and Food Quality Assessment, Faculty of Food Science and Biotechnology, University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland
* Correspondence: katarzyna.skrzypczak@up.lublin.pl; Tel.: +48-81-462-33-08

Featured Application: This study provides insight into changes in the profile of the protein–peptide fraction, including the potential reduction of allergenic factors (such as non-specific lipid-transfer proteins). The investigation indicates possibilities for further elimination of anti-nutritional and allergenic components in legume-derived food products. The investigation also shows the quantitative and qualitative composition of bacteria present in the red lentil matrix after 48 h of spontaneous fermentation, which may constitute a reservoir of microorganisms with potentially desirable technological properties.

Abstract: Pulses have desirable nutritional properties and a wide range of applications in the food industry as meat-free, casein-free, gluten-free, and functional food products. Unfortunately, the legume raw material contains some anti-nutrients and allergenic agents; nonetheless, fermentation processes may reduce some of these undesirable compounds. Therefore, the objective of the preliminary investigation was to determine the profile of bacteria occurring after spontaneous fermentation of *Lens culinaris* Medik. and detect changes in the protein–peptide pattern, including potential modifications of Len c3, i.e., a non-specific lipid-transfer protein (nsLTP) recognized as an important allergen. This study involved MALDI TOF/TOF, Illumina next-generation sequencing, and FT-IR spectroscopy analyses. Sixteen different species were identified in the plant-based material after 48-h spontaneous fermentation. The most abundant species were *Lactococcus taiwanensis* and *Pediococcus pentosaceus* (54.95% and 25.34%, respectively). The performed initial analysis revealed that after spontaneous fermentation had occurred the degradation of proteins (~10 kDa) and peptides (6–8 kDa), as well as the decomposition of proteins in the mass range that might be attributed to allergenic nsLTP. The preliminary findings encourage further research into the functional and technological properties of the isolated bacteria and in-depth analyses of the possibility of the removal of allergenic compounds from red lentils through fermentation carried out by the isolates.

Keywords: red lentils; *Lens culinaris* Medik.; spontaneous fermentation; FT-IR

check for updates

Citation: Skrzypczak, K.; Michalak, K.; Wyrostek, J.; Jabłońska-Ryś, E.; Sławińska, A.; Radzki, W.; Gustaw, W. Bacterial Profile and Changes in the Protein–Peptide Fraction in Spontaneously Fermented *Lens culinaris* Medik. *Appl. Sci.* **2022**, *12*, 8916. https://doi.org/10.3390/app12178916

Academic Editor: Piotr Minkiewicz

Received: 11 August 2022
Accepted: 3 September 2022
Published: 5 September 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

Fermentation is recognized as one of the oldest methods of food preservation and has been used for centuries in many regions of the globe. It is often part of the traditional way of food preparation (especially in many types of foodstuffs with ethnoreligious significance). Fermentation is also perceived as a biopreservation method contributing to the enhancement of the health-promoting properties and increasing the bioavailability of many nutrient components present in raw material [1,2].

Originally, fermentation was applied in food production as a spontaneous process carried out by naturally resident microorganisms in raw materials. However, on an industrial scale, fermentation is relatively difficult to control and the achievement of repeatable results in each batch is very challenging. Therefore, selected starter cultures in a defined formulation are used nowadays in food technology [3,4]. However, it has already been indicated that autochthonous microorganisms, rather than allochthonous starters, are able to perform more efficient fermentation processes and yield more desirable, specific features of the final product [2,5]. Therefore, technologically attractive potential starter strains are usually selected from the food matrix, in which they are to be used as an inoculum for fermentation [2,6,7]. Furthermore, recent research indicates that wild-type fermented fruits and vegetables can be an ample source for the isolation of lactic acid bacteria (LAB), not only exhibiting a favorable technological potential but also producing desired health effects in the human body [8,9]. It is also suggested that the fermentation process may contribute to the reduction of anti-nutrients and allergenic factors, including some proteins present in raw plant material [1]. This is of paramount importance, especially in the context of the changes in nutritional trends observed nowadays and the current needs for modification in food production. The trends include the increasing demand for developing new substitutes for milk and meat products as well as products without (or with reduced levels of) factors that cause food allergies, intolerances, or digestive discomfort.

Pulses exhibit favorable nutritional properties due to their content of protein, carbohydrates, and fiber as well as the high concentration of such bioactive components as phytosterols or polyphenols [10]. It has also been reported that legumes can be a proper raw material suitable for the fermentation process enhancing the beneficial properties of the plant-derived food matrix [11,12]. Furthermore, it is suggested that the formulation of novel food products based on this type of plant (with particular regard to fermented ones) may significantly expand the range of alternative food products (or substitutes for various foodstuffs) suitable for individuals with special food requirements or suffering from certain digestive disorders. This is particularly important because the prevalence of such disorders as lactose intolerance or allergies to cow's whey and milk protein is constantly increasing in contemporary society [13–15]. Consequently, the process of the fermentation of pulses has recently been of great interest to the food industry and is an object of various interdisciplinary scientific studies. Nevertheless, the spontaneous fermentation of red lentils has not been fully explored and characterized so far. Therefore, the objective of the preliminary study was to determine the profile of bacteria occurring in the spontaneously fermented material of *Lens culinaris* Medik. Another important cognitive objective of this investigation was to detect modifications in the protein—peptide pattern after spontaneous fermentation of red lentils resulting in some potential changes in the content of Len c3, which belongs to the group of non-specific lipid-transfer proteins (nsLTP) recognized as important allergens.

2. Materials and Methods

2.1. Preparation of Raw Material for the Fermentation Process

Organic (EU organic production logo—PL-EKO-01) red lentil seeds (*Lens culinaris* Medik.) were purchased from a local market and used as raw material in the research. The characteristics of the raw material are presented in Table 1.

The dry red lentil seeds were milled to obtain homogeneous powder (flour) with a Knife Mill GM 200 laboratory grinder (Retsch GmbH, Haan, Germany). The spontaneous fermentation of the material was performed as in [9], with some modifications. Briefly, three grams of milled lentil seeds were transferred (in sterile conditions) into falcon tubes that contained 30 mL of sterile distilled water. Afterward, the samples were tightly sealed, thoroughly mixed (Multi-Speed Vortex MSV-3500, BioSan, Riga, Latvia), and incubated anaerobically at 37 °C for 48 h (all procedures were performed in sterile conditions). Control samples were prepared in the same way as described above but were not subjected to incubation and spontaneous fermentation.

Table 1. Specification of the plant raw material [1].

Nutritional Values in 100 g of the Product	
Energy	1332 kJ/316 kcal
Fat in total	1.7 g
Saturated fatty acids	0.4 g
Carbohydrates	37.8 g
Sugars	0.2 g
Fiber	21.1 g
Protein	26.9 g
Salt	0.0 g

[1] According to the distributor's declaration (Jeronimo Martins Poland S.A.). Reference intake value for an average adult (8400 kJ/2000 kcal).

Depending on the type of further analysis (described below), the samples of the fermented matrix were collected after 12-h fermentation. After 48 h (final products), they were frozen at −80 °C and lyophilized (Labconco, Kansas City, MO, USA).

The lyophilized samples of the tested material were ground using the laboratory mill (Retsch ZM 200, Retsch, Haan, Germany) and sieved to obtain homogenous powders with particle sizes below 500 µm.

2.2. FT-IR Spectroscopy Analysis

The spectra of each lyophilized sample were measured in the wavenumber region from 4000 to 400 cm^{-1} using an Alpha II FTIR spectrometer (Bruker Corporation, Bremen, Germany) coupled to a Platinum Diamond ATR with a single reflection diamond crystal. An air spectrum was used as the background of the sample. Background and sample spectra were taken at room temperature with a spectral resolution of 4 cm^{-1}, and 24 scans were taken for each measurement. The OPUS 8.7 10 (20200710 x 64) ALPHA SYSTEM (Bruker Optic GmbH 2020, Bruker, Bremen, Germany) software was used to analyze the spectra.

2.3. Analysis of Peptide Fractions in Spontaneously Fermented Lentil Material

Changes in peptide fractions in the spontaneously fermented lentil matrix were identified using the MALDI TOF technique. Briefly, 0.3 g of each lyophilized sample was subjected to cryogenic grinding with liquid nitrogen. Afterward, 0.25 g of the grounded sample was placed in an Eppendorf tube, covered by 20 mM TRIS HCl solution, and homogenized for 10 min. Glass beads were added to the homogenized sample, and sonification was carried out for 20 min at 10 °C. The samples obtained were centrifuged (5000× g, 20 min, 4 °C) and supernatants were collected.

The supernatants were subjected to purification and concentration by ZipTip with 0.6 µL C18 resin using 100% acetonitrile (ACN) as the wetting solution. The samples were supplemented with 0.1% trifluoroacetic acid (TFA). In the analysis, 0.1 TFA in Mili-Q was used as an equilibration solution and a wash solution, whereas 0.1 TFA/50% ACN was used as an elution solution. Concentrated peptide and protein mixtures were spotted on an Anchor Chip MALDI plate (Bruker, Bremen, Germany) and covered with 1 µL of the α-cyano-4-hydroxycinnamic acid matrix (HCCA, Bruker, Bremen, Germany). Simultaneously, a standard solution (Peptide Calibration Standard II, Bruker, Bremen, Germany) was applied to the calibration spots. Mass spectra were recorded using an Ultraflextreme MALDI TOF/TOF (Bruker, Bremen, Germany) spectrometer in two linear modes to detect as many peaks as possible: LP1 (linear detector gain: 26 × 2998 V, frequency: 1000 Hz, 2 Gs/s, mass range: 4000 to 20,000 m/z) and LP2 (linear detector gain: 42 × 3058 VV, frequency: 500 Hz, 0.50 Gs/s, mass range: 4.500 to 8500 m/z), and the RP-reflector mode to acquire spectra for lower masses (reflector detector gain: 8.8 × 2531 V, frequency: 1000 Hz, 4 GS/s, mass range: 700 to 4000 m/z).

The spectra were smoothed and baseline corrected in flexAnalysis 3.0 software (Bruker, Bremen, Germany). The peptide and protein masses shown on the spectra were compared with Standard Protein BLAST for lentil plants and assigned to the respective proteins.

2.4. Determination of the Profile of Microorganisms

The samples of the spontaneously fermented red lentil matrix collected directly after the 48-h incubation (without the freezing and lyophilization processes) were intended for genetic analysis. The analysis was performed by NEXBIO Sp. z o.o. (Lublin, Poland) applying the Illumina Sequencer Platform using the Library Kit (Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2) and a developed library protocol (16S Metagenomic Sequencing Library Preparation Part # 15044223 Rev. B). The Illumina NGS (next-generation sequencing) workflow included the following stages: sample preparation (after DNA extraction and preparation of quality control, qualified samples were selected for library construction), library construction (the sequencing library was prepared by random fragmentation of the DNA sample followed by 5′ and 3′ adapter ligation; the adapter-ligated fragments were then PCR amplified and gel purified), and the sequencing procedure.

Four terminator-bound dNTPs were present during each sequencing cycle (natural competition minimizes incorporation bias and reduces raw error rates compared to other technologies).

3. Results

3.1. FT-IR Spectroscopy Analysis

The FT-IR spectra (4000–400 cm^{-1}) of the tested lentil samples from different stages of fermentation are displayed in Figure 1.

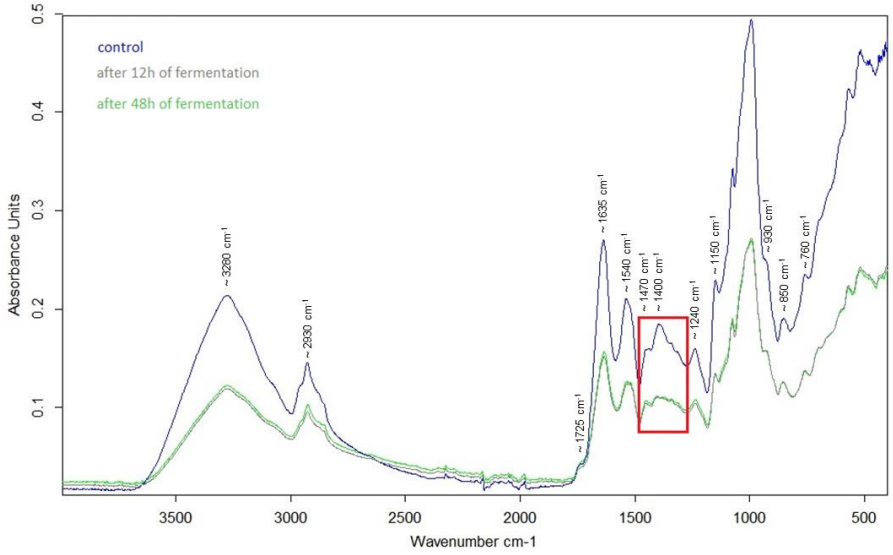


Figure 1. Comparison of the raw FT-IR spectra of the tested samples (the area of spectra in which the differences between fermented and control samples are the most noticeable is marked with a red rectangle).

The analysis of the spectra of the legume material revealed that the curves were similar to each other (Figure 1). Particular similarity was exhibited by the samples of the plant material (red lentils) after the 12- and 48-h spontaneous fermentation. Nevertheless, the fragment of the spectrum at 1200–1500 cm^{-1} (marked by the red area in Figure 1) indicates strong differences between the plant material samples after the spontaneous fermentation and the non-fermented lentils constituting the control sample. In this area, a discernible peak signal at 1395.78 cm^{-1} was noticed in the control sample, whereas this signal was not detected in the other samples.

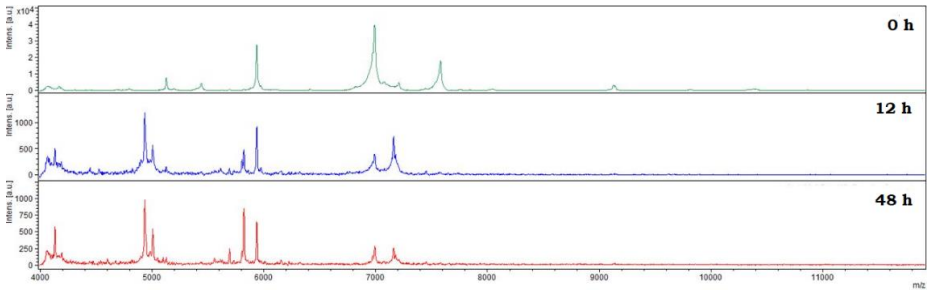
3.2. Analysis of Peptide Fractions in Spontaneously Fermented Lentil Material

The analysis of the MALDI TOF spectra was carried out using two linear mode methods (LP1 and LP2) to visualize as many masses as possible (Figure 2a–g). The spectra

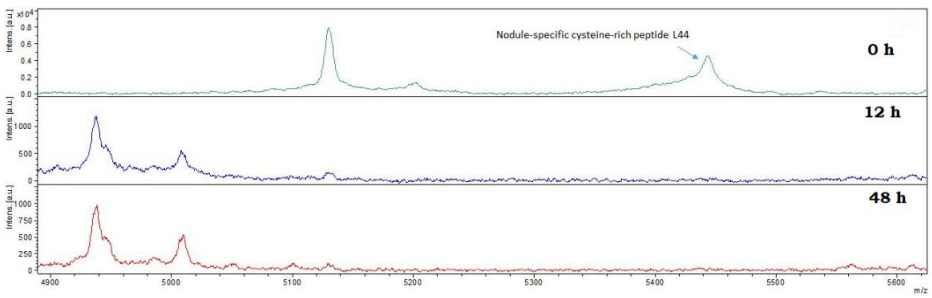
obtained using LP1 demonstrated evident changes in the fermented legume-based material increasing with incubation time (12 h and 48 h) in comparison with the control sample (0 h). The degradation of proteins with lower molecular weight (~10 kDa) and 6–8 kDa peptides was evident (Figure 2a). Moreover, the close-up of the LP spectrum (Figure 2b) revealed apparent degradation of the peptide at 5130 and 5443 m/z, which may be attributed to the nodule-specific cysteine-rich peptide L44. After the fermentation process (12 h and 48 h), the decomposition of proteins (Figure 2d) occurred in the mass range from approximately 11,840 to 11,900 m/z. Moreover, during the spontaneous fermentation of the lentils a decrease in the nascent polypeptide-associated complex subunit beta peak can be observed, and the creation of new signals (~5800 m/z) increasing over the process is clear to see (Figure 2c). A similar situation took place in the mass range of 4500–8500 m/z. Three main peaks on the 0 h spectrum during the fermentation decreased (~5900 m/z, ~7000 m/z) or decomposed (~7600 m/z). The appearance of new peaks in the range of 4800–5100 m/z obtained from initial peptides or proteins from the 0 h sample can be observed after 12 and 48 h (Figure 2e).

The spectra acquired with the LP2 method demonstrated that the peak of 5822 m/z was more intensive in the analyzed fermented plant material in the longer incubation period (Figure 2f). Furthermore, the degradation of the peptide from the region of about 6993 m/z (which can be attributed to the nodule-specific cysteine-rich peptide L53) and the occurrence of a new peptide (7162 m/z) in the fermented samples were detected in samples of spontaneously fermented red lentils (Figure 2g). As a result, compared to the 0 h samples, spectra obtained after fermentation indicated changes in the protein–peptide profile. The appearance of signals with a lower m/z value was most probably the result of fragmentation of higher mass peaks.

The RP spectra showed the slightest differences depending on the incubation time. In this case, the differences were associated with changes in the intensity of peaks, indicating the fragmentation of masses to smaller ones which were impossible to detect by the MALDI TOF technique (Figure 3).

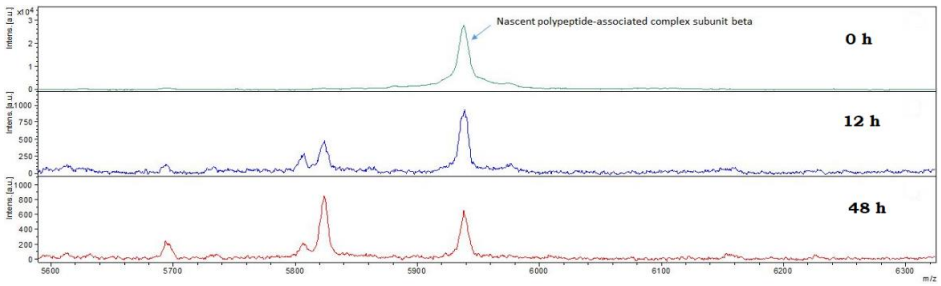


(**a**)

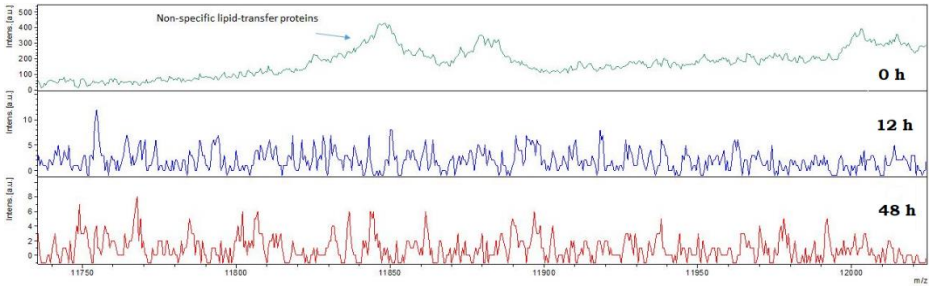


(**b**)

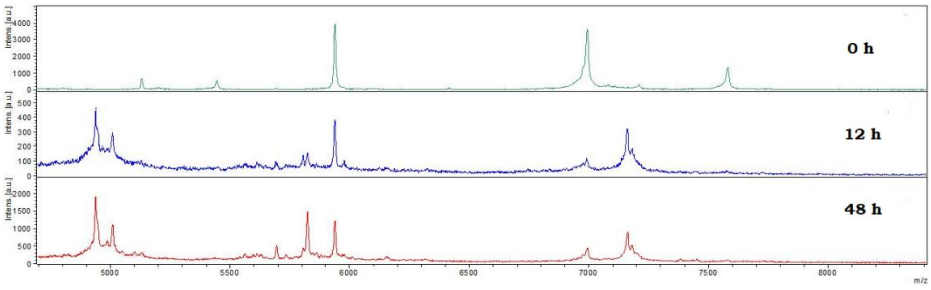
Figure 2. *Cont.*



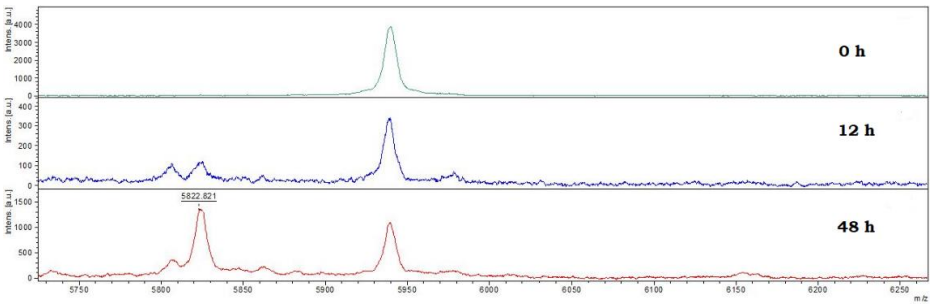
(**c**)



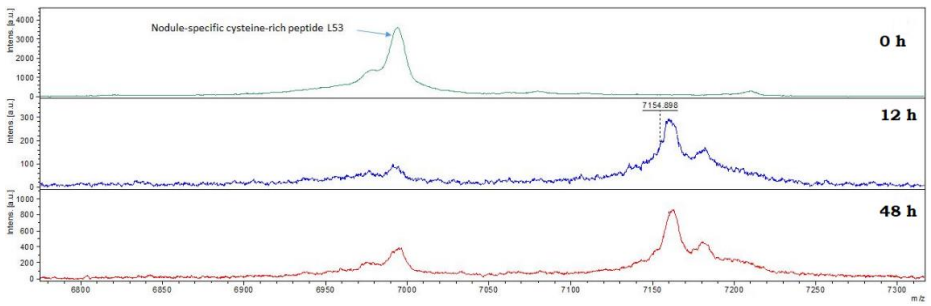
(**d**)



(**e**)



(**f**)



(**g**)

Figure 2. Comparison of the peptide fractions of *Lens culinaris* material through the analysis of selected MALDI TOF spectra with the LP1 (**a**–**d**) and LP2 (**e**–**g**) method.

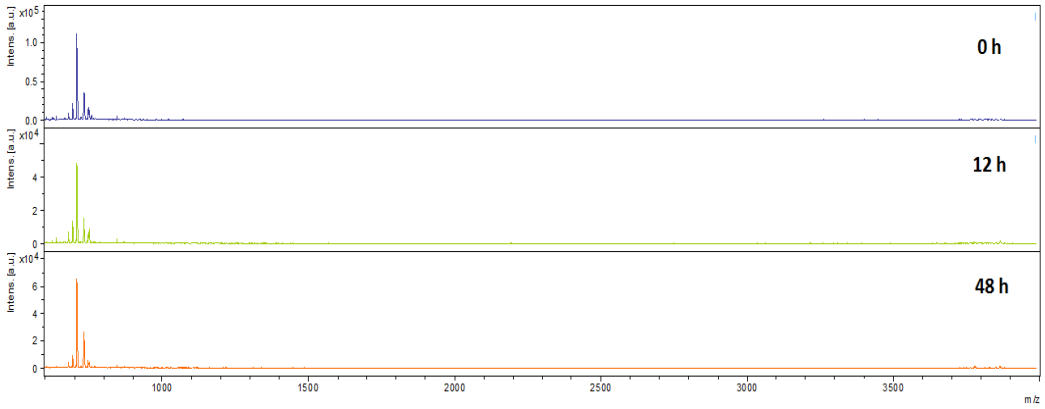


Figure 3. Comparison of the peptide fractions of *Lens culinaris* material through the analysis of selected MALDI TOF spectra with the RP method.

3.3. Determination of the Profile of Microorganisms

The method of determining the microbial profile yields results with highly accurate base-by-base sequencing (and eliminates sequence-context-specific errors even within repetitive sequence regions and homopolymers).

The analysis revealed the presence of sixteen different species in the fermented lentil matrix after 48 h of spontaneous fermentation (Figure 4). *Lactococcus taiwanensis* and *Pediococcus pentosaceus* were the most abundant species (54.95 and 25.34%, respectively).

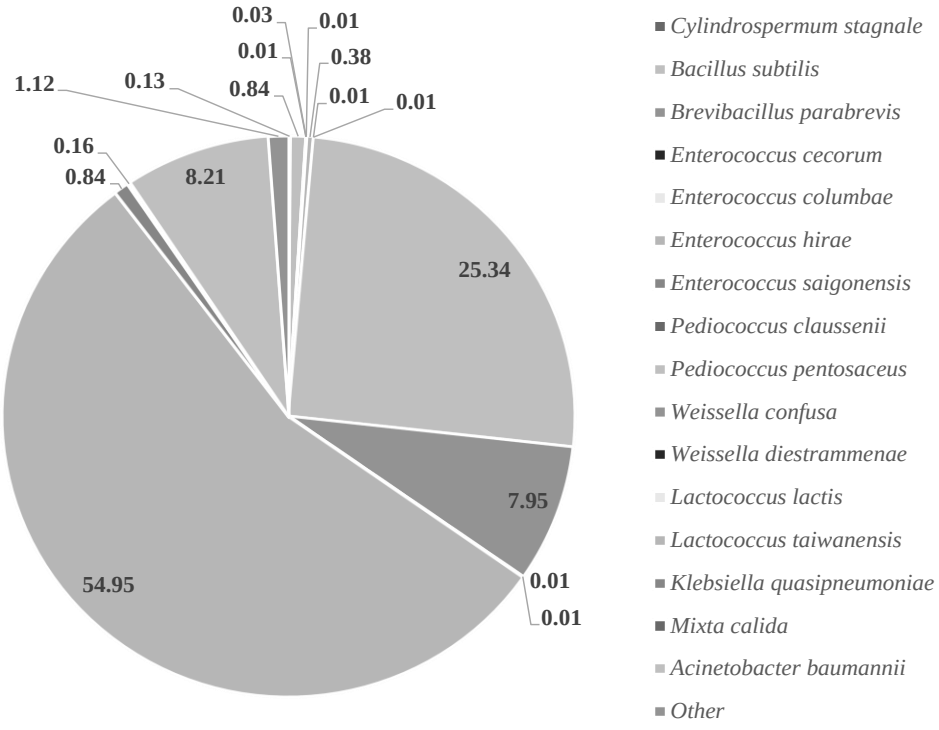


Figure 4. Bacterial profile (composition expressed in %) detected in the spontaneously fermented *Lens culinaris* Medik. material.

In the composition of the microflora in the tested fermented food matrix, the smallest contribution (about 0.01%) was represented by *Brevibacillus parabrevis*, *Enterococcus columbae*, *Enterococcus saigonensis*, *Pediococcus claussenii*, *Weissella diestrammenae*, and *Lactococcus lactis*. Potential pathogens (*Klebsiella quasipneumoniae* and *Acinetobacter baumannii)* were detected as well.

4. Discussion

FT-IR spectroscopic analysis allowed the detection of functional groups in different matrices, including plant-based foodstuffs. It also facilitated the determination of some phytochemical changes in the tested material. The broad bands at 3280 cm^{-1} and 2930 cm^{-1} can be attributed to the stretching vibrations of O-H, N-H, and C-H, respectively. In turn, the signal at ~1725 cm^{-1} is the result of C=O stretching and suggests the presence of fat [16,17].

The spectra exhibited two strong signals at ~1635 cm^{-1} (Amide I) and ~1540 cm^{-1} (Amide II). The former is the result of the C=O stretching mode, while the peak at ~1540 cm^{-1} indicates the presence of the bending vibrations of N-H groups. These two signals can be attributed to the presence of protein in the samples.

The bands between 1450 cm^{-1} and 1300 cm^{-1} can be assigned to the bending vibrations of CH_2, O–H, and C–O–H [17]. The signal at ~1400 is noticeably stronger in the sample that was not subjected to lactic acid fermentation. It may be attributed to the presence of aromatic groups. This is in accordance with [18] who indicated that aromatic groups were inferred based on the band around 1407 cm^{-1} (C-C stretch) and the peak near 798 cm^{-1}. Moreover, it was suggested that the changes in the region (absence of the peak in the area near 1400 cm^{-1} in the fermented legume material) may be related to biochemical modifications (particularly in phenolic and aromatic groups) occurring through the fermentation process and microbial activity.

Three signals at ~760 cm^{-1}, ~850 cm^{-1}, and 930 cm^{-1} indicate the presence of α-glycosidic links and may result from the presence of starch [19].

The FT-IR spectra of the tested lentil samples are similar to the results for water extracts of red lentils described by [20]. It has been suggested that some slight changes in spectra of fermented legume samples in the 750–1200 cm^{-1} zone (a fingerprint region characteristic for carbohydrate components) and the amide I zone (presented also in this analysis) may be related to starch and protein [18]. Further, as indicated by [18], these changes contribute to various conversions of anti-nutritional components, e.g., tannins, which are of particular importance in the case of food production based on plant raw materials.

Moreover, the visible band region 1657–1632 cm^{-1} (characteristic for proteins and peptides) attributed to amide I (C=O stretching) [21,22] was noted in all the tested legume samples. The changes may be an effect of protein and peptide hydrolysis occurring during the process of spontaneous fermentation with the participation of bacterial proteolytic enzymes. The differences within the protein–peptide fraction in the fermented samples (in comparison to the control variant) were also reflected in the images of the spectra obtained after the MALDI TOF/TOF analysis.

Certain proteins present in legumes are described as strong allergens that may lead to adverse reactions in the human body. They may also lead to weaker absorption of other pulse-derived proteins in the gut [23,24]. Hitherto, through the use of immunoblot analysis and mass spectrometry-based platforms, a subfamily of six lentil LTP isomers (designated as Lc-LTP1-6) has been identified and verified as immunologically potential allergens [2,25–27]. One of them (the earliest identified lentil allergen) is γ-vicilin (50 kDa), considered the major allergen of lentils and designated Len c1 [28]. It is worth emphasizing that vicilin (also known as 7S globulin) belongs to the major storage proteins of legumes [29]. Another important protein is Len c2 with a molecular weight of about 66 kDa [30], while Len c3, belonging to the prolamin super-family, is a lipid transfer protein (LTPs) with a relatively small molecular weight (9 kDa).

Non-specific lipid transfer proteins (nsLTPs) in plants are responsible for non-specific exchange (between membranes) of hydrophobic molecules and lipids [31]. Moreover, nsLTPs are involved in many biochemical processes such as plant development, germination, sexual reproduction, cuticle formation, defense against pathogens, responses to stress factors, and many others [32–37]. Nevertheless, non-specific lipid transfer proteins are perceived as important factors of food and pollen allergy in humans. They exhibit high resistance to temperature treatments and digestive enzymes [38,39].

The promising findings from the conducted preliminary study suggest that the process of spontaneous fermentation may contribute to the reduction in the level of some types of allergenic factors present in the red lentil food matrix (the mass range from approximately 11,840 to 11,900 m/z is a region that may be attributed to allergenic non-specific lipid-transfer proteins). The present results also indicate changes in the protein profile after 48-h spontaneous fermentation of red lentils due to presumable proteolysis induced by microbial enzymes. It has already been revealed that fermentation may contribute to the partial hydrolysis of proteins, improving their digestibility (and bioavailability); therefore, fermentation can be considered as a relatively inexpensive bioprocess reducing the level of allergenicity of legume-derived food products [40]. Moreover, Aguirre, et al. [41] indicated that soybean protein isolate subjected to fermentation conducted by different LAB bacteria caused a significant reduction of allergens, including β-conglycinin, glycinin, and glycin. The results described by Yang et al. [42] indicated that the process of fermentation of soybean food products with the application of *B. subtilis* and *Lb. casei* microbial combination and yeast contributed to the degradation of major protein allergens. The results of the present preliminary study suggest a possibility to reduce the level of some allergens in the tested red lentil material through spontaneous fermentation conducted by the detected bacteria, which should be the object of further investigations, especially given the fact that *Klebsiella quasipneumoniae* were detected in the fermented red lentil matrix as well. Nevertheless, it has been indicated that the bacteria are characterized by a low prevalence of virulence genes and exhibit higher rates of susceptibility to many commonly tested antimicrobials [43]. Therefore, this preliminary study needs a continuation with a more extensive scope of analysis focusing on indigenous bacteria in the analyzed raw material and the ability of individual bacteria to hydrolyze allergenic proteins naturally present in legumes. Moreover, their functional properties and technological usability (i.e., as starter cultures) should be assessed. The relevance of the research to the possibility of using indigenous bacteria from spontaneously fermented legume seeds in food production is supported by the research carried out by Sáez et al. [44], where four different kidney bean varieties were subjected to spontaneous fermentation. The results indicated that autochthonous lactic acid bacteria were represented in kidney beans by four genera and eight species: *Enterococcus durans, E. faecium, E. mundtii, E. casseliflavus, Lactobacillus rhamnosus, Lactococcus garvieae, Weissella cibaria*, and *W. paramesenteroides*. Moreover, based on the analysis of the properties (gallate decarboxylase, growth ability, acidification rate, proteolytic activities, and antimicrobial potential) exhibited by the identified bacteria, two strains (*Enterococcus durans* CRL 2178 and *Weissella paramesenteroides* CRL) were selected as a functional starter culture useful for fermentation of legume flours. In the spontaneously fermented red lentil material, four *Enterococcus* species (*Enterococcus cecorum, Enterococcus saigonensis, Enterococcus hirae, Enterococcus columbae*) and two *Weissella* species (*Weissella diestrammenae* and *Weissella confuse*) were detected. There are currently ongoing investigations to explore and characterize the properties of the microorganisms detected in spontaneously fermented *Lens culinaris* Medik. in terms of their potential use in the food industry.

5. Conclusions

This short communication (although very preliminary) may have potentially high scientific value, as it is the first insight into the composition of autochthonous bacteria in red lentils available on the Polish market. After fermentation, visible changes in the protein–peptide profile were observed in the analyzed plant matrix. Given the changes in the protein–peptide fraction, it may be assumed that the modifications of the structure of allergenic components occurring during the fermentation process depend on native microorganisms of the plant-based matrix. Nonetheless, there are currently no sufficient data to explicitly define the influence of individual bacterial strains on allergens, including a group of non-specific lipid-transfer proteins (such as Len c3). Furthermore, the results encourage further analysis to elucidate in detail the mechanisms of changes in the structure of allergenic proteins in red lentils induced by the process of spontaneous fermentation.

The findings prompt the continuation of the investigation for effective identification and selection of novel microorganisms isolated from the fermented food matrix with high technological potential relevant for food production. Moreover, the results of this work may serve as the background for evaluating the technological and functional properties of the identified bacteria and encourage further investigations of bacterial activity leading to a decrease in the content of allergenic components in *Lens culinaris* Medik. And other legumes.

Author Contributions: Conceptualization: K.S. and K.M.; methodology: K.M., J.W. and K.S.; software: W.R. and A.S.; validation: A.S. and E.J.-R.; formal analysis: K.S., K.M. and J.W. investigation: J.W., K.M., K.S. and A.S.; resources: A.S. and W.G.; data curation: W.R. and W.G.; writing—original draft preparation: K.S. and K.M.; writing—review, and editing: K.S.; visualization: K.M.; supervision: W.G.; project administration: K.S.; funding acquisition: K.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the subsidy of the Ministry of Science and Higher Education (in Poland) according to the Order No. 68 of the Rector of the University of Life Sciences in Lublin (of 21 December 2018) as financial support awarded for the development of young scientists (research grant ID: VKR/MN-6/TŻ/20).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; nor in the decision to publish the results.

References

1. Curiel, J.A.; Coda, R.; Centomani, I.; Summo, C.; Gobbetti, M.; Rizzello, C.G. Exploitation of the nutritional and functional characteristics of traditional Italian legumes: The potential of sourdough fermentation. *Int. J. Food Microbiol.* **2015**, *196*, 51–61. [CrossRef] [PubMed]
2. Torres, S.; Verón, H.; Contreras, L.; Isla, M.I. An overview of plant-autochthonous microorganisms and fermented vegetable foods. *Food Sci. Hum. Wellness* **2020**, *9*, 112–123. [CrossRef]
3. Wood, B.J.B. Fermentation, Origins and Applications. In *Reference Module in Food Science*; Elsevier: Amsterdam, The Netherlands, 2016.
4. Peñas, E.; Martinez-Villaluenga, C.; Frias, J. Chapter 24—Sauerkraut: Production, Composition, and Health Benefits. In *Fermented Foods in Health and Disease Prevention*; Frias, J., Martinez-Villaluenga, C., Peñas, E., Eds.; Academic Press: Boston, MA, USA, 2017; pp. 557–576.
5. Di Cagno, R.; Coda, R.; De Angelis, M.; Gobbetti, M. Exploitation of vegetables and fruits through lactic acid fermentation. *Food Microbiol.* **2013**, *33*, 1–10. [CrossRef] [PubMed]
6. Coda, R.; Rizzello, C.G.; Gobbetti, M. Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of a functional bread enriched of γ-aminobutyric acid (GABA). *Int. J. Food Microbiol.* **2010**, *137*, 236–245. [CrossRef]
7. Coda, R.; Rizzello, C.G.; Pinto, D.; Gobbetti, M. Selected lactic acid bacteria synthesize antioxidant peptides during sour-dough fermentation of cereal flours. *Appl. Environ. Microbiol.* **2012**, *78*, 1087–1096. [CrossRef]
8. Naeem, M.; Ilyas, M.; Haider, S.; Baig, S.; Saleem, M. Isolation, characterization and identification of lactic acid bacteria from fruit juices and their efficacy against antibiotics. *Pak. J. Bot.* **2012**, *44*, 323–328.
9. Benavides, A.B.; Ulcuango, M.; Yépez, L.; Tenea, G.N. Assessment of the in vitro bioactive properties of lactic acid bacteria isolated from native ecological niches of Ecuador. *Rev. Argent. Microbiol.* **2016**, *48*, 236–244. [CrossRef]
10. Aviles-Gaciola, S.; Chuck-Hernandez, C.; Salvadar, S. Inactivation Methods of Trypsin Inhibitor in Legumes: A Review. *J. Food Sci.* **2018**, *83*, 17–29. [CrossRef]
11. Petruláková, M.; Valík, L. Legumes as potential plants for probiotic strain *Lactobacillus rhamnosus* GG. *Acta Univ. Agric. Silv.* **2015**, *63*, 1505–1511. [CrossRef]
12. Cichońska, P.; Ziarno, M. Legumes and Legume-Based Beverages Fermented with Lactic Acid Bacteria as a Potential Carrier of Probiotics and Prebiotics. *Microorganisms* **2022**, *10*, 91. [CrossRef]
13. Tangyu, M.; Muller, J.; Bolten, C.J.; Wittmann, C. Fermentation of plant-based milk alternatives for improved flavour and nutritional value. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 9263–9275. [CrossRef] [PubMed]
14. McClements, D.J. Development of Next-Generation Nutritionally Fortified Plant-Based Milk Substitutes: Structural Design Principles. *Foods* **2020**, *9*, 421. [CrossRef] [PubMed]

15. Aydar, E.F.; Tutuncu, S.; Ozcelik, B. Plant-based milk substitutes: Bioactive compounds, conventional and novel processes, bioavailability studies, and health effects. *J. Funct. Foods* **2020**, *70*, 103975. [CrossRef]
16. Tas, O.; Ertugrul, U.; Grunin, L.; Oztop, M.H. An Investigation of Functional Quality Characteristics and Water Interac-tions of Navy Bean, Chickpea, Pea, and Lentil Flours. *Legum. Sci.* **2022**, *4*, e136. [CrossRef]
17. Socrates, G. *Infrared and Raman Characteristic Group Frequencies*, 3rd ed.; John Wiley & Sons: Chichester, UK, 2001.
18. Toor, B.S.; Kaur, A.; Sahota, P.P.; Kaur, J. Antioxidant Potential, Antinutrients, Mineral Composition and FTIR Spectra of Legumes Fermented with *Rhizopus oligosporus*. *Food Technol. Biotechnol.* **2021**, *59*, 530–542. [CrossRef]
19. Radzki, W.; Ziaja-Sołtys, M.; Nowak, J.; Topolska, J.; Bogucka-Kocka, A.; Sławińska, A.; Michalak-Majewska, M.; Jabłońska-Ryś, E.; Kuczumow, A. Impact of Processing on Polysaccharides Obtained from Button Mushroom (*Agaricus Bisporus*). *Int. J. Food Sci. Technol.* **2019**, *54*, 1405–1412. [CrossRef]
20. Dıblan, S.; Kadiroğlu, P.; Aydemir, L.Y. FT-IR spectroscopy characterization and chemometric evaluation of legumes extracted with different solvents. *Food Health* **2018**, *4*, 80–88. [CrossRef]
21. Guerrero, P.; Garrido, T.; Leceta, I.; De La Caba, K. Films based on proteins and polysaccharides: Preparation and physical-chemical characterization. *Eur. Polym. J.* **2013**, *49*, 3713–3721. [CrossRef]
22. Singh, R.K.; Kukrety, A.; Sharma, O.P.; Baranwal, S.; Atray, N.; Ray, S.S. Study of a novel phenolic-ester as antioxidant additive in lube, biodiesel and blended diesel. *J. Ind. Eng. Chem.* **2016**, *37*, 27–31. [CrossRef]
23. Untersmayr, E.; Jensen-Jarolim, E. The role of protein digestibility and antacids on food allergy outcomes. *J. Allergy Clin. Immunol.* **2008**, *121*, 1301–1308. [CrossRef]
24. Licandro, H.; Ho, P.H.; Nguyen, T.K.C.; Petchkongkaew, A.; Van Nguyen, H.; Chu-Ky, S.; Nguyen, T.V.A.; Lorn, D.; Wa-ché, Y. How fermentation by lactic acid bacteria can address safety issues in legumes food products? *Food Control* **2020**, *110*, 106957. [CrossRef]
25. Bogdanov, I.; Finkina, E.; Balandin, S.; Melnikova, D.; Stukacheva, E.; Ovchinnikova, T. Structural and functional characterization of recombinant isoforms of the lentil lipid transfer protein. *Acta Nat.* **2015**, *7*, 65–73. [CrossRef]
26. Shaheen, N.; Halima, O.; Akhter, K.T.; Nuzhat, N.; Rao, R.S.P.; Wilson, R.S.; Ahsan, N. Proteomic characterization of low molecular weight allergens and putative allergen proteins in lentil (*Lens culinaris*) cultivars of Bangladesh. *Food Chem.* **2019**, *297*, 124936. [CrossRef] [PubMed]
27. Finkina, E.I.; Melnikova, D.N.; Bogdanov, I.V.; Matveevskaya, N.S.; Ignatova, A.A.; Toropygin, I.Y.; Ovchinnikova, T.V. Impact of Different Lipid Ligands on the Stability and IgE-Binding Capacity of the Lentil Allergen Len c 3. *Biomolecules* **2020**, *10*, 1668. [CrossRef] [PubMed]
28. López-Torrejón, G.; Salcedo, G.; Martín-Esteban, M.; Díaz-Perales, A.; Pascual, C.Y.; Sánchez-Monge, R. Len c 1, a major allergen and vicilin from lentil seeds: Protein isolation and cDNA cloning. *J. Allergy Clin. Immunol.* **2003**, *112*, 1208–1215. [CrossRef]
29. Finkina, E.I.; Melnikova, D.N.; Bogdanov, I.V.; Ovchinnikova, T.V. Plant Pathogenesis-Related Proteins PR-10 and PR-14 as Components of Innate Immunity System and Ubiquitous Allergens. *Curr. Med. Chem.* **2017**, *24*, 1772–1787. [CrossRef]
30. Sánchez-Monge, R.; Pascual, C.Y.; Díaz-Perales, A.; Fernández-Crespo, J.; Martín- Esteban, M.; Salcedo, G. Isolation and characterization of relevant allergens from boiled lentils. *J. Allergy Clin. Immunol.* **2000**, *106*, 955–961. [CrossRef]
31. Nazeer, M.; Waheed, H.; Saeed, M.; Ali, S.Y.; Choudhary, M.I.; Ul-Haq, Z.; Ahmed, A. Purification and Characterization of a Nonspecific Lipid Transfer Protein 1 (nsLTP1) from Ajwain (*Trachyspermum ammi*) Seeds. *Sci. Rep.* **2019**, *11*, 4148. [CrossRef]
32. Pighin, J.A.; Zheng, H.; Balakshin, L.J.; Goodman, I.P.; Western, T.L.; Jetter, R.; Kunst, L.; Samuels, A.L. Plant cuticular lipid export requires an ABC transporter. *Science* **2004**, *306*, 702–704.
33. Edstam, M.M.; Blomqvist, K.; Eklof, A.; Wennergren, U.; Edqvist, J. Coexpression patterns indicate that GPI-anchored non-specific lipid transfer proteins are involved in accumulation of cuticular wax, suberin and sporopollenin. *Plant Mol. Biol.* **2013**, *83*, 625–649. [CrossRef]
34. Guo, C.; Ge, X.; Ma, H. The rice OsDIL gene plays a role in drought tolerance at vegetative and reproductive stages. *Plant Mol. Biol.* **2013**, *82*, 239–253. [CrossRef] [PubMed]
35. Fich, E.A.; Segerson, N.A.; Rose, J.K. The plant polyester cutin: Biosynthesis, structure, and biological roles. *Annu. Rev. Plant Biol.* **2016**, *67*, 207–233. [CrossRef] [PubMed]
36. Gangadhar, B.H.; Sajeesh, K.; Venkatesh, J.; Baskar, V.; Abhinandan, K.; Yu, J.W.; Prasad, R.; Mishra, R.K. Enhanced Tolerance of Transgenic Potato Plants Over-Expressing Non-specific Lipid Transfer Protein-1 (StnsLTP1) against Multiple Abiotic Stresses. *Front Plant Sci.* **2016**, *7*, 1228. [CrossRef] [PubMed]
37. Renault, H.; Alber, A.; Horst, N.A.; Basilio Lopes, A.; Fich, E.A.; Kriegshauser, L.; Wiedemann, G.; Ullmann, P.; Herrgott, L.; Erhardt, M.; et al. A phenol-enriched cuticle is ancestral to lignin evolution in land plants. *Nat. Commun.* **2017**, *8*, 14713. [CrossRef]
38. Palacín, A.; Gómez-Casado, C.; Rivas, L.A.; Aguirre, J.; Tordesillas, L.; Bartra, J.; Blanco, C.; Carrillo, T.; Cuesta-Herranz, J.; de Frutos, C.; et al. Graph based study of allergen cross-reactivity of plant lipid transfer proteins (LTPs) using microarray in a multicenter study. *PLoS ONE*. **2012**, *7*, 50799.
39. Van Winkle, R.C.; Chang, C. The biochemical basis and clinical evidence of food allergy due to lipid transfer proteins: A comprehensive review. *Clin. Rev. Allergy Immunol.* **2014**, *46*, 211–224. [CrossRef]
40. Emkani, M.; Oliete, B.; Saurel, R. Effect of Lactic Acid Fermentation on Legume Protein Properties, a Review. *Fermentation* **2022**, *8*, 244. [CrossRef]

41. Aguirre, L.; Garro, M.S.; De Giori, G.S. Enzymatic hydrolysis of soybean protein using lactic acid bacteria. *Food Chem.* **2008**, *111*, 976–982. [CrossRef]
42. Yang, A.; Zuo, L.; Cheng, Y.; Wu, Z.; Li, X.; Tong, P.; Chen, H. Degradation of major allergens and allergenicity reduction of soybean meal through solid-state fermentation with microorganisms. *Food Funct.* **2018**, *9*, 1899–1909. [CrossRef]
43. Chew, K.L.; Octavia, S.; Lai, D.; Lin, R.; Teo, J. Genomic Characterization of *Klebsiella quasipneumoniae* from Clinical Specimens in Singapore. *Antimicrob. Agents Chemother.* **2021**, *65*, e0041221. [CrossRef]
44. Sáez, G.D.; Hébert, E.M.; Saavedra, L.; Zárate, G. Molecular identification and technological characterization of lactic acid bacteria isolated from fermented kidney beans flours (*Phaseolus vulgaris* L. and *P. coccineus*) in north-western Argentina. *Food Res. Int.* **2017**, *102*, 605–615. [CrossRef]